Diagnosis of Tuberculosis: Nanodiagnostic Approaches

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Abstract

Tuberculosis (TB) remains one of the most devastating infectious diseases worldwide. The burden of TB is alarmingly high in developing countries, where diagnosis latent TB infection (LTBI), Extra-pulmonary tuberculosis (EPTB), drug-resistant tuberculosis (DR-TB), HIV-associated TB, and paediatric TB is still a challenge. This is mainly due to delayed or misdiagnosis of TB, which continues to fuel its worldwide epidemic. The ideal diagnostic test is still unavailable, and conventional methods remain a necessity for TB diagnosis, though with poor diagnostic ability. The nanoparticles have shown potential for the improvement of drug delivery, reducing treatment frequency and diagnosis of various diseases. The engineering of antigens/antibody nanocarriers represents an exciting front in the field of diagnostics, potentially flagging the way toward development of better diagnostics for TB. This chapter discusses the presently available tests for TB diagnostics and also highlights the recent advancement in the nanotechnology-based detection tests for \textit{M. tuberculosis}.

Keywords

\textit{M. tuberculosis} · Nano diagnostics · Nanoparticles

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11.1 Introduction

Tuberculosis (TB), caused by an aerobic, acid-fast bacillus, that is, *Mycobacterium tuberculosis* (*M. tuberculosis*), to humans, and it is still a major public health problem worldwide (Kerantzas and Jacobs 2017). For many decades, it has continued to pose a significant threat to human health (WHO 2017). The situation becomes critical by the increasing incidence of multidrug-resistant (MDR) forms of *M. tuberculosis*, that is, resistance to both isoniazid (INH) and rifampicin (RIF) and now, extensively Drug-Resistant Tuberculosis (XDR-TB) strains, that is, MDR TB strains plus resistance to any fluoroquinolone and at least one of three injectable second-line drugs (amikacin, kanamycin, or capreomycin) that is virtually untreatable (CDC 2006). It has been estimated that almost billions of peoples will be newly affected with TB between 2000 and 2020. WHO estimated 10.4 million new cases and more than 1.3 million deaths in 2016 (WHO 2017). Among the estimated 10.4 million incident cases, 10% were children and 35% were female. In 2016, 153,119 cases of multidrug-resistant TB and rifampicin-resistant TB (MDR/RR-TB) were reported to the World Health Organization (WHO) globally (WHO 2017). Most of the TB cases reported in 2016 occurred in Asia (56%) and the African Region (29%). The smaller proportions of cases were reported in the Eastern Mediterranean Region (8%), European Region (4%), and the Region of the Americas (3%).

A fast and reliable laboratory diagnosis would help in the control of TB especially in the high burden countries. Current TB diagnostics mostly depend on the identification of *M. tuberculosis* by acid-fast bacilli (AFB) staining directly from clinical specimens, culture, and molecular tests. Although, smear microscopy permits the rapid detection of mycobacteria in clinical samples, it has comparatively low sensitivity and requires at least $5 \times 10^3$ bacilli/ml in the specimen (Desikan 2013). Also, it has a higher failure rate in children and immuno-compromised groups such as Acquired Immuno Deficiency Syndrome (AIDS) (Desikan 2013; Tuberculosis Division 2005).

The solid culture method is based on the visible appearance of growth on the medium, but it takes very long time for detection (up to 60 days), while automated liquid culture system, that is, Mycobacterial Growth Indicator Tube (MGIT) 960 system (Recommended by WHO for liquid culture and drug susceptibility test) is slightly better than solid culture due to reduced time duration required (42 days) for bacilli detection (Chihota et al. 2010; Lawson et al. 2013). However, it requires longer duration to obtain the results and also specific laboratory facilities, which may be unreachable by resource-limited or poor countries. Immunological approaches, such as the Tuberculin Skin Test (TST) and IFN-Gamma Release Assay (IGRA) have been developed for the detection of TB/latent TB infection (LTBI) (Lagrange et al. 2013, 2014; Pai et al. 2004). However, both TST and IGRA failed to distinguish between latent TB and active TB infection in the high burden countries (Chegou et al. 2009; Ra et al. 2011).

Current nucleic acid amplification-based tests (NAAT), that is, Polymerase Chain Reaction (PCR) (Gopinath and Singh 2009), Xpert MTB/Rif assay (Rufai et al. 2017), Loop-mediated isothermal amplification (LAMP) (Kumar et al. 2014)
and Line Probe Assay (LPA) (Rufai et al. 2014) are able to detect \textit{M. tuberculosis} within few hours to days in suspected TB patients than culture methods and play an important role in the patient care and TB control programmes.

Despite all advances in TB diagnosis landscape, there is no accurate, rapid, inexpensive, point-of-care assay available for \textit{M. tuberculosis} detection, well-matched for children, extrapulmonary TB (EPTB) and HIV associated TB (HIV-TB)(Kozel and Burnham-Marusich 2017). Furthermore, in developing countries, like India and Pakistan, where resources are very limited and the requirement of sophisticated, costly instruments becomes an extraburden due to the requirement of trained technicians to perform the tests, which directly or indirectly increases the diagnostic cost. Therefore, an improvised version and/or new diagnostic test/techniques are urgently required for the prevention and treatment of \textit{M. tuberculosis} infection to fulfil the unmet demands (Singh et al. 2015). From these viewpoints, diagnostic test based on nanotechnology can offer fast and efficient alternative methods for TB detection (Caliendo et al. 2013).

Nanotechnology, known as general purpose technology, utilizes nanoscale molecules ranging from 1 nm to100 nm. It plays a key role in the development of many fields such as automotive, textile, electronics, food, healthcare, and due to its unique characteristics, it is useful in optical, mechanical, magnetic, catalytic, and electrical perspectives (Chaturvedi et al. 2012). For the past several decades, biomedical applications such as tissue engineering, drug delivery, bioimaging, and nanodiagnostics have been developed by utilizing the concept of nanotechnology. Among these applications, nanodiagnostics-based rapid test has drawn more and more attention for infectious diseases due to its unique characteristics in the early detection with high sensitivity and specificity (Wang et al. 2017).

These potential of nanodiagnostics opened the door for development of portable, robust, and affordable POCs, which can detect infectious diseases very efficiently (Sharma and Bhargava 2013; Wang et al. 2017; Singh et al. 2017). In this direction, various innovative and efficient nanodiagnostics have been developed by researchers for infectious diseases including TB. Laksanasopin et al. (2015) developed a smartphone-based POC to diagnose infectious diseases by connecting traditional immunoassay into a smartphone via accessories such as dongle (Laksanasopin et al. 2015). Hence, nanodiagnostics-based POCs are promising tools for rapid detection of infectious diseases and could be exploited in the near future for different clinical requirements.

In this chapter, we highlight prospects of the advances in the nanotechnology-based diagnostic methods that can offer better solutions for diagnosis of \textit{M. tuberculosis} infections.

### 11.2 Diagnosis of Tuberculosis

The TB diagnostic method can be divided into three categories: conventional methods, immunological methods, and new diagnostic methods.
11.2.1 Conventional Methods

Microscopy is possibly the earliest and most rapid procedure that can be performed in the laboratory to detect the presence of AFB, Adenosine Deaminase Activity (ADA), culture (egg-based solid media like Lowenstein–Jensen medium, agar-based medium like Middlebrook 7H10), which is shown in Table 11.1.

11.2.2 Immunological Methods

Enzyme-linked immunosorbent assay (ELISA), tuberculin skin test (TST), interferon-gamma determination, and tuberculin test are discussed in Table 11.1 above.

11.2.3 New Diagnostic Methods

Automated culture methods (BECTEC 460 TB (Aggarwal et al. 2008), BECTEC MGIT™ 960 (Rodrigues et al. 2009), Versa TREK and BacT/ALERT 3D (Mirrett et al. 2007), Nucleic acid amplification methods (amplified MTD, amplified M. tuberculosis direct test (AMTD) (Goessens et al. 2005; Reischl et al. 1998), transcriptase-mediated amplification system and amplicor MTB test (Wang and Tay 1999), Multiplex Polymerase Chain Reaction (PCR) (Gopinath and Singh 2009), LAMP (Yadav et al. 2017), Real-time PCR (Watanabe Pinhata et al. 2015), LPA (Desikan et al. 2017), Xpert MTB/RIF assay (Osman et al. 2014). Genetic identification methods: PCR restriction-enzyme analysis, RFLP (Gómez Marín et al. 1995), Spoligo typing (Mistry et al. 2002), DNA probes (Badak et al. 1999) and DNA sequencing (Brown et al. 2015), etc. are shown in Table 11.1. Other molecular tests that are under development or under evaluation have been mentioned in Table 11.2.

11.3 Diagnostic Gaps Between Existing Technologies and Its Unmet Clinical Need

M. tuberculosis was identified more than a century ago, and its diagnosis in the developing world still remains a major healthcare issue owing to a number of challenges, listed below. First, M. tuberculosis is a slow-growing bacterium, and therefore it cannot provide direction for on-site patient care. Second, the PTB patient do not develop symptoms at the early stage of infection, which lead to delays in seeking patient care (Parsons et al. 2011; Kritski et al. 2013). Third, even the active PTB cases often exhibit low bacteria count of sputum thus making it difficult to detect with smear microscopy and other commonly used POC diagnostic tests in the developing world. Fourth, use of sputum and other invasive body fluids in the diagnosis
### Table 11.1 Summary of the available diagnostic tests/methods for TB

| Technology, test | Stage of development | Developer(s)/supplier(s) | Level of the health system | DST utility |
|------------------|----------------------|--------------------------|-----------------------------|-------------|
| **A: Conventional method** |
| **Direct visualization (Microscopy)** |
| Conventional microscopy with acid-fast staining | In routine use | Multiple | Microscopy | No |
| Fluorescent microscopy with nonspecific cell-wall staining | In routine use | Multiple | Microscopy | No |
| Fluorescent microscopy with LED light source | In routine use | Various | Microscopy | No |
| **Growth-based detection (Culture)** |
| Conventional solid media | Commercialized reagents and prepared media | Multiple | Referral | Yes |
| LJ, Middlebrook 7H10/7H11 agar, 7H9/7H12/Dubos medium | | | | |
| Automated liquid culture systems | Commercialized, under study for feasibility and impact of use in resource-limited settings | BD, BioMerieux, Thermofisher | Referral | Yes |
| MGIT 960, BacT/ALERT 3D, VersaTREK Myco, etc. | | | | |
| MODS assay, thin-layer culture | Academic evaluations published | Non-commercial testing methods | Referral | Yes |
| Phage-based detection | Commercialized, improved test in development | Biotec | Referral | Yes |
| **B: Immunological method** |
| **Latent Tuberculosis Infection detection** |
| Tuberculin skin test with PPD | Commercialized | Multiple | Microscopy | No |
| Whole-blood IFN-γ release assay | Commercialized; in evaluation for disease-endemic countries | Cellestis | Referral | No |
| ELISPOT IFN-γ release assay | Commercialized; in evaluation for disease-endemic countries | Oxford Immunotech | Referral | No |
| **Antigen detection (Immunodiagnosis)** |
| TB-derived antigen detection in urine or other clinical material | In development | Various | Research centre | No |

(continued)
### Table 11.1 (continued)

| Technology, test | Stage of development | Developer(s)/ supplier(s) | Level of the health system | DST utility |
|------------------|----------------------|---------------------------|----------------------------|-------------|
| TB-derived antigen detection in exhaled air/breath | In evaluation | Rapid Biosensor Systems | Health centre | No |

#### Antibody detection (Immunodiagnostic)

| Detection of diagnostic antibody responses to TB | WHO banned all existing commercial test | Various | Health centre | No |

#### C: Molecular detection

| Automated, non-integrated NAAT | Commercialized | GenProbe, Roche, | Referral | No |
| Automated, integrated NAAT | Commercialized | Cepheid | Referral | Yes |
| Simplified manual NAAT (LAMP) | In evaluation | Eiken | Referral | No |
| Non-amplified probe detection | In development | Investigen, | Microscopy | No |
| GeneXpert | Commercialized | Cepheid, USA | Referral | Yes |

Modified from Pai and O’Brien (2008)

### Table 11.2  Tuberculosis diagnostics pipeline (2016): Products in later-stage development or on track for evaluation by WHO

#### New molecular diagnostics

| S. No. | Test | Type | Developer(s)/ supplier(s) | Status | Comments |
|--------|------|------|---------------------------|--------|----------|
| 1.     | BD MAX MTB assay | qPCR for MTB in automated BD MAX | Becton, Dickinson | 100% sensitivity and 97.1% specificity with smear-positive samples (Rocchetti et al. 2016) | Under the evaluation stage |
| 2.     | EasyNAT | Isothermal DNA amplification / lateral flow to detect MTB | Ustar | Poor sensitivity, especially for smear-negative specimens, in Tanzanian field study (Bholla et al. 2016; Mhimbira et al. 2015) |
| 3.     | FluoroType MTB | Semi-automated direct MTB detection; PCR in a closed system; results in 3 h | Hain Lifescience | Sensitivity 88%, specificity 98% (Bwanga et al. 2015; Obasanya et al. 2017) | Marketed |

(continued)
### Table 11.2 (continued)

New molecular diagnostics

| S. No. | Test Type | Developer(s)/ supplier(s) | Status | Comments |
|--------|-----------|----------------------------|--------|----------|
| 4.     | GeneChip RT-PCR for RIF + INH DR | CapitalBio | MarkedBio | CCDCP and University of Georgia published a paper on 1400 samples from SW China (Sensitivity 83–94.6%, specificity 91.3–98%) (Zhang et al. 2018; Zhu et al. 2015) | Marketed |
| 5.     | Genedrive MTB/RIF Portable RT-PCR for MTB + RIF resistance | Epistem | Lower sensitivity (45.4%) (Shenai et al. 2016) | Marketed in India |
| 6.     | GenoType MTBDRplus Line probe assay for RIF + INH resistance | Hain Life science | (Sensitivity 90.3% and specificity 98.5%) (Nathavitharana et al. 2016) | WHO recommended |
| 7.     | LiPA pyrazinamide Line probe assay for PZA resistance | Nipro | High sensitivity (65.9–100%) and specificity (98.2–100%) (Rienthong et al. 2015) | Marketed |
| 8.     | LiPA MDR-TB Line probe assay for RIF + INH resistance | Nipro | Sensitivity 89% and 99.4% Specificity (Havumaki et al. 2017) | Marketed |
| 9.     | REBA MTB-MDR Line probe assay for RIF + INH resistance | YD Diagnostics | (Havumaki et al. 2017) | Marketed |
| 10.    | REBA MTB-XDR Line probe assay for FQ + SLID DR | YD Diagnostics | Initial study 2015 (Jaksuwan et al. 2018; Lee et al. 2015) | Marketed |
| 11.    | MeltPro TB/INH Closed-tube RT-PCR for INH DR | Zeesan Biotech | 3-site evaluation of 1096 clinical isolates (Liang et al. 2018; Pang et al. 2016) | Chinese FDA-approved |
| 12.    | MeltPro TB/STR Closed-tube RT-PCR for streptomycin DR | Zeesan Biotech | 3-site evaluation of 1056 clinical isolates (Zhang et al. 2015) | WHO guidance pending |
| 13.    | PURE-LAMP Manual NAAT by LAMP for MTB detection | Eiken | Eddabra and AitBenhassou (2018) | WHO review |

(continued)
of TB with existing techniques is more complex compared to blood and urine samples (Sharma et al. 2015). The unavailability of accurate and validated biomarkers (for Active TB and LTBI infection) either derived from host or pathogen are due to inadequate knowledge of the host–pathogen interaction, pathogenesis, and protected immune response generated by *M. tuberculosis* during infection, which limited utility of rapid diagnostic test of TB (Goletti et al. 2016).

Despite exiting technologies, development of simple POCs test in the near future is still challenging in the current TB diagnostics pipeline (Pai and Nathavitharana 2014). Although Xpert MTB provides same-day detection, its use is limited by its cost and poor detection rate in extra-pulmonary tuberculosis (EPTB) (Rufai et al. 2017). Hence, there is an urgent need of inexpensive TB diagnostic test for resource-limited settings to miniaturize TB diagnosis, which can be done by using a novel nanotechnology approach.

### 11.4 Nanotechnology

#### 11.4.1 Nanoparticles

Nanoparticle (NP) is a small particle less than 100 nm in diameter. The unique property depends on the size and composition of the particles compared to atoms and other materials. These properties includes: (1) large to volume ratio (metal NP, in
particular gold NP), (2) surface plasmon resonance, (3) Surface-Enhanced Raman Scattering (SERS), (4) super-magnetization or ferromagnetic nanoparticles (e.g. iron oxide), (5) enhanced photoluminescence (semiconductor quantum dots), (6) high electric and heat conductivity, (7) potent surface catalytic activity (Gatoo et al. 2014; Khan et al. 2019). The combination of nanoparticles with biology has led to the development of various diagnostic test/devices, contrast agents, analytical tools, physical therapy, and drug delivery systems. Since biomolecules and cellular organelles lie in the nanosized range, NPs can be altered with various biomolecules, such as antibodies, nucleic acid, peptides (Jacob and Deigner 2018; Wang and Wang 2014). Such manipulations enable NPs to be extremely useful in both in vivo and in vitro biomedical research and applications (Curtis and Wilkinson 2001). A schematic presentation of a core/shell nanoparticle for multipurpose biomedical applications is shown in Fig. 11.1.

11.4.2 Types of Nanoparticle-Based Platforms

The designing of nanodiagnoses are based on the binding of a labelled nanoparticle or probe to the target biomolecule, generates a quantifiable electric signal characteristic of the target biomolecules (Alharbi and Al-sheikh 2014). The most promising approaches include nanoparticles (carbon and gold nanoparticles), nanotubes, nanoshells, nanopores, quantum dots (QDs), and nanocantilever technologies, which display promising activity in the diagnostic applications (Capek 2016;
Table 11.3  Types of nanodevices used in clinical applications

| S. No | Nonodevices                  | Applications                                      |
|-------|------------------------------|---------------------------------------------------|
| 1.    | Cantilevers                  | High thoughtful screening                          |
|       |                              | Protein biomarkers detection                       |
|       |                              | SNPs                                              |
|       |                              | Gene expression detection                         |
| 2.    | Carbon Nanotubes             | SNPs                                              |
|       |                              | Protein biomarkers detection                       |
| 3.    | Dendrimers                   | Image contrast agents                              |
| 4.    | Nanocrystals                 | Improved formulation for poorly soluble drugs     |
| 5.    | Nanoparticles                | Target drug delivery                              |
|       |                              | MRI, USG image contrast agents                     |
|       |                              | Reporters of apoptosis                             |
| 6.    | Nanoshell                    | Tumour-specific imaging                            |
| 7.    | Nanowires                    | High thoughtful screening                          |
|       |                              | Disease protein biomarkers detection              |
|       |                              | SNPs                                              |
|       |                              | Gene expression analysis                          |
| 8.    | Quantum dots                 | Optical detection of genes and proteins in animal |
|       |                              | Cell assays                                       |
|       |                              | Visualization of tumour and lymph node in human   |

Mancebo 2009). The QDs are semiconductor nanocrystals which are characterized by strong light absorbance, and they can be used as fluorescent labels/tag for the detection of biomolecules. The cantilevers and QDs are the most promising nanostructures, which are mainly characterized by high photostability, single-wavelength excitation, and size-tunable emission (Azzazy et al. 2006; Rizvi et al. 2010). Different types of nanostructure or nanodevices that are used for specific purposes are listed in Table 11.3.
11.4.3 Nanoparticle-Based Diagnostics

Nano diagnostics, referred to as the use of nanotechnology in diagnostic applications, has been widely explored for the development of diagnostic tests with high sensitivity and prior detection of infection. The nanoscale size and high surface-to-volume ratio of nanoparticles make this field superior and indispensable in multifield of human action. The unique properties of nanomaterials or nanostructures deliberate the nanodiagnostic platforms and ability of rapid detection by utilizing very small volumes of clinical samples (Jackson et al. 2017). The technology itself is variegated, and several options are available, for instance, nanosuspensions, nanoemulsions, niosomes (nonionic surfactant-based vesicles). Therefore, nanodiagnostic approaches have strong potential to be cost-effective, user-friendly, and robust (Azzazy et al. 2006; Kumar et al. 2011; Wang et al. 2017).

The significant progress has been made in the field of nanotechnology in the last two decades, which showed its wide potential and advantageous applications in the field of biomedicine, biotechnology, human and animal health including nanodiagnostics and nanomedicines. Majority of the nanodiagnostic work has been carried out in the field of cancer diagnostics, but this technique has also been contributed significantly to the diagnosis of various infectious diseases presently (Kumar et al. 2011; Yukuyama et al. 2017). Most of the infectious disease-causing agents such as bacteria (*M. tuberculosis*), virus (SARS), and fungi may sometimes cause an epidemic outbreak, resulting in higher morbidity and mortality (Mathuria 2009; Nasiruddin et al. 2017; Xu et al. 2018). Thus, initiation of nano-based diagnostic platforms in a clinical setting is gaining importance these days. This is because of the ability of nanodiagnostics to achieve consistency, quick conclusions with simple and movable devices by using various body fluids, such as blood, sputum, or urine samples from patients (Banyal et al. 2013; Wang et al. 2017).

In addition, the highly sensitive nanodiagnostics platforms, with strong potential must be robust, cost effective, and reproducible and could be extremely applicable for the diagnosis of infectious diseases, especially in resource-limited areas in the developing countries.

11.4.4 Gold Nanoparticle (AuNPs)-Based Diagnostics for TB

The gold nanoparticles (AuNPs) pose unique physiochemical (inert and nontoxic) and optical characteristics making them most appropriate nanomaterial for clinical diagnosis, treatments, and other multidisciplinary research. The optical property of AuNPs with antibody or antigen and other biomolecules enable their utility in the diagnosis of various pathogens. Moreover, AuNPs do not disturb the functional activity even after antigen immobilization (Choi and Frangioni 2010; Sonawane and Nimse 2016). The antibody–antigen reaction is enhanced by the surface functionalization of gold nanoparticles, thereby increasing immunoassay signals, which ultimately increase the test sensitivity (Kim et al. 2018). It offers an easy, low-cost assay, which allows simultaneously numerous sample testing. The assay has been
found to be very specific and produce reliable results even with tiny amount of mycobacterial DNA. The colorimetric detection of target gene/sequence from test DNA samples via AuNP probes (thiol-linked single-stranded DNA, or ssDNA, modified gold nanoparticles) offer a low-cost alternative method for detection (Chandra et al. 2010; Cordeiro et al. 2016).

The utilization of AuNPs was firstly reported in TB diagnosis by (Baptista et al. 2008), which utilized DNA probes (oligonucleotide derived from the gene sequence of the *M. tuberculosis* RNA polymerase subunit) coupled with AuNPs for the colorimetric detection of *M. tuberculosis*. Principally, at wavelength 526 nm, if the complementary DNA is present, the nanoprobe solution remains pink in colour (no DNA probe aggregation), while the solution turns purple (due to nanoprobe aggregation at a high NaCl concentration) in the absence of complementary DNA in the samples. The method is more accurate when compared to other diagnostic methods, that is, InnoLiPA-Rif-TB, which gave 100% concordance (Baptista et al. 2008). The test was proved to be more sensitive than smear microscopy and can be simply visualized for detection. The major advantage of this method is that the chances of contamination is very less (carried out in a single tube reducing contamination), rapid (takes approximately 15 min per sample).

Subsequently, activity of this method was also compared with automated liquid culture system (BACTEC™ MGIT™) and semi-nested PCR, which shows greater sensitivity and specificity of the test in the detection of *M. tuberculosis* complex (Baptista et al. 2008; Cordeiro et al. 2016). Insertion sequence (IS6110) of *M. tuberculosis* was also used to increase the sensitivity of this test along with microfluidics technology, which utilized calorimetric detection of AuNPs coupled with IS6110 sequence (Tsai et al. 2017).

Surface Plasmon Resonance (SPR) has attracted much attention for novel metal (Au), which gives a red colour to the AuNPs colloid. The method is based on the real-time monitoring of changes happening in the surface refractive index, formed by association or dissociation of the molecules from the sensor (Khan et al. 2019). The major advantage of the SPR-based test is its optical sensor sensitivity making it capable of detecting even tiny amount of disease-specific analyte from the complex fluid without any specific procedure (Masson 2017; Nguyen et al. 2015; Wang and Fan 2016). Due to these advantages, SPR has emerged as a powerful optical tool, which can provide valuable data in the analysis of biomedical and chemical analyses. The SPR-based CFP-10 antigen detection system was developed in clinical samples by Yang et al. (2014), which showed reputable usefulness in TB diagnostics (Hsieh et al. 2012; Yang et al. 2014).

Zhu et al. (2017) developed AuNPs modified indium tin oxide (ITO) electrode for the direct detection of *M. tuberculosis* using genomic DNA (gDNA) isolated directly from clinical samples. The method utilized two probes: capture probe and gold nanoprobe coupled with alkaline phosphatase (ALP) enzyme as detection probe. First, ITO probe is activated via capture probe, then activated probe is immersed in the gDNA containing hybridization buffer to form double strand DNA (dsDNA) via hybridization of probe and target nucleotide sequence. Finally, ITO is placed as electrode in the buffer containing detection probe to generate
11.4.5 AuNP-Mediated Dipstick Assay

The colloidal AuNPs were coated with the *M. tuberculosis* antigen using alkanethiols derivatives and anti-MTB rabbit antibodies. These antigen-coated AuNPs act as a counter or detector reagent in this assay. The serum samples or antibody immobilized on the nitrocellulose (NC) membrane binds to the *M. tuberculosis* antigen coated on AuNPs. Resultant binding could be visually detected by naked eye, due to the development of the red colour formed by the gold nanoparticles on the nitrocellulose membrane (NC) (Stephen et al. 2015).

11.4.6 Silica Nanoparticles-Based Detection

The application of mesoporous silica nanoparticle (SiO₂NPs) has been reported in the various fields, that is, imaging, drug delivery, and biosensors (Sun et al. 2015). The indirect immunofluorescence microscopy has been developed by utilizing nanoparticle coupled with fluorescent dye for the detection of *M. tuberculosis*. The technology consists of SYBR Green I mediated assay, which stained only bioconjugated fluorescent silica nanoparticles. The intensity of fluorescent signals is five-fold higher than conventional fluorescence isothiocyanate (FITC)-based detection method. This assay gives promising results within 2 h and therefore is considered to be a promising method for the rapid detection of *M. tuberculosis* (Qin et al. 2007).

11.4.7 Magnetic Nanoparticles-Based Detection

The magnetic nanoparticles (MNPs), nanoscale-sized molecules are present in nature. They harbour favourable features for their usage in the nano-biomedicine, that is, imaging therapy (Akbarzadeh et al. 2012). The surface of MNPs can be easily modified with recognition moieties, that is, antibodies, antibiotics, and carbohydrate, which enable their use for bacterial detection. Super paramagnetic iron oxide nanoparticle (Iron oxide nanoparticles [IONPs], composed of magnetite [Fe₃O₄] or maghemite [γ-Fe₂O₃] nanoparticles) is commonly used in the field of drug therapy, cell tracking, drug delivery by magnetic resonance imaging (MRI) (Cristea et al. 2017; Sabale et al. 2017). Using IONPs coupled with IgG has allowed enhanced detection limit (10⁴ CFU/mL) of bacterial cells significantly by using nano-MALDI platforms (Chiu, 2014). Various studies have reported the use of diagnostic magnetic resonance (DMR) along with iron oxide nanoparticles for the detection of *M. tuberculosis* DNA (Kaittanis et al. 2010; Vallabani and Singh 2018).
Engstrom and his co-workers developed a novel platform using streptavidin tagged magnetic nanobeads labelled with biotin, for the detection of rifampicin mutation in the rpoB gene of M. tuberculosis. The assay comprised of 11 padlock probes (PLPs) targeting 23S ITS region of M. tuberculosis (Engstrom et al. 2013). Of which, one probe was for MTBC detection and another PLP for wild-type and a remaining mixture of nine PLPs are designed for identification of a common mutation in the RRDR-rpoB gene. The detection system is based on the Brownian relaxation principal, and signal is detected via AC susceptometry (Engström et al. 2013).

Efficacy of super-paramagnetic iron oxide (SPIO) nanoparticles has also been tried for improvement of the sensitivity and specificity of MRI systems in TB detection (Sabale et al. 2017). This method is more effective for the diagnosis of TB at the molecular level and also provides a valuable tool for the analysis of antibody–antigen and parasite–host interactions. The procedure includes activation of SPIO nanoparticles using an anti-MTB surface antibody to form conjugates. Then, conjugate was incubated with mycobacterium followed by MRI imaging, which reveals specific target recognition by reducing signal intensity. This method is more specific for the detection of EPTB (Musculoskeletal TB, Central nervous system TB, abdominal TB) (Skoura et al. 2015).

11.4.8 Quantum Dots-Based Detection System

Quantum dots, also known as semiconductor nanocrystals, possess unique optical and physical properties making them suitable for diagnostics developments (Kairdolf et al. 2013; Smith and Nie 2010). The broad absorption spectra, narrow emission spectra, slow excited-state decay rates, and broad absorption cross-sections are major advantages of quantum dots over other fluorescence-based methods (Rizvi et al. 2010). Also, it can identify multiple targets at the same time, which makes it a much sought after application in the identification of various pathogens in single clinical samples (Rizvi et al. 2010).

The hybrid detection system (Quantum dots and magnetic beads) uses M. tuberculosis-specific molecular probes for TB detection. One probe binds to the 23S rRNA gene of the mycobacterium very precisely and the second probe precisely recognizes IS900 conserved sequence in mycobacterium, which was treated on sulphurous acid chromium quantum dots. Subsequently, sandwich is formed after hybridization with target gene sequences of mycobacterium DNA, isolated from suspected samples of TB patients. Then, quantum dot-magnetic bead conjugates are exposed to ultraviolet (UV) light, which emits red fluorescence (visible to the naked eye).

These conjugate detection systems are also highly versatile molecular probes, which can be easily modified according to their diagnostic utility/purposes. The method can identify the unamplified DNA of M. tuberculosis complex directly from clinical samples (Gazouli et al. 2012). A similar study was reported by Liandris and his co-workers who utilized quantum dots of CdSeO3 coupled with streptavidin and species-specific probes, which detect surface antigen of mycobacterium species.
The gDNA of mycobacterial was targeted by a sandwich hybridization, which consisted of two biotinylated probes that would recognize and detect the target DNA specifically. The detection limit is approximately 10^4 cell/mL of the sample (Liandris et al. 2011).

### 11.4.9 Magnetic Barcode Assays

The principal of magnetic barcode (MB) assays is more or less similar to QDs. The assay used specific complementary DNA sequences of *M. tuberculosis* as probes for TB detection (Liong et al. 2013; Wang et al. 2017).

The major differences are the necessity for DNA extraction and PCR amplification, which are not required in quantum dots assay. After DNA is captured by probes, the resultant conjugate is then marked by complementary magnetic nanoparticle probes, which is then detected by nuclear magnetic resonance (NMR) techniques (Chen et al. 2017) (Fig. 11.2).

### 11.4.10 Biosensors-Based Detection System

A biosensor is an analytical system developed for the detection of presences/absences of a specific biological analyte via integrating a bio-recognition element (transduction system, amplifiers, and display unit). The biosensor consists of an analytical device coupled with biological analytes, which report physio-chemical changes in the sensing area (Bhalla et al. 2016; van den Hurk and Evoy 2015; Mehrotra 2016). The biosensor is based on the detection of short nucleotide sequences of *M. tuberculosis* DNA. TB biosensor can be divided into one of the following categories: mass/piezoelectric, biochemical, electrical, and optical sensors (Table 11.4). These sensing platforms are based on the detecting antibody–antigen interaction, nucleic acid hybridization, and whole mycobacterium bacilli (Lim et al. 2015; Prabhakar et al. 2008; Zhou et al. 2011).

![Fig. 11.2 Framework of the procedure of magnetic barcode (MB) assays](image)
Conclusion and Future Perspectives

Presently, approximately 40–50% of TB cases still remain undetected either due to non-availability of diagnostic services, poor awareness in the masses or due to scanty or absence of tubercle bacilli in the clinical samples. In such conditions, tests tare-getting the whole bacilli in clinical samples are missing many cases due to their poor detection sensitivity. This suggested that detection of bacillary by-products or detection of triggered changes in the host-immune response might be an alternative diagnostic approach for TB detection. Although several attempts have been made in this direction, none of these attempts has displayed clear clinical utility.

Nanotechnology is a fast progressing field, which attracts multi-disciplinary teams to target various healthcare challenges in the diagnosis and treatment of infectious diseases, cancer, and cardiovascular diseases. These technologies have contributed significantly in the diagnosis of various bacterial and viral diseases. Most significantly the nano-based technologies help miniaturizing the diagnostic devices and implants. In the field of tuberculosis, which is one of the major killer disease, the application of nanobiotechnology can help management of TB with added advantage of rapidity, ease of performing test, at cheaper rates, and especially useful for resource-limited countries.

### Table 11.4 Comparison of different biosensors developed for *M. tuberculosis* detection

| S. No | Technology                              | Biomarkers                  | Detection Limit               | References                                      |
|-------|-----------------------------------------|-----------------------------|-------------------------------|------------------------------------------------|
| 1.    | QCM                                     | Whole MTB bacilli           | $10^5$ CFU/mL                 | Kaewphinit et al. (2010)                        |
| 2.    | MSPQC                                   | NH3 & CO2 absorption        | $10^2$ CFU/mL                 | Mi et al. (2012)                                |
| 3.    | RBS breath analyser                     | Ag85 B antigen              | 94% sensitivity 79% specificity | Camilleri (2015) and McNerney et al. (2010)    |
| 4.    | Interferometric biosensor               | 38 kDa antigen              | _                             | Wang et al. (2013)                              |
| 5.    | SPR                                     | ssDNA                       | 115 ng/mL (28fM ssDNA)        | Hsu et al. (2013) and Prabowo et al. (2016)    |
| 6.    | SPCE                                    | Ag360 & Ag231               | 1 ng/mL                       | Wang et al. (2013)                              |
| 7.    | Enzymatic sensor                        | Mycolic acid antibody       | _                             | Wang et al. (2013)                              |
| 8.    | Electro-osmosis microchip sensor         | Whole *M. tuberculosis* Bacilli | 100 CFU/mL                   | Hiraiva et al. (2015) and Khairulina et al. (2017) |

Abbreviations: *QCM* quartz crystal microbalance, *MSPQC* multi-channel series piezoelectric quartz crystal, *RBS* rapid biosensor system, *SPR* surface plasmon resonance, *SPCE* screen-printed carbon electrode, *BES* bioelectric sensor, *CFU* colony forming unit
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