Advances in Applied Nano-Bio Technologies

J. Adv. Appl. NanoBio Tech.
ISSN: 2710-4001

Journal web link:
http://www.AANBT.dormaj.com
https://doi.org/10.47277/AANBT/2(4)14

Challenge of biosensors in *Mycobacterium Tuberculosis*

Sargol Mazraedoost[1], Reza Masoumzade[2], Zahra Javidi[1]

[1] Biotechnology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[2] Department of Medical, Shiraz University of Medical Sciences, Shiraz, Iran.

Received:10/07/2021 Accepted:27/07/2021 Published: 20/12/2021

Abstract

Tuberculosis (TB) is a bacterial illness caused by *Mycobacterium tuberculosis* (MTB), and it is a long-term public health risk due to several biological and societal factors. *Tuberculosis* (TB) is a bacterial infection that most commonly affected the lungs and can damage the kidneys, brain, and spine. *Tuberculosis* (TB) is a fatal human illness that has been prevalent for a long time. It is also known as "consumption" or "phthisis."*M. tuberculosis* is thought to have killed more people than any other bacterial infection. As a result, early detection of this bacterial infection is critical for patients to get prompt and suitable therapy. In underdeveloped countries, more than 98% of *tuberculosis* cases are recorded. Effective diagnosis approaches based on biosensors are required for these bacteria due to a shortage of well-equipped and specialized diagnostic facilities.

Keywords: *Tuberculosis Diagnosis, Mycobacterium Tuberculosis, Detection, Biosensors*

1 Introduction

Tuberculosis (TB) is an ancient bacterial illness that has infected people for over 40 000 years and has developed from animal domestication [1]. A total of 7,163 CASES of *tuberculosis* illness were recorded in 2020. In the face of the extensive use of a diminished live vaccine and numerous medicines, *tuberculosis* (TB), one of the first recognized human illnesses, is among the most common causes of mortality among infectious diseases. To stop the worldwide TB pandemic, which kills two million people per year, new vaccinations and medicines are needed. It is critical to understand the physiology and genetics of *M. TB* and similar *mycobacteria* to design antitubercular novel drugs rationally [2-4]. It is also crucial to comprehend the *M. tuberculosis*-host interface to determine if these bacteria get past the host's immune system and cause sickness [5-8]. Although *tuberculosis* is primarily a pulmonary infection produced by the deposition of *M. TB* onto lung alveolar surfaces through aerosol particles, it can harm bone, the central nerve system, and other organ systems. The disease can take several different paths, all of which are primarily dictated by the immune system's reaction to the host. Internal variables such as immune system genetics and external factors such as immune system insults, the host's nutritional and physiological condition impact the efficacy of this reaction [9-11].

Corresponding author: Sargol Mazraedoost, Biotechnology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
Sargol.mazraedoost7@gmail.com

Furthermore, the pathogen might be responsible for disease development; meanwhile, various *M. TB* strains are more infectious than others; infected persons have higher morbidity and mortality, as demonstrated by increased transmissibility. Regardless of the widespread use of an attenuated live vaccination and a variety of treatments, TB diagnoses are higher than ever, prompting the development of novel vaccines and therapies, as well as more precise and quick diagnostics. Researchers are using data from *Mycobacterium tuberculosis'* complete genome sequence, innovative genetic and physiological approaches to classify objects in *M. TB* that will assist in developing these much-looked-for antitubercular medicines [12-14]. The rise of multidrug resistance (MDR), drug resistance, and Pan drug-resistant bacteria (PDR or XDR) is a severe issue in the fight against *tuberculosis*. Roughly resistant bacterial strains have no known therapies, and they might spread fast throughout the planet [15-17].

The countries with the highest frequency of MDR TB infection include China, Eastern Europe, and Iran [18]. The World Health Organization (WHO) has recommended the reporting incidents treatment, a short course method that effectively prevents drug resistance in *tuberculosis* [19]. Current pulmonary *tuberculosis* treatment methods need clinical processing, laboratory materials, and techniques, including culture methods, X-radiography, sputum smear microscopy, and the tuberculin skin test. Furthermore, modern extrapulmonary TB diagnostic techniques, such as biopsy of various bodily parts, should be done regularly to vouch for preliminary results in instances with extrapulmonary TB [19]. So, the progress of rapid, accurate, easy, and sensitive detection methods is serious [2-4, 9-11, 13, 14, 16, 20-42].

The countries with the highest frequency of MDR TB infection include China, Eastern Europe, and Iran [18]. The World Health

Organization (WHO) has recommended the reporting incidents treatment, a short course method that effectively prevents drug resistance in *tuberculosis* [19]. Current pulmonary *tuberculosis* treatment methods need clinical processing, laboratory materials, and techniques, including culture methods, X-radiography, sputum smear microscopy, and the tuberculin skin test. Furthermore, modern extrapulmonary TB diagnostic techniques, such as biopsy of various bodily parts, should be done regularly to vouch for preliminary results in instances with extrapulmonary TB [19]. So, the progress of rapid, accurate, easy, and sensitive detection methods is serious [2-4, 9-11, 13, 14, 16, 20-42].

2 *Tuberculosis* Types

The illness is divided into two types:

Latent TB. Your body includes bacteria; however, your immune system prevents them from spreading. You are not infectious and do not have any symptoms. Nevertheless, the bacteria is still alive and might resurface at any time. Your doctor will prescribe medications to prevent active TB if you have HIV, have had an infection in the previous two years, have an abnormal chest X-ray, or have a compromised immune system.

Active TB. The bacteria proliferate and cause you to become ill. You can spread the disease to others. In adults, 90% of active cases are caused by latent *tuberculosis* infection.
Whether latent or active, a drug-resistant TB infection means that some treatments will not work in contrast to the bacterium [43]:

***Tuberculosis* Signs and Symptoms**
There are no signs of latent *tuberculosis*. A blood test or skin can discover it.

3 Signs of active TB disease contain:
- A cough that lasts more than three weeks
- Night sweats
- Chest pain
- Feeling tired all the time
- Weight loss
- Coughing up blood
- Loss of appetite
- Fever
- *Tuberculosis* Tests and Diagnosis
- Chills

4 For *M. tuberculosis*, there are two standard tests:

- **Skin test**. The Mantoux tuberculin skin test is another name for this. A tiny quantity of fluid is injected into your lower arm's skin by a technician. They will assess your arm for swelling after 2 or 3 days. If your findings are positive, you are most likely infected with *tuberculosis*. However, a false positive is possible. If you have had the *bacillus Calmette-Guerin (BCG) tuberculosis* vaccination, The test may show that you have *tuberculosis* when you don't. If you have a relatively new infection, the findings can also be false negative, indicating that you do not have *tuberculosis* when you have. This exam may be given to you more than once.
- **Blood test**. While TB proteins are combined with a small amount of your blood, these tests, also known as interferon-gamma release assays (IGRAs), assess the reaction.

These tests will not reveal whether your infection is dormant or active. Your doctor will figure out which kind you have if you get a positive blood test or skin:
- A CT scan or chest X-ray to evaluate for lung changes
- Acid-fast bacillus (AFB) checks for *tuberculosis* bacteria in sputum or cough mucus.

5 *M. Tuberculosis* Treatment

The type of infection you have will determine how you are treated:

Your doctor will prescribe medications to kill the bacteria and prevent the infection from becoming active if you have latent TB. Rifapentine, Isoniazid, or Rifampin can be used individually or in combination. You may have to take medicine for up to 9 months. Active *tuberculosis* is also treated with a mix of medications. Ethambutol, pyrazinamide, isoniazid, and rifampin are the furthermost frequent. You will be taking them for 6 to 12 months. Your doctor may prescribe one or more medicines if you have drug-resistant TB. You could have to take them for a longer time, up to 30 months, and you might have more serious side effects.
Even if you feel better, it is critical to finish all of your prescriptions, regardless of the type of illness. If you stop using the medications too soon, the bacteria may grow resistant to them. The severe worldwide public health burden of *tuberculosis* necessitates the development of different quick and sensitive detection methods. As yet, numerous procedures and methods have been established for rapid detection of *M. TB*, for instance, polymerase chain reaction (PCR) [44-47], latex agglutination [48], enzyme-linked immunosorbent assay (ELISA) [49-51], radiometric detection [52], Gen-Probe amplified *M. TB* direct test (AMTDT) [53], TB rapid cultivation detection method, for instance, MB/Bact system, BactecMGIT 960 system [54, 55] and flow cytometry [56]. As appeared in **Table 1**, Traditional microbial culture-based techniques are less sensitive and time-consuming than modern methods [57]. Furthermore, because complicated apparatus and highly skilled technical people are essential, they cannot offer detection findings in real-time. Furthermost of these approaches are centralized in an extensive stationary research laboratory. As a result, developing portable, rapid, sensitive, real-time, and accurate techniques for detecting *M. TB* is critical for successfully preventing TB infection [39, 40, 58, 59].

6 Detection of *M. tuberculosis* using traditional screening approaches

Signs, chest radiography findings, sputum smear microscopy, and *M. TB* culture, which in this situation is considered the "gold standard," might all be used to diagnose pulmonary *tuberculosis* [60]. Also, the Ziehl–Neelsen (ZN) technique is regularly worked to discover acid-fast bacilli (AFB). In low-income countries, it is highly regarded for its simplicity, cost efficiency, high specificity, speed, and positive predictive value [15]. The bacterial load per milliliter of sputum determines the method's sensitivity. It is correspondingly distinguished that accurate consequences on acid-fast staining involve an extreme bacterial load for sputum (5,000 AFB/ml;[61]). This technique has been found to have poor detection capabilities in immunocompromised individuals (e.g.,

Table 1: The numerous mentioned non-biosensing methods for bacteria detection [57]

Technique type	Samples analysed	Detection limit	References
MB/Bact system	*Mycobacteria*	—	Cambau et al. [55]
ELISA	*M. tuberculosis*	with the true positivity of 68%	Delacourt et al. [51]
The AMTDT	*M. tuberculosis*	with the sensitivity of 94.3%	Gamboa et al. [53]
MB/Bact system	*M. tuberculosis*	—	Horvath et al. 2004
Latex agglutination	*M. tuberculosis*	with the true positivity of 73.6%	Krambovitis et al. [48]
Radiometric detection	*M. tuberculosis*	—	Middlebrook et al. [52]
Flow cytometry	*M. tuberculosis*	3.5×10^3 cells/mL	Qin et al. [56]
PCR	*M. tuberculosis*	with the true positivity of 95.5%	Thomson et al. [45]

HIV infection), pediatric TB, extrapulmonary TB, and may generate false-negative findings [62].

Alternatively, acid-fast staining fails to discriminate live and dead bacilli and *M. TB* from NTM and resistant/susceptible strains [63]. The second class of techniques used to identify *M. TB* in clinical samples is culture-based approaches. They are regarded as the "gold standard" techniques for the diagnosis of *tuberculosis* [64]. Solid and broth media can be used for culturing; the solid egg-based Lowenstein–Jensen (LJ) medium is a popular option.

The detection limit of culture methods is about 10 viable bacilli per milliliter of clinical samples. However, compared to acid-fast staining, LJ medium culture has a higher sensitivity (80–85%). Because of the extended incubation period (4–6 weeks), the findings must be accessed over a long period [65]. As a result, an alternate method for the quick and reliable identification of *M. TB* is necessary. Although no worldwide recommendations have advised its use, For decades, serological techniques for identifying antibodies against *M. TB* antigens have been presented [66]. A commercial technique for detecting immunoglobulin G against the antigen 85 complex (Ag85 complex) in serum samples is the enzyme-linked immunosorbent assay (ELISA) [67]. PCR-based diagnostic molecular testing includes real-time PCR, strand displacement amplification methods, line probe tests, and loop-mediated isothermal amplification; these procedures have excellent accuracy but disadvantages, including high cost. Furthermore, they need complex tools, and specialized people have precluded widespread use, mainly in low-income populations **Table 1**[68].

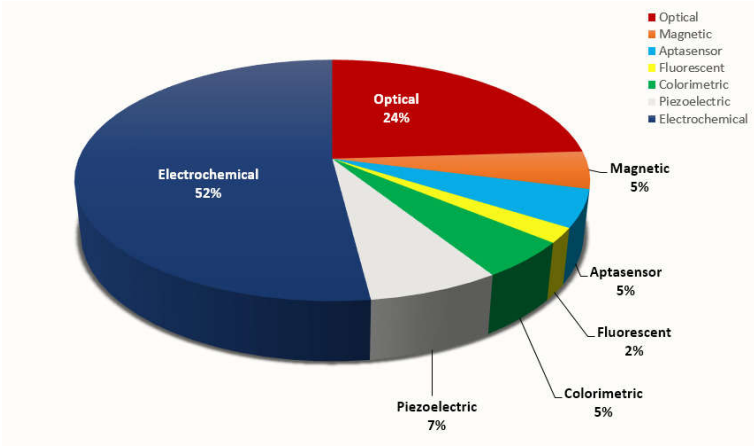

Figure 1. The number of reported biosensors for detecting *M. TB* (optimal, aptamer, magnetic, electrochemical, piezoelectric, and immunosensors) [20]

Table 2: Nucleic acid-based methods for detecting *M. TB* in the lungs are compared [20]

Method	Specificity	Sensitivity	Limit of detection	Assay time	References
GTMD	~58%	~97%	NM	up to 3 hours	[69, 70]
q real-time PCR	62.5%	97.02%	NM	up to 2 h	[71]
Xpert MTB/RIF (Cepheid, USA)	99% (Adults) 62% (Children)	89% (Adults) 98% (Children)	50-150 cfu/ml	2 h	[66, 72]
Conventional PCR	71.4% (pulmonary) 28.6% (Extra pulmonary)	91.7% (pulmonary) 82.2%(Extra-pulmonary)	NM	120 min	[73, 74]
Line probe assays	99.2%	100% (smear-positive) 68.6% (smear-negative)	10000 cfu/ml	up to 150 min	[75-77]
LAMP	100%	~99%	NM	<1 h	[78]

Abbreviations: cfu, colony-forming unit; GTMD assay, GenoType Mycobacteria Direct assay; LAMP, loop-mediated isothermal amplification; q real-time PCR, quantitative real-time PCR.

7 Alternatives to *M. tuberculosis* detection using biosensing methods

The majority of the existing diagnostic techniques for TB detection are insufficiently effective for the early diagnosis of the illness. Furthermore, they lack sufficient sensitivity and/or specificity to distinguish between latent and active types of *M. TB*. Current TB detection techniques are limited by the extended development time of *M. TB* (minimum of 17 days), the onset of symptoms only in the later stages of the illness (pulmonary TB), and the low bacterial load of sputum even in the active form of TB [79]. Consequently, there is an immediate essential for quick, sensitive, cost-effective, and simple approaches to distinguish between active and latent TB forms, perform medication susceptibility testing, and be deployed in underserved regions. Reports on creating point-of-care testing strips that use biosensors to identify bacteria in clinical and biological samples have been reported [80-87]. Numerous TB biosensors were planned, for example, piezoelectric, electrochemical, fluorescent, colorimetric, magnetic biosensors, and optical **Figure 1**.

8 Electrochemical biosensors

A solid electrode serves as the primary electrode in electrochemical biosensors, transmitting signals via the detecting process and converting them to electrical signals like potential, current, coulometry signals, and impedance [84, 86, 88-92]. The reported electrochemical biosensors for *M. TB* diagnosis have been classified into electrochemical DNA and immunological biosensors based on the various biological targets of *M. TB* detection [83, 93-95]. The most prevalent use for electrochemical biosensors (particularly electrochemical DNA biosensors) is detecting infectious pathogens such as *M. TB* [96, 97]. In a study in this regard, [98], A sophisticated target sandwich creation technique was used to create an electrochemical DNA biosensor for *M. TB* discovery. The DNA target amplification in this biosensor was done with thermophilic helicase-dependent isothermal primers, and a 190bp segment of the IS6110 gene was aimed for the *M. TB* complex.

Furthermore, two different Thiolated DNA probes were utilized to functionalize the dextrin-coated gold nanoparticles and the amine-coated magnetic particles. The hybridized sandwich complex formed between the two functionalized probes was pulled and separated using a magnet before being transferred to an SPCE. By dropping the presence of the gold ions, the differential pulse voltammograms [Au^{3+}] The gold nanoparticles were produced by dissolving them in an HCl solution, and this is achieved within the possible range of 0.00-1.25 V. In addition, the discovery limit of future biosensors is an amplified target (105 bp) of 0.01 ng/µl.

Another investigation found that [99], For detecting *M. TB* in human serum samples, researchers utilized an immunosensor device based on reformed graphene oxide TB specific antigens on a gold electrode. Graphene oxide was changed for protein attachment with N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride/N-hydroxysuccinimide.

An additional report found that [100] established an electrochemical DNA biosensor to *discover M. TB* target based on that DNA reduced gold nanoparticles and graphene oxide nanoribbons **Figure 2**. In this work, the electrode was produced by immobilizing gold nanoparticles on RGONPs; Following that, the gold nanoparticles were covalently modified using [5′-SH-ssDNA (ssDNA/Au/RGONR)]. Cyclic voltammetry and chronoamperometry were utilized throughout the testing. As shown in **Figure 3**, the DNA biosensor showed enough sensitivity and selectivity (up to 0.1 fM) for DNA *M. TB*; the response mechanism of the proposed biosensor is described by the hybridization and immobilization of ssDNA probes. Due to the repulsion between the [Fe $[CN]_6]^{3-/4-}$ ions in the solution and the phosphate backbone of DNA, the combination of ssDNA and Au/RGO bioelectrodes hybridization complementary target ssDNA to form dsDNA leads to electrons decreased transfer kinetics.

In addition, the medication time for bacteria tests was reduced from 9 months to 6 months (e.g., TB), numerous antibiotics (e.g., rifampicin; [101, 102] and pyrazinamide (PZA;[103]) were used. In PZA, just susceptible *M. TB* strains released pyrazinoic acid (POA; **Figure 4**). The quantitative measurement of POA is used as a predictor of PZA resistance in this procedure. In an investigation in this regard, [103] **Figure 5** shows two electrochemical biosensors made of gold and platinum wires that measure the current produced when a potential difference is applied to different concentrations of POA. POA is a pyrazinamide prodrug working process catalyzed by pyrazinamidase (PZase), an enzyme produced by the *M. TB* pncA gene. When a mutation in the pncA gene of *M. TB* occurs, the pyrazinamidase enzyme is weakened, and the bacterium develops resistance to the prodrug PZA. The supernatant of negative liquid-based microscopy was used to test these biosensors, and drug susceptibility culture was seen to detect PZA, POA, and their combination in the study stated. Furthermore, the suggested biosensors' detection limits were predicted to be 40 and 1 µM of POA for the gold and platinum working electrodes, respectively.

9 Fluorescent biosensors

Fluorescent chemical compounds, proteins, nanoparticles, or mixtures are commonly used in biosensors [104]. These biosensors have several significant benefits, including the ability to scan and use excellent spatial and temporal resolution in live cells and assess protein activity and small-molecule signals [99, 105]. Typically, because neither the receptor nor the binding molecules are fluorescent, they must be mixed, for instance, green fluorescent protein (GFP; [43, 105, 106]. Fluorescent labels are usually working in fluorescence spectroscopy [107]. Tryptophan, phenylalanine, tyrosine, and the proteins that include these amino acids (nucleic acids, flavin nucleotides, GFP, and NADH) are reactive species.

The majority of lipids and saccharides, on the other hand, are nonfluorescent [108]. Fluorescence resonance energy transfer (FRET) biosensors are based on the physical FRET procedure; these biosensors utilize lower-energy radiation from a donor fluorophore to an acceptor fluorophore to detect viral and bacterial infections [109-111]. Fluorescent nanomaterials with a minimum of one dimension (100 nm) and organic dyes might be utilized to improve the design and production of fluorescent biosensors, according to the study subject [112]. In this regard, [113] suggested a nano biosensor detection technique that integrates the benefits of cadmium telluride quantum dots (CdTeQDs) and gold nanoparticles onto the platform surface and two probes based on the conserved genomic sections of the ESAT6 antigen. This is seen in **Figure 6**. Using two sequence-specific oligonucleotide probes, QDs and AuNPs were shown to construct a selective and sensitive sandwich from a FRET-based biosensor (p1 and p2). The proposed biosensor was tested using

culture, PCR, and nested PCR methods to verify it. As a consequence of their collaboration, a sandwich shape fluorescence resonance energy transfer sensing technique with a detection limit of ten was developed. The goal of these probes was to identify BCG and other Mycobacterium species at the same time, which might lead to false positives and cross-reactivity during the detection procedure.

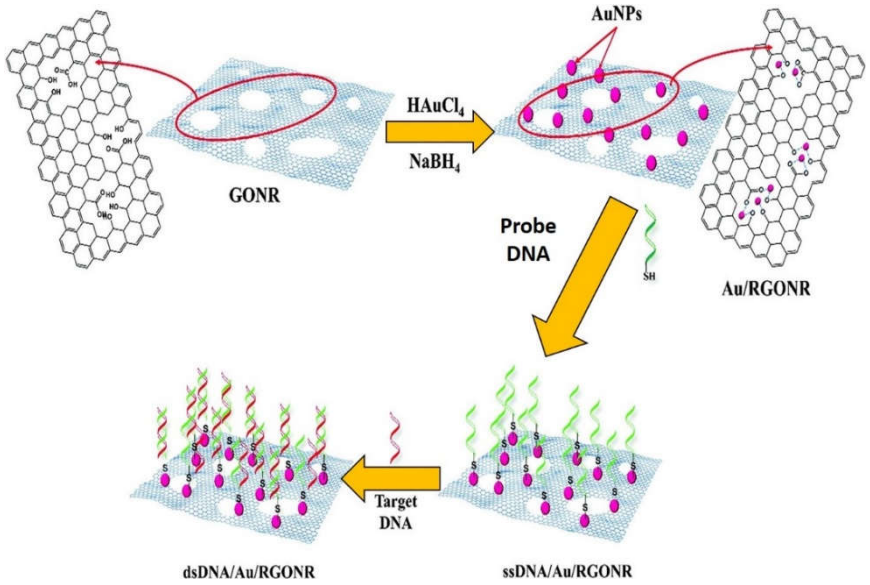

Figure 2. The synthesis of AuNPs and RGONRs to detect *M. TB* target DNA is schematically [20, 100].

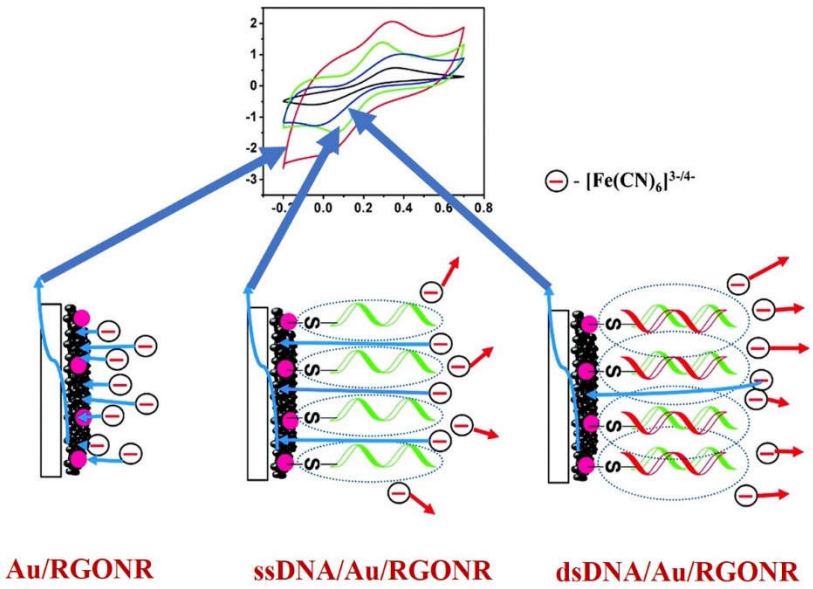

Figure 3. Schematic representation of the ssDNA / Au / RGONR bioelectrode [100]. reduced graphene oxide nanoribbon (RGONR); double-stranded DNA (dsDNA); single-stranded DNA (ssDNA) [20].

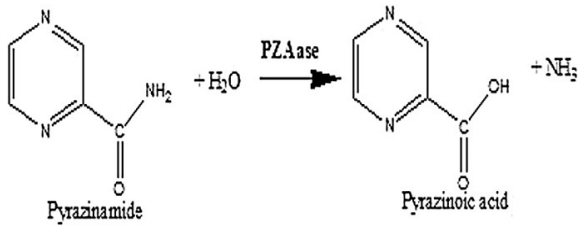

Figure 4: Production of ammonia (NH3) and pyrazinoic acid from water (H2O) and pyrazinamide by the action of the enzyme pyrazinamidase [20].

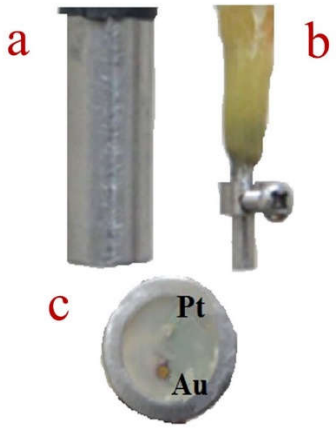

Figure 5: a: Outer diameter 13.2 mm in excellent electrochemical sensor, **b:** Outer diameter 4 mm in small electrochemical sensor, **c**: Large electrochemical sensor including platinum (Pt) and gold (Au) electrodes [20, 98].

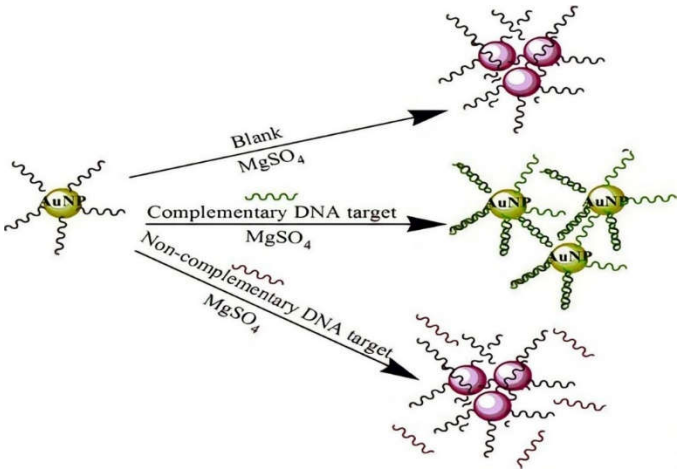

Figure 6. Schematic image of Sandwich-shaped fluorescence resonance energy transfer (FRET) based biosensor. In the presence of the objective, the P1/QDs segment is optically turned off by the AuNPs / P2 segment; Fluorescence receptors near P1/QDs are made of P2/AuNPs in the presentment target molecules, leading to a FRET signal. [113] quantum dot (QD); fluorescence resonance energy transfer (FRET); gold nanoparticle (AuNP) [20].

10 Colorimetric biosensors

Light resistance etching techniques are used to build color biosensors on rigid surfaces such as polymers, glass substrates, and plastics; Finally, a deposit of thin dielectric film is formed on the glass layer [114]. It can be said that light resistance material is a substance that is sentient to light. Howbeit bio-color sensors have received much consideration, they have limited clinical diagnostic applications and relatively little sensitivity [115]. The combination of colorimetric biosensors with LAMP is used to detect *M. TB*, which is highly sensitive [116]. A polymer network near gold nanoparticles was created in related research by combining two target DNA probes on gold nanoparticles. In addition, in a single tube, the pink color turned purple. A complementary target strand at the reaction site inhibits aggregation and prevents pink to purple discoloration. In a dilute sample containing mycobacterial DNA (10 microliters), the recognition range of the DNA biosensor is approximately 18.70 ng. A similar study designed a different colorimetric DNA biosensor using LAMP products based on AuNPs connected to a thiol reformed ssDNA probe based on the *M.TB's* IS-6110 gene for M.TB detection [110]. **Figure 7** shows that the change from red to purple has a positive reaction, and the accumulation of AuNPs produces a purple / blue color from red. LAMP-AuNP detects approximately 5 pg of genomic DNA. A recent study identified *M. TB* strains with point mutations in the katG 315 (G → C) gene and isoniazid-resistant with a single biological LAMP turbidity sensor [117]. Actual specimens like multidrug resistance *tuberculosis* (MDR-TB) DNA are detected in less than 60 minutes using the proposed LAMP biosensor.

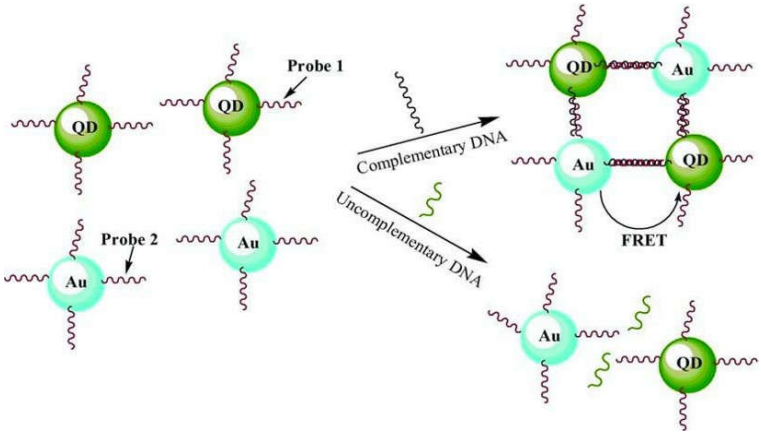

Figure 7. Probes LAMP-AuNPs are shown schematically based on the colorimetric method. Before and after aggregation of AuNP probes, the test solutions were compared visually; **A:** AuNPs probes single as empty; **B:** AuNPs probes in the presentment of a non-supplementary DNA specimen as negative; **C:** AuNPs probes in the presentment of a supplementary DNA specimen as positive [113]., quantum dot (QD); loop-mediated isothermal amplification (LAMP); gold nanoparticle (AuNP) [20].

11 Piezoelectric biosensors

Piezoelectric biosensors come in various models, including interactive, unlabelled, and direct, which take full advantage of the system [118, 119]. Kaewphinit et al. Improved the piezoelectric DNA biosensor, which contains a particular

digested DNA sequence of IS6110 M.tb (by BtgI enzyme) fixed by a supplementary DNA probe quartz [120]. Examination of 200 clinical specimens using biosensors and PCR showed similar results. Also, high specificity was found in experiments on microorganisms such as *Escherichia coli* and *Mycobacterium* complex. The ability to repeat more than 10 times is another strength of the biosensor. The Quartz Piezoelectric Series (MSPQC) is another biosensor designed by Mi, X. et al. to detect *M. TB* based on the natural growth-sensitive conductivity of *M. smegmatis* [121]. *M. smegmatis* was detected during normal growth due to phage killing of D29 due to the action of ammonium iron sulfate (FAS) in the medium. In contrast, in this instance, *M. TB* was not detectable in the medium. The principle of recognition of *M. TB* is shown schematically in **Figure 8** of the diagram. In the sample solution containing *M. TB*, phase D29 was step up, and incubation took 1 hour. Then to the inactive phase D29, 4% FAS solution was added and larger than *M. TB* to remove the duty of FAS, the specimen dilution was attenuate 10 times to exclude the role of FAS and placed in a cell containing smegmatis, a diagnostic cell, in the assay medium. Subsequently, the frequency curve was recorded using MSPQC, and nil *M. TB* destroyed phage D-29 in the reconnaissance environment. The initial microbial value of *M. TB* with this biosensor can be measured a maximum of 102 CFU/ml in a clinical specimen by a rotation period of fewer than 30 hours [122]. Drug-resistant *M. TB* cells can be identified by the recently introduced indirect series piezoelectric system (ISP). There are three parts III, II, and I of the ISP systems. The first part consisted of a quartz piezoelectric, crystal chamber, and culture plus 32 sensors array to initiate the *M. TB* development reaction; the data collection device forms the second part. The third part of the system chamber is constructed **Figure 8**. The sensitive part of the ISP system is the first part of the biosensor, which response to modification in electrical components in the *M. TB* broth growth environment. Isolated *M. TB* (1%) and control room without anti-*tuberculosis* drugs form the third part. The standard and critical density of anti-*tuberculosis* antibiotics is ethambutol, ethionamide, isoniazid, rifampin, the amino acid, caproemycin, streptomycin, rifabutin, salicylic acid, and hydrochloride, prepared by the laboratory and the standard institution. In the ISP system, the detection chamber absorbs and measures the CO_2 and NH_3 products produced by the growth of *M. TB* cells in the diagnostic environment.

12 Optical biosensors

These biosensors are common sensors that use light tracking from the reaction with target analytes as a source. There is scope for extensive future research on optical biosensors and their relationship to bioelectronics [124]. Features such as easy sample preparation processes and low sample size lead to the use of these materials compared to other biosensors [125]. Optical biosensor systems such as reflectometric interference spectro- scopy, bioluminescent optical fiber biosensors, SPR-based biosensors, and evanescent wave fluorescence (RIfS; [126]). The other two optical sensors are label-based and Label-free optical biosensors [127]. In unlabelled optical biosensors, a straight reaction is produced among the converter and the analyte. Still, in optically labelled biosensors, the optical signal is produced by fluorescence, luminescence, or colorimetric method [81, 128]. In this regard, [129] detected *tuberculosis* antigens, including Ag85 LAM and ESAT6, by a flat-wave-based optical sensor in sputum and spiked urine, respectively. Also, a reporter antibody labelled fluorophore, antigen, and a biotinylated receptor antibody were used on the biosensor. The combination of optical sensors and ELISA increases recognition reliability and sensitivity. In a similar study by Hong, S.C., et al. in TB patient's tissue fluids, they apply an SPR light sensor to discover new composition TB antigen (CFP10) [130, 131]. Cystamine absorbed the monoclonal antibody (anti-CFP10) by direct chemical method to immobilize the nude gold. The correlation between CFP10 density (span, 0.1-1.0 μg / ml) and SPR angle change led to a diagnostic limit of 100 ng/ml. Clinical urine samples are the most commonly used samples in the proposed optical biosensor for measuring CFP10 levels in *tuberculosis* patients. [130, 131]. Depending on the degree of infection, the group of 55 patients was divided according to the AFB system (stages 0–4). Subsequently, between different studies for recombinant CFP10, a quantitative correlation between AFB steps and SPR angle change was surveyed with plotting a calibration curve. The results showed a direct connection among CFP10 density and the numeral of bacteria in AFB staining. Also, there was a large difference between infected and healthy samples. Therefore, the study's main strength was the classification of TB patients based on the degree of the infection. In another study, an SPR biosensor system by the capability to simultaneously diagnose DNA subsequence from complex *M. gordonae* and *M. TB* cells (target ssODNs) was introduced as a portable multi-channel based on probe-ssODNs [132].

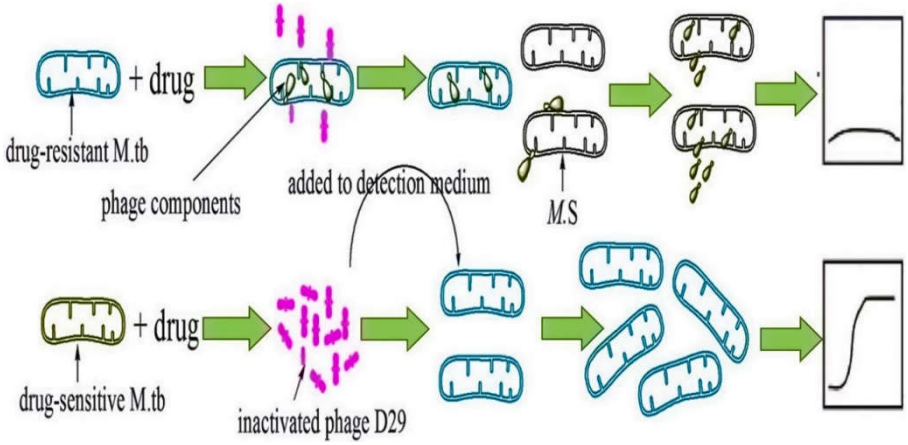

Figure 8. Flowchart of PA-MSPQC for *M.tb* detection [20, 123]

The recommended biosensor detection limit for DNA hybridization is a maximum of 0.05 μM at 30 ng / μl. Therefore, according to the results, it was found that the efficiency of the SPR method is premier toward ELISA. Another research presented the waveguide biosensor as an optical detector that detects *tuberculosis* with special antibodies. Alter in the evanescent field dispensation and the interaction of antibodies and *tuberculosis* antigens were generated and received by the optical detector [123]. Also, a diagnosis bound of 30 ng/microliter was proposed for the DNA biosensor, which is used as an optical SPR genosensor to detect oligonucleotide strains with specific point mutations rpoB gene and resistance to rifampin [123, 133]. The hybridization diagnosis limit and performance of the genosensor were approximated at 10 nM and 1012×5 molecules/cm^{-2} (30%), respectively. Another major study was performed to recognize epitope areas (based on the SPR sensory method) on this antigen to interact with specific monoclonal antibodies and characterize the new composition Ag-85B affinity [134]. In online and unlabelled measurements, the most suitable option is SPR biosensors [122]. Therefore, the design and production of unique and new vaccines based on current biomarkers of different pathogens can be based on optical biosensors **Figure 9**.

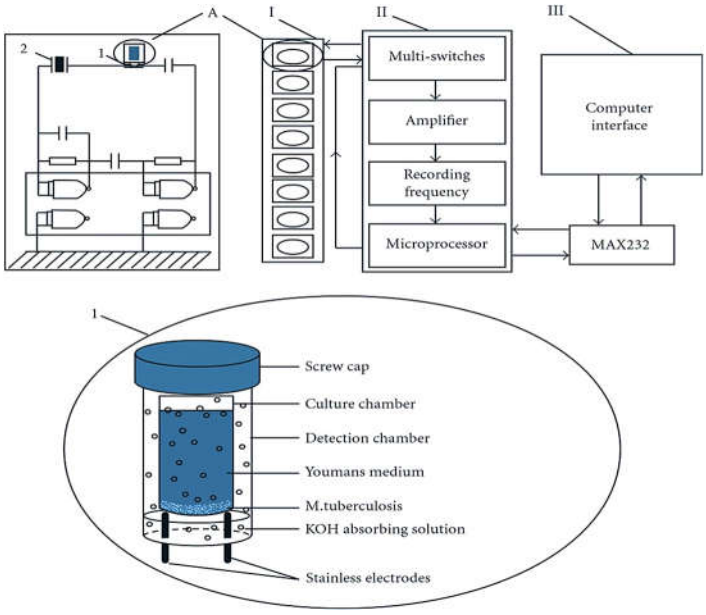

Figure 9. There are three essential components to an indirect piezoelectric system: **A:** 32 speciman of the diagnosis system; 1, 9 MHz AT-cut piezoelectric quartz crystals; 2, diagnose cell), **B:** microprocessor system, and **C:** control system and data output [20, 57, 135]

13 Magnetic biosensors

Nanoparticles can do the etiology of infection sickness [20, 136], such as *M. TB* with magnetic properties [137]. The reaction in a suspicious sample between a specific bio-probe on the scheme of magnetic composite and its supplement creates a biomolecular detection system used for the biological diagnose of *M. TB* and a combination of a magnetoresistive biochip and a sandwich - immunoassay [136, 138]. Anti-*M. TB* labelling was performed using streptavidin-coated magnetic nanoparticles. *Mycobacterium Bovis* BCG cells and biotinylated tube antibodies were used to replace *M. TB* [136]. During magnetic recording, the functional biochip surface collided with the target bacteria to carry them, and the anti-M reacted with the magnetically labelled *M. TB* cells, which carried the TB antibodies through a functional sensor on the magnetic biochip surface with Sandwich safety test methods were identified **Figure 10** [139]. A magnetic biosensor to detect resistance to rifampin, wild-type strains as well as *M. TB* nucleic acids. They were designed based on magnetic barcodes. In this study, total RNA analysis was obtained, and the sample was analysed from *M. TB* cells. Amplification of the 16S-rRNA sequence was performed with asymmetric real-time PCR. The ssDNAs were fixed on connected beads, in search for reaction with MNPs, and covered to create a magnetic sandwich composition by a detection probe. The identification procedure of these collections was augmented by passing through microfluidic pipes and its cost efficiency was significant [139].

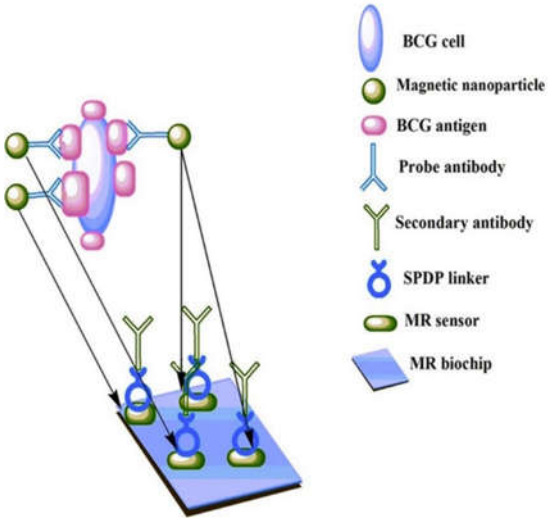

Figure 10. Schematic allegory of ELISA-sandwich assay in magnetoresistive biochip apply in the experience task [20, 136]

14 DNA biosensors and *M. tuberculosis* detection

DNA biosensors are sensors with many applications in diagnosing infectious diseases, are highly sensitive, low cost, and are easy to operate [138]. Inexpensive automated testing devices increase demand that could deal with the miniaturization of the trial ideology motivated by the vast expansion of DNA biosensors [138]. These sensors are based on DNA combination detection and labelled and unlabelled DNA biosensors [138]. Labels on DNA biosensors include nanoparticles, dye molecules (such as methylene blue), and electrically active labels [138]. Guanine or adenine oxidation signals are used in unlabelled DNA biosensors, while among DNA biosensors, electrochemical DNA biosensors have more different usages [138]. This article found that in *M. TB*, the most critical diagnostic target for researchers is the protected areas of the IS6110 insertion sequence. Laboratory diagnosis of *M. TB* by PCR is performed by identifying IS6110 b61 length. A typical diagnostic sequence among complex *M. TB* species acts as diagnostic targets while different regions of this sequence can be identified [138, 140, 141].

Table 3: Sequences and genes of DNA probes for *M. TB* bio-sensory diagnosis [20]

Genome	Subsequences [5′-3′]	Ref
sequence IS-6110	10-mer: 5′-GGTGAGGTCT-3′	[142]
DNA genome M.tb	22-mer: 5′- CCA ACT TTG TTG TCA TGC ACC C-3′	[132]
DNA genome M.tb	24-mer: 5′-CGGTGGCGTGTTCTTTGTGCAATA-3′	[143]
Not in reference	20-mer: 5′-CTCGTCCAGCGCCGCTTCGG-3′	[144]
sequence IS-6110	5′-SH-$[CH2]_6$-TTT TTT GTG GCC ATC GTG GAA GCG A-3′	[120]
sequence IS-6110	15-mer: 5′-ATCCGGCCACAGCCC-3′	[145]
Rpo-B	15-mer: 5′-GATACTTCTATCACC-3′	[101]
Not in reference	21-mer: 5′-GGTCTTCGTGGCCGGCGTTCA-3′	[100]
DNA genome M.tb	20-mer: 5′-CTCGTCCAGCGCCGCTTCGG-3′	[146]
DNA genome M.tb	20-mer: 5′-CTC GTC CAG CGC CGC TTC GG-3′	[147]
Not in reference	21-mer: 5′-IACIIICAATCCAIIIC-3′	[148]
Not in reference	21-mer: 5′- GGTCTTCGTGGCCGGCGTTCA-3′	[149]
Rpo-B	21-mer: 5′- ACCCACAAGCGCCGACTGTTG-3′	[133]
ESAT-6 antigen' Genomic region	Probe 1: 5′-NH2-[CH2]6- GTA AGT AAG GGA GGA AC-3 ′Probe 2: 5′- TGC TCC CCT TCG TCA GG -[CH2]6-SH-3′	[113]
MPT-64 antigen	77-bp aptamer: 5′-[Btn]-GTA CAA ACG ACG GCC AGT CCT TGG GAT GAT TCA AGC AAA GCC TCA CGC CTA CGG CTA AGT CAT AGC TGT CTC TCC TG-3′	[150, 151]
Not in reference	21-mer: 5′-GGTCTTCGTGGCCGGCGTTCA-3′	[152]
sequence IS-6110	18-mer: 5′-GAGCGTAGGCGTCGGTGA-3′	[98]
IFN-γ	37-bp aptamer: 5′- GGG GTT GGT TGT GTT GGG TGT TGT GTC CAA CCC CCC C-3′	[153]
DNA genome M.tb	25-mer: 5′- biotin GAC CAA ATA GGT ATC GGC GTG TTC A-3′	[154]
Not in reference	15-mer: 5′--GATACTTCTATCACC-3′	[102]

15 Biosensors and antibiotic resistance of *M. tuberculosis*

Designing standard and appropriate treatment regimens with consideration of resistant strains are one of the critical principles in antimicrobial monitoring of patients. According to *M. TB*, antibiotic susceptibility testing is performed using user-friendly and common drug allergy testing methods, usually in endemic TB regions worldwide, especially in the middle- and low-income regions [155, 156]. Therefore, it is necessary to identify resistant strains by accurate, fast, and cost-effective methods with potential multifunctional features. Also, long-term antimicrobial treatment of *tuberculosis* plays a critical duty in preventing the clinical complications of *tuberculosis*. Treatment of *tuberculosis* is due to common mutations and the high intrinsic resistance of *M. TB* to antibiotics [147]. Magnetic barcode strategy is one of the new sensory methods widely used to detect *M. TB* drug-resistant strains [139]. Bio structural detection components as mononucleotide mutant detectors can be detected by constructing and designing new biostructures to providing great sensitivity and specificity.

In contrast, in the clinical setting, the *M. TB* antibiotic susceptibility pattern using Specific high-velocity probes detect mutations for common native drug resistance. Diagnosis of the resistor to first and second-line medicines, isoniazid [117], pyrazinamide [103], and rifampin [101, 102, 133], is done through sensory-based diagnostic systems. Ethynamide, re-fabutin, the amino acid salicylic acid, capreomycin, rifampin, hydrochloride, streptomycin, and ethambutol with; [122] the motivation that these systems are becoming more advanced.

16 Conclusion

Successful TB control worldwide requires solving the problem of drug-resistant TB. According to the WHO, in developing countries, more than 95% of *tuberculosis* cases occur due to incorrect resource adjustments, high transmission of drug-resistant strains, and high rates of TB infection in children. Therefore, providing cost-effective biosensors as routine analytical experiments plays an essential role in preventing transmission and mortality from this disease.

Ethical issue

Authors are aware of and comply with, best practices in publication ethics, specifically about authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests, and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that no conflict of interest would prejudice the impartiality of this scientific work.

Authors contribution

All authors of this study have a complete contribution for data collection, data analyses, and manuscript writing.

References

1. Wirth, T. and F. Hildebrand, *lix-Beguec C, Wolbeling F, Kubica T, Kremer K, et al.* Origin, spread and demography of the Mycobacterium tuberculosis complex. PLoS Pathog, 2008. **4**: p. e1000160.
2. Hashemi, S.A., et al., *Integrated polyaniline with graphene oxide-iron tungsten nitride nanoflakes as ultrasensitive electrochemical sensor for precise detection of 4-nitrophenol within aquatic media.* Journal of Electroanalytical Chemistry, 2020. **873**: p. 114406.
3. Mousavi, S.M., et al., *Green synthesis of supermagnetic Fe3O4–MgO nanoparticles via Nutmeg essential oil toward superior anti-bacterial and anti-fungal performance.* Journal of Drug Delivery Science and Technology, 2019. **54**: p. 101352.
4. Gholami, A., et al., *3D nanostructures for tissue engineering, cancer therapy, and gene delivery.* Journal of Nanomaterials, 2020. **2020**.
5. Bloom, B.R. and P.E. Fine, *The BCG experience: implications for future vaccines against tuberculosis.* Tuberculosis: pathogenesis, protection, and control, 1994: p. 531-557.
6. Friedman, L.N., *Tuberculosis: current concepts and treatment.* 2000.
7. Grange, J., *Mycobacteria and human disease 2nd ed.* Arnold ISBN 0, 1996. **340**(64563): p. 6.
8. Funnye, A.S., K. Ganesan, and T.T. Yoshikawa, *Tuberculosis in African Americans: clinical characteristics and outcome.* Journal of the National Medical Association, 1998. **90**(2): p. 73.
9. Mousavi, S.M., et al., *Recent Advancements in Polythiophene-Based Materials and their Biomedical, Geno Sensor and DNA Detection.* International Journal of Molecular Sciences, 2021. **22**(13): p. 6850.
10. Mousavi, S.M., et al., *Recent Progress in Electrochemical Detection of Human Papillomavirus (HPV) via Graphene-Based Nanosensors.* Journal of Sensors, 2021. **2021**.
11. Hashemi, S.A., et al., *Picomolar-level detection of mercury within non-biological/biological aqueous media using ultra-sensitive polyaniline-Fe 3 O 4-silver diethyldithiocarbamate nanostructure.* Analytical and Bioanalytical Chemistry, 2020. **412**: p. 5353-5365.
12. Smith, I., *Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence.* Clinical microbiology reviews, 2003. **16**(3): p. 463-496.
13. Mousavi, S.M., et al., *Data on cytotoxic and antibacterial activity of synthesized Fe3O4 nanoparticles using Malva sylvestris.* Data in brief, 2020. **28**: p. 104929.
14. Mousavi, S.M., et al., *Development of hydrophobic reduced graphene oxide as a new efficient approach for photochemotherapy.* RSC Advances, 2020. **10**(22): p. 12851-12863.
15. Palomino, J., *Nonconventional and new methods in the diagnosis of tuberculosis: feasibility and applicability in the field.* European Respiratory Journal, 2005. **26**(2): p. 339-350.
16. Mousavi, S.M., et al., *Gold nanostars-diagnosis, bioimaging and biomedical applications.* Drug metabolism reviews, 2020. **52**(2): p. 299-318.
17. Mazraedoost, S. and N. Banaei, *Biochemical composition properties of Kombucha SCOBY: Mini Reviews.* Advances in Applied NanoBio-Technologies, 2020. **1**(4): p. 99-104.
18. Organization, W.H. and S.T. Initiative, *Treatment of tuberculosis: guidelines*. 2010: World Health Organization.
19. Frieden, T., *Directly observed treatment, short-course (DOTS): ensuring cure of tuberculosis.* Indian journal of pediatrics, 2000. **67**(2 Suppl): p. S21-7.
20. Salimiyan Rizi, K., et al., *The overview and perspectives of biosensors and Mycobacterium tuberculosis: A systematic review.* Journal of Cellular Physiology, 2021. **236**(3): p. 1730-1750.
21. Hashemi, S.A., et al., *Polythiophene silver bromide nanostructure as ultra-sensitive non-enzymatic electrochemical glucose biosensor.* European Polymer Journal, 2020. **138**: p. 109959.
22. Mousavi, S.M., et al., *Recent biotechnological approaches for treatment of novel COVID-19: from bench to clinical trial.* Drug Metabolism Reviews, 2021. **53**(1): p. 141-170.
23. Mousavi, S.M., et al., *Development of clay nanoparticles toward bio and medical applications*. 2018: IntechOpen.
24. Hashemi, S.A., et al., *Coupled graphene oxide with hybrid metallic nanoparticles as potential electrochemical biosensors for precise detection of ascorbic acid within blood.* Analytica chimica acta, 2020. **1107**: p. 183-192.
25. Bahrani, S., et al., *Zinc-based metal–organic frameworks as nontoxic and biodegradable platforms for biomedical applications: review study.* Drug metabolism reviews, 2019. **51**(3): p. 356-377.
26. Tech, J.E.T., *Investigating the Activity of Antioxidants Activities Content in Apiaceae and to Study Antimicrobial and Insecticidal Activity of Antioxidant by using SPME Fiber Assembly Carboxen/Polydimethylsiloxane (CAR/PDMS).* Journal of Environmental Treatment Techniques, 2020. **8**(1): p. 214-24.
27. Mazraedoost, S. and G. Behbudi, *Nano materials-based devices by photodynamic therapy for treating cancer applications.* Advances in Applied NanoBio-Technologies, 2021. **2**(3): p. 9-21.
28. Goudarzian, N., et al., *Enhancing the Physical, Mechanical, Oxygen Permeability and Photodegradation Properties of Styrene-acrylonitrile (SAN), Butadiene Rubber (BR) Composite by Silica Nanoparticles.* Journal of Environmental Treatment Techniques, 2020. **8**(2): p. 718-726.

29. Mazraedoost, S. and G. Behbudi, *Basic Nano Magnetic Particles and Essential Oils: Biological Applications.* Journal of Environmental Treatment Techniques, 2021. **9**(3): p. 609-620.
30. Goudarzian, N., et al., *Evalouation of Styrene Acrylo Nitrile (SAN), Butadiene Rubber (BR), Nano-silica (Nano SiO2) Blend and Nanocomposite in the Presence of Oxoperoxidant Study.* Journal of Environmental Treatment Techniques, 2020. **9**(1): p. 24-32.
31. Mousavi, S.M., et al., *Development of graphene based nanocomposites towards medical and biological applications.* Artificial cells, nanomedicine, and biotechnology, 2020. **48**(1): p. 1189-1205.
32. Mousavi, S.M., et al., *Nanosensors for chemical and biological and medical applications.* Med Chem (Los Angeles), 2018. **8**(8): p. 2161-0444.1000515.
33. Mousavi, S., M. Zarei, and S. Hashemi, *Polydopamine for biomedical application and drug delivery system.* Med Chem (Los Angeles), 2018. **8**: p. 218-29.
34. Hashemi, S.A., et al., *Ultra-sensitive viral glycoprotein detection NanoSystem toward accurate tracing SARS-CoV-2 in biological/non-biological media.* Biosensors and Bioelectronics, 2021. **171**: p. 112731.
35. Mousavi, M., et al., *Erythrosine adsorption from aqueous solution via decorated graphene oxide with magnetic iron oxide nano particles: kinetic and equilibrium studies.* Acta Chimica Slovenica, 2018. **65**(4): p. 882-894.
36. Avval, Z.M., et al., *Introduction of magnetic and supermagnetic nanoparticles in new approach of targeting drug delivery and cancer therapy application.* Drug metabolism reviews, 2020. **52**(1): p. 157-184.
37. Takmil, F., et al., *Nano-magnetically modified activated carbon prepared by oak shell for treatment of wastewater containing fluoride ion.* Advanced Powder Technology, 2020. **31**(8): p. 3236-3245.
38. Azhdari, R., et al., *Decorated graphene with aluminum fumarate metal organic framework as a superior non-toxic agent for efficient removal of Congo Red dye from wastewater.* Journal of Environmental Chemical Engineering, 2019. **7**(6): p. 103437.
39. Hashemi, S.A., et al., *Superior X-ray radiation shielding effectiveness of biocompatible polyaniline reinforced with hybrid graphene oxide-iron tungsten nitride flakes.* Polymers, 2020. **12**(6): p. 1407.
40. Ahmadi, S., et al., *Anti-bacterial/fungal and anti-cancer performance of green synthesized Ag nanoparticles using summer savory extract.* Journal of Experimental Nanoscience, 2020. **15**(1): p. 363-380.
41. Mousavi, S.M., et al., *Asymmetric membranes: a potential scaffold for wound healing applications.* Symmetry, 2020. **12**(7): p. 1100.
42. Gholami, A., et al., *Current trends in chemical modifications of magnetic nanoparticles for targeted drug delivery in cancer chemotherapy.* Drug metabolism reviews, 2020. **52**(1): p. 205-224.
43. Bajar, B.T., et al., *A guide to fluorescent protein FRET pairs.* Sensors, 2016. **16**(9): p. 1488.
44. Rodríguez-Lázaro, D., et al., *Real-time PCR-based methods for detection of Mycobacterium avium subsp. paratuberculosis in water and milk.* International journal of food microbiology, 2005. **101**(1): p. 93-104.
45. Thomson, L.M., et al., *An extremely rapid and simple DNA-release method for detection of M. tuberculosis from clinical specimens.* Journal of microbiological methods, 2005. **63**(1): p. 95-98.
46. Choi, Y.J., Y. Hu, and A. Mahmood, *Clinical significance of a polymerase chain reaction assay for the detection of Mycobacterium tuberculosis.* American journal of clinical pathology, 1996. **105**(2): p. 200-204.
47. Durmaz, R., et al., *Sensitivity of two-stage PCR amplification for detection of Mycobacterium tuberculosis in paraffin-embedded tissues.* Journal of microbiological methods, 1997. **29**(2): p. 69-75.
48. Krambovitis, E., et al., *Rapid diagnosis of tuberculous meningitis by latex particle agglutination.* The Lancet, 1984. **324**(8414): p. 1229-1231.
49. Tamminen, M., et al., *Screening of lactic acid bacteria from fermented vegetables by carbohydrate profiling and PCR–ELISA.* Letters in Applied Microbiology, 2004. **39**(5): p. 439-444.
50. Nassau, E., E. Parsons, and G. Johnson, *The detection of antibodies to Mycobacterium tuberculosis by microplate enzyme-linked immunosorbent assay (ELISA).* Tubercle, 1976. **57**(1): p. 67-70.
51. Delacourt, C., et al., *Value of ELISA using antigen 60 for the diagnosis of tuberculosis in children.* Chest, 1993. **104**(2): p. 393-398.
52. Middlebrook, G., Z. Reggiardo, and W.D. Tigertt, *Automatable radiometric detection of growth of Mycobacterium tuberculosis in selective media.* American Review of Respiratory Disease, 1977. **115**(6): p. 1066-1069.
53. Gamboa, F., et al., *Detection and identification of mycobacteria by amplification of RNA and DNA in pretreated blood and bone marrow aspirates by a simple lysis method.* Journal of clinical microbiology, 1997. **35**(8): p. 2124-2128.
54. Liu, Z., X. Shi, and X. Wu, *The method of Mycobacterium tuberculosis rapid cultivation fluorescence detection.* Chinese Journal of Clinical Laboratory Science, 2001. **19**: p. 347.
55. Cambau, E., et al., *Evaluation of the new MB Redox system for detection of growth of mycobacteria.* Journal of clinical microbiology, 1999. **37**(6): p. 2013-2015.
56. Qin, D., et al., *Using fluorescent nanoparticles and SYBR Green I based two-color flow cytometry to determine Mycobacterium tuberculosis avoiding false positives.* Biosensors and Bioelectronics, 2008. **24**(4): p. 626-631.
57. Zhou, L., et al., *Biosensing technologies for Mycobacterium tuberculosis detection: status and new developments.* Clinical and developmental immunology, 2011. **2011**.
58. Griffiths, D. and G. Hall, *Biosensors—what real progress is being made?* Trends in Biotechnology, 1993. **11**(4): p. 122-130.
59. Owen, V.M., *Market requirements for advanced biosensors in healthcare.* Biosensors & bioelectronics, 1994. **9**(6): p. xxix-xxxv.
60. Gholoobi, A., et al., *Comparison of culture and PCR methods for diagnosis of Mycobacterium tuberculosis in different clinical specimens.* Jundishapur journal of microbiology, 2014. **7**(2).
61. Swai, H.F., F.M. Mugusi, and J.K. Mbwambo, *Sputum smear negative pulmonary tuberculosis: sensitivity and specificity of diagnostic algorithm.* BMC research notes, 2011. **4**(1): p. 1-6.
62. Desikan, P., *Sputum smear microscopy in tuberculosis: is it still relevant?* The Indian journal of medical research, 2013. **137**(3): p. 442.
63. Srivastava, S.K., C.J. Van Rijn, and M.A. Jongsma, *Biosensor-based detection of tuberculosis.* RSC advances, 2016. **6**(22): p. 17759-17771.
64. Asmar, S. and M. Drancourt, *Rapid culture-based diagnosis of pulmonary tuberculosis in developed and developing countries.* Frontiers in microbiology, 2015. **6**: p. 1184.
65. Muddaiah, R.K., P.M. James, and R.K. Lingegowda, *Comparative study of Smear Microscopy, Rapid Slide Culture, and Lowenstein-Jensen culture in cases of pulmonary tuberculosis in a tertiary care hospital.* Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences, 2013. **18**(9): p. 767.

66. Steingart, K.R., et al., *Serological tests for the diagnosis of active tuberculosis: relevance for India.* The Indian journal of medical research, 2012. **135**(5): p. 695.
67. Imaz, M.S., et al., *Serodiagnosis of tuberculosis: specific detection of free and complex-dissociated antibodies anti-mycobacterium tuberculosis recombinant antigens.* Brazilian Journal of Infectious Diseases, 2008. **12**(3): p. 234-244.
68. Foulds, J. and R. O'brien, *New tools for the diagnosis of tuberculosis: the perspective of developing countries.* The International Journal of Tuberculosis and Lung Disease, 1998. **2**(10): p. 778-783.
69. de Luna, F.F.-A., et al., *Evaluation of the GenoType Mycobacteria Direct assay for detection of Mycobacterium tuberculosis complex and four atypical mycobacterial species in clinical samples.* Journal of clinical microbiology, 2006. **44**(8): p. 3025-3027.
70. Neonakis, I.K., et al., *Evaluation of GenoType mycobacteria direct assay in comparison with Gen-Probe Mycobacterium tuberculosis amplified direct test and GenoType MTBDRplus for direct detection of Mycobacterium tuberculosis complex in clinical samples.* Journal of clinical microbiology, 2009. **47**(8): p. 2601-2603.
71. Lv, Z., et al., *Utility of real-time quantitative polymerase chain reaction in detecting Mycobacterium tuberculosis.* BioMed research international, 2017. **2017**.
72. Detjen, A.K., et al., *Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: a systematic review and meta-analysis.* The lancet respiratory medicine, 2015. **3**(6): p. 451-461.
73. Chawla, K., et al., *Role of PCR in the diagnosis of pulmonary and extra-pulmonary tuberculosis.* National Journal of Laboratory Medicine, 2015. **4**(4): p. 64-67.
74. Sarmiento, O.L., et al., *Assessment by meta-analysis of PCR for diagnosis of smear-negative pulmonary tuberculosis.* Journal of Clinical Microbiology, 2003. **41**(7): p. 3233-3240.
75. Farooqi, J., et al., *Utility of Line Probe Assay for diagnosis of extrapulmonary tuberculosis.* International Journal of Mycobacteriology, 2015. **4**: p. 110.
76. Luetkemeyer, A.F., et al., *Evaluation of two line probe assays for rapid detection of Mycobacterium tuberculosis, tuberculosis (TB) drug resistance, and non-TB Mycobacteria in HIV-infected individuals with suspected TB.* Journal of clinical microbiology, 2014. **52**(4): p. 1052-1059.
77. Singh, B.K., et al., *Diagnostic utility of a line probe assay for multidrug resistant-TB in smear-negative pulmonary tuberculosis.* PLoS One, 2017. **12**(8): p. e0182988.
78. Sharma, G., et al., *A loop-mediated isothermal amplification assay for the diagnosis of pulmonary tuberculosis.* Letters in applied microbiology, 2019. **68**(3): p. 219-225.
79. Srivastava, S.K., *Biosensor based detection of tuberculosis biomarkers*. 2014: Wageningen University.
80. Fani, M., et al., *Correlation of human papillomavirus 16 and 18 with cervical cancer and their diagnosis methods in Iranian women: A systematic review and meta-analysis.* Current problems in cancer, 2020. **44**(1): p. 100493.
81. Fani, M., et al., *Current approaches for detection of human T-lymphotropic virus Type 1: A systematic review.* Journal of cellular physiology, 2019. **234**(8): p. 12433-12441.
82. Khorasani, M.Y., et al., *The role of curcumin and its derivatives in sensory applications.* Materials Science and Engineering: C, 2019. **103**: p. 109792.
83. Rezayi, M., et al., *A comparison of analytical methods for measuring concentrations of 25-hydroxy vitamin D in biological samples.* Analytical Methods, 2018. **10**(47): p. 5599-5612.
84. Rezayi, M., et al., *Titanium (III) cation selective electrode based on synthesized tris (2pyridyl) methylamine ionophore and its application in water samples.* Scientific reports, 2014. **4**(1): p. 1-8.
85. Said, N.R., et al., *A new N-heterocyclic Carbene Ionophore in plasticizer-free Polypyrrole membrane for determining Ag+ in tap water.* Electrochimica Acta, 2016. **197**: p. 10-22.
86. Said, N.R., et al., *A novel potentiometric self-plasticizing polypyrrole sensor based on a bidentate bis-NHC ligand for determination of Hg (II) cation.* RSC advances, 2015. **5**(93): p. 76263-76274.
87. Sany, S.B.T., et al., *An overview of detection techniques for monitoring dioxin-like compounds: latest technique trends and their applications.* RSC advances, 2016. **6**(60): p. 55415-55429.
88. Abraham, A.A., et al., *A novel potentiometric sensor based on 1, 2-Bis (N'-benzoylthioureido) benzene and reduced graphene oxide for determination of lead (II) cation in raw milk.* Electrochimica Acta, 2015. **165**: p. 221-231.
89. Ahmadzadeh, S., et al., *Highly selective detection of titanium (III) in industrial waste water samples using meso-octamethylcalix [4] pyrrole-doped PVC membrane ion-selective electrode.* Electrochimica Acta, 2015. **178**: p. 580-589.
90. Ahmadzadeh, S., et al., *Cesium selective polymeric membrane sensor based on p-isopropylcalix [6] arene and its application in environmental samples.* RSC Advances, 2015. **5**(49): p. 39209-39217.
91. Rezayi, M., et al., *Potentiometric chromate anion detection based on Co (SALEN) 2 ionophore in a PVC-membrane sensor.* Journal of The Electrochemical Society, 2014. **161**(6): p. B129.
92. Rezayi, M., et al., *A novel polymeric membrane sensor for determining titanium (III) in real samples: Experimental, molecular and regression modeling.* Sensors and Actuators B: Chemical, 2016. **224**: p. 805-813.
93. Fani, M., et al., *The role of microRNAs in the viral infections.* Current pharmaceutical design, 2018. **24**(39): p. 4659-4667.
94. Mahmoodi, P., et al., *Early detection of cervical cancer based on high-risk HPV DNA-based genosensors: A systematic review.* Biofactors, 2019. **45**(2): p. 101-117.
95. Rasouli, E., et al., *Advancements in electrochemical DNA sensor for detection of human papilloma virus-A review.* Analytical biochemistry, 2018. **556**: p. 136-144.
96. Huang, Y., et al., *Disease-related detection with electrochemical biosensors: a review.* Sensors, 2017. **17**(10): p. 2375.
97. Ronkainen, N.J., H.B. Halsall, and W.R. Heineman, *Electrochemical biosensors.* Chemical Society Reviews, 2010. **39**(5): p. 1747-1763.
98. Torres-Chavolla, E. and E.C. Alocilja, *Nanoparticle based DNA biosensor for tuberculosis detection using thermophilic helicase-dependent isothermal amplification.* Biosensors and Bioelectronics, 2011. **26**(11): p. 4614-4618.
99. Zhang, P., et al. *Electrochemical biosensor based on modified graphene oxide for tuberculosis diagnosis*. in *2011 9th IEEE International Conference on ASIC*. 2011. IEEE.
100. Mogha, N.K., et al., *Reduced graphene oxide nanoribbon immobilized gold nanoparticle based electrochemical DNA biosensor for the detection of Mycobacterium tuberculosis.* Journal of Materials Chemistry B, 2018. **6**(31): p. 5181-5187.

101. Miodek, A., et al., *E-DNA sensor of Mycobacterium tuberculosis based on electrochemical assembly of nanomaterials (MWCNTs/PPy/PAMAM).* Analytical chemistry, 2015. **87**(18): p. 9257-9264.
102. Zribi, B., et al., *A microfluidic electrochemical biosensor based on multiwall carbon nanotube/ferrocene for genomic DNA detection of Mycobacterium tuberculosis in clinical isolates.* Biomicrofluidics, 2016. **10**(1): p. 014115.
103. Rueda, D., et al., *A novel inexpensive electrochemical sensor for pyrazinoic acid as a potential tool for the identification of pyrazinamide-resistant Mycobacterium tuberculosis.* International journal of mycobacteriology, 2018. **7**(3): p. 275.
104. VanEngelenburg, S.B. and A.E. Palmer, *Fluorescent biosensors of protein function.* Current opinion in chemical biology, 2008. **12**(1): p. 60-65.
105. Newman, R.H., M.D. Fosbrink, and J. Zhang, *Genetically encodable fluorescent biosensors for tracking signaling dynamics in living cells.* Chemical reviews, 2011. **111**(5): p. 3614-3666.
106. Vacaru, A.M., et al., *Generation of transgenic mouse fluorescent reporter lines for studying hematopoietic development*, in *Mouse genetics*. 2014, Springer. p. 289-312.
107. Gaddam, R.R., R. Narayan, and K. Raju, *Fluorescence spectroscopy of nanofillers and their polymer nanocomposites*, in *Spectroscopy of polymer nanocomposites*. 2016, Elsevier. p. 158-180.
108. Stich, M.I., L.H. Fischer, and O.S. Wolfbeis, *Multiple fluorescent chemical sensing and imaging.* Chemical Society Reviews, 2010. **39**(8): p. 3102-3114.
109. Lu, X., et al., *A gold nanorods-based fluorescent biosensor for the detection of hepatitis B virus DNA based on fluorescence resonance energy transfer.* Analyst, 2013. **138**(2): p. 642-650.
110. Villalobos, P., et al., *The application of polymerized lipid vesicles as colorimetric biosensors for real-time detection of pathogens in drinking water.* Electronic Journal of Biotechnology, 2012. **15**(1): p. 4-4.
111. Zadran, S., et al., *Fluorescence resonance energy transfer (FRET)-based biosensors: visualizing cellular dynamics and bioenergetics.* Applied microbiology and biotechnology, 2012. **96**(4): p. 895-902.
112. Manasreh, M.O., *Introduction to nanomaterials and devices*. Vol. 1. 2012: Wiley Online Library.
113. Shojaei, T.R., et al., *Development of sandwich-form biosensor to detect Mycobacterium tuberculosis complex in clinical sputum specimens.* Brazilian Journal of Infectious Diseases, 2014. **18**(6): p. 600-608.
114. Yang, F., G. Yen, and B.T. Cunningham, *Voltage-tuned resonant reflectance optical filter for visible wavelengths fabricated by nanoreplica molding.* Applied Physics Letters, 2007. **90**(26): p. 261109.
115. Xiao, L., et al., *Colorimetric biosensor for detection of cancer biomarker by au nanoparticle-decorated Bi2Se3 nanosheets.* ACS applied materials & interfaces, 2017. **9**(8): p. 6931-6940.
116. Ckumdee, J., et al., *Development of Au-nanoprobes combined with loop-mediated isothermal amplification for detection of Isoniazid resistance in Mycobacterium tuberculosis.* Journal of Chemistry, 2016. **2016**.
117. Ckumdee, J., S. Santiwatanakul, and T. Kaewphinit. *Development of a rapid and sensitive DNA turbidity biosensor test for diagnosis of katG gene in isoniazid resistant Mycobacterium tuberculosis*. in *2017 IEEE SENSORS*. 2017. IEEE.
118. Pohanka, M., *Overview of piezoelectric biosensors, immunosensors and DNA sensors and their applications.* Materials, 2018. **11**(3): p. 448.
119. Williams, A.L. and W.J. Brown, *Piezoelectric motor*. 1948, Google Patents.
120. Kaewphinit, T., et al., *Detection of non-amplified Mycobacterium tuberculosis genomic DNA using piezoelectric DNA-based biosensors.* Sensors, 2010. **10**(3): p. 1846-1858.
121. Mi, X., et al., *Novel phage amplified multichannel series piezoelectric quartz crystal sensor for rapid and sensitive detection of Mycobacterium tuberculosis.* Analytical chemistry, 2012. **84**(2): p. 939-946.
122. Ren, J., et al., *Simultaneous and early detection of Mycobacterium tuberculosis resistance to antituberculosis drugs using an indirect series piezoelectric system.* Biosensors and Bioelectronics, 2013. **43**: p. 115-119.
123. Yan, R., et al., *Waveguide biosensor with integrated detector array for tuberculosis testing.* Applied Physics Letters, 2011. **98**(1): p. 013702.
124. Dey, D. and T. Goswami, *Optical biosensors: a revolution towards quantum nanoscale electronics device fabrication.* Journal of Biomedicine and Biotechnology, 2011. **2011**.
125. Ligler, F.S. and C.R. Taitt, *Optical biosensors: today and tomorrow*. 2011: Elsevier.
126. Martins, T.D., et al., *New insights on optical biosensors: techniques, construction and application.* State of the art in biosensors—General aspects, 2013: p. 112-139.
127. Heydari, M., et al., *Aptamers as potential recognition elements for detection of vitamins and minerals: A systematic and critical review.* Critical reviews in clinical laboratory sciences, 2020. **57**(2): p. 126-144.
128. Damborský, P., J. Švitel, and J. Katrlík, *Optical biosensors.* Essays in biochemistry, 2016. **60**(1): p. 91-100.
129. Mukundan, H., et al., *Rapid detection of Mycobacterium tuberculosis biomarkers in a sandwich immunoassay format using a waveguide-based optical biosensor.* Tuberculosis, 2012. **92**(5): p. 407-416.
130. Hong, S.C., et al., *Ultrasensitive immunosensing of tuberculosis CFP-10 based on SPR spectroscopy.* Sensors and Actuators B: Chemical, 2011. **156**(1): p. 271-275.
131. Hong, S.C., et al., *Clinical immunosensing of tuberculosis CFP-10 in patient urine by surface plasmon resonance spectroscopy.* Sensors and Actuators B: Chemical, 2011. **160**(1): p. 1434-1438.
132. Duman, M. and E. Piskin, *Detection of Mycobacterium tuberculosis complex and Mycobacterium gordonae on the same portable surface plasmon resonance sensor.* Biosensors and Bioelectronics, 2010. **26**(2): p. 908-912.
133. Rachkov, A., et al., *Discrimination of single base mismatched oligonucleotides related to the rpoB gene of Mycobacterium tuberculosis using a surface plasmon resonance biosensor.* Biotechnology and applied biochemistry, 2013. **60**(4): p. 453-458.
134. Rinaldi, F., et al., *Epitope and affinity determination of recombinant Mycobacterium tuberculosis Ag85B antigen towards anti-Ag85 antibodies using proteolytic affinity-mass spectrometry and biosensor analysis.* Analytical and bioanalytical chemistry, 2019. **411**(2): p. 439-448.
135. Xiang, Y., et al., *Real-time monitoring of mycobacterium genomic DNA with target-primed rolling circle amplification by a Au nanoparticle-embedded SPR biosensor.* Biosensors and Bioelectronics, 2015. **66**: p. 512-519.
136. Barroso, T.R., et al. *Detecting antibody-labeled BCG MNPs using a magnetoresistive biosensor and magnetic labeling technique*. in *Journal*

of Nano Research. 2016. Trans Tech Publ.
137. Wang, Y., et al., *Application of nanodiagnostics in point-of-care tests for infectious diseases.* International journal of nanomedicine, 2017. **12**: p. 4789.
138. Vargas, G., et al., *Applications of magnetotactic bacteria, magnetosomes and magnetosome crystals in biotechnology and nanotechnology: mini-review.* Molecules, 2018. **23**(10): p. 2438.
139. Liong, M., et al., *Magnetic barcode assay for genetic detection of pathogens.* Nature communications, 2013. **4**(1): p. 1-9.
140. Gonzalo-Asensio, J., et al., *New insights into the transposition mechanisms of IS 6110 and its dynamic distribution between Mycobacterium tuberculosis Complex lineages.* PLoS genetics, 2018. **14**(4): p. e1007282.
141. Sankar, S., et al., *Analysis of sequence diversity among IS6110 sequence of Mycobacterium tuberculosis: possible implications for PCR based detection.* Bioinformation, 2011. **6**(7): p. 283.
142. Liu, C., et al., *An electrochemical DNA biosensor for the detection of Mycobacterium tuberculosis, based on signal amplification of graphene and a gold nanoparticle–polyaniline nanocomposite.* Analyst, 2014. **139**(21): p. 5460-5465.
143. Hsu, S.-H., et al., *Mycobacterium tuberculosis DNA detection using surface plasmon resonance modulated by telecommunication wavelength.* Sensors, 2014. **14**(1): p. 458-467.
144. Issa, R., N. Hamdan, and M. Noh, *Differential pulse voltammetric determination of DNA hybridization using methylene blue on screen printed carbon electrode for the detection of Mycobacterium tuberculosis.* Biotechnology, 2010. **9**(3): p. 304-311.
145. Kanayeva, D., I. Bekniyazov, and Z. Ashikbayeva, *Detection of tuberculosis using biosensors: Recent progress and future trends.* Sensors & Transducers, 2013. **149**(2): p. 166.
146. Mohamad, F.S., et al., *Synthesis and characterization of polyaniline/graphene composite nanofiber and its application as an electrochemical DNA biosensor for the detection of Mycobacterium tuberculosis.* Sensors, 2017. **17**(12): p. 2789.
147. Nguyen, L., *Antibiotic resistance mechanisms in M. tuberculosis: an update.* Archives of toxicology, 2016. **90**(7): p. 1585-1604.
148. Nurmalasari, R., S. Gaffar, and Y.W. Hartati, *Label-free electrochemical DNA biosensor for the detection of Mycobacterium tuberculosis using gold electrode modified by self-assembled monolayer of thiol.* Procedia Chemistry, 2015. **17**: p. 111-117.
149. Prabhakar, N., et al., *Peptide nucleic acid immobilized biocompatible silane nanocomposite platform for Mycobacterium tuberculosis detection.* Electroanalysis, 2010. **22**(22): p. 2672-2682.
150. Thakur, H., et al., *Electrochemical determination of M. tuberculosis antigen based on Poly (3, 4-ethylenedioxythiophene) and functionalized carbon nanotubes hybrid platform.* Talanta, 2017. **171**: p. 115-123.
151. Thakur, H., et al., *Aptamer based voltammetric biosensor for the detection of Mycobacterium tuberculosis antigen MPT64.* Microchimica Acta, 2017. **184**(7): p. 1915-1922.
152. Torati, S.R., et al., *Electrochemical biosensor for Mycobacterium tuberculosis DNA detection based on gold nanotubes array electrode platform.* Biosensors and Bioelectronics, 2016. **78**: p. 483-488.
153. Liu, Y., et al., *Aptamer-based electrochemical biosensor for interferon gamma detection.* Analytical chemistry, 2010. **82**(19): p. 8131-8136.
154. Yesil, M., S. Donmez, and F. Arslan, *Development of an electrochemical DNA biosensor for detection of specific Mycobacterium tuberculosis sequence based on poly (L-glutamic acid) modified electrode.* Journal of Chemical Sciences, 2016. **128**(11): p. 1823-1829.
155. Miotto, P., et al., *A standardised method for interpreting the association between mutations and phenotypic drug resistance in Mycobacterium tuberculosis.* European Respiratory Journal, 2017. **50**(6).
156. Morency-Potvin, P., D.N. Schwartz, and R.A. Weinstein, *Antimicrobial stewardship: how the microbiology laboratory can right the ship.* Clinical microbiology reviews, 2017. **30**(1): p. 381-407.