Review

Quantum Dot–Based Nanosensors for in Vitro Detection of Mycobacterium Tuberculosis

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Quantum Dot–Based Nanosensors for In Vitro Detection of Mycobacterium tuberculosis

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Abstract: Despite the existing effective treatment methods, tuberculosis (TB) is the second most deadly infectious disease globally, its carriers in the latent and active phases accounting for more than 20% of the world population. An effective method to control TB and reduce the mortality is regular population screening and diagnosis of the latent form of TB in order to take preventive and curative measures. Numerous methods allow diagnosing TB and directly detecting *Mycobacterium tuberculosis* (*M.tb*) biomarkers, including *M.tb* DNA, proteins, and specific metabolites, as well as antibodies produced by the host immune system in response to *M.tb.* PCR, ELISA, immunofluorescence and immunochemical analyses, flow cytometry, and other methods allow the detection of *M.tb* biomarkers or the host immune response to *M.tb* by recording the optical signal of fluorescent or colorimetric dyes included in the diagnostic tools. Current research in biosensors is aimed at increasing the sensitivity of detection, which can be achieved by using brighter and more photostable optical tags containing fluorescent quantum dots. Here, we review current methods for detection of *M.tb* biomarkers using optical sensor systems, primarily quantum dot–based nanosensors, and summarize *M.tb* biomarkers whose detection can be made significantly more sensitive by using quantum dot–based nanosensors.

Keywords: quantum dot; nanosensor; tuberculosis; diagnostics

1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and most often affecting the lungs. In 2022, there were 10.6 million TB cases worldwide, including 5.8 million men, 3.5 million women, and 1.3 million children; a total of 1.3 million people died from TB (including 167,000 people with concomitant HIV infection). Globally, TB is the second leading cause of death among infectious diseases after COVID-19, with death rates higher than those of AIDS [1]. TB can have particularly severe consequences for women, especially those of reproductive age and during pregnancy, and is one of the top five killers of women aged 20–59 years [2]. Recent estimates demonstrate that about 1.7 billion people are latently infected by *M.tb* [3]. At the same time, traditional diagnostic methods, such as chest X-ray and tuberculosis skin tests, do not have a sufficient level of sensitivity and specificity to reliably diagnose latent forms of TB [4], especially against the background of other diseases or pathological conditions [5]. The risk of progression of latent TB infection to the active form is estimated at 10% [6]. One of the health-related targets of the United Nations Sustainable Development Goals (SDGs) is to end the TB epidemic by 2030 [1]. To attain this goal, it is necessary not only to combat active forms of TB, but also to carry out prevention

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and therapy for patients with latent TB, which requires methods for diagnosing *M.tb* infection at the earliest stages.

2. Current Tuberculosis Diagnostic Methods

TB can be diagnosed in two ways: direct detection of *M.tb* in a clinical specimen or detection of the biomarkers associated with *M.tb* infection. Currently, numerous methods are widely used for TB diagnosis [7–9]. At the moment, there are four major groups of routine clinical methods used for this purpose: rapid molecular diagnostic tests, cultural methods, provocation tests, and diagnosis using optical methods. The characteristics of these groups of methods are presented in Table 1. With exception of skin tests, all the procedures are performed *in vitro.*

Table 1. The major groups of routine clinical methods used for tuberculosis diagnosis.

Assay	Biomaterial analyzed	Time of analysis	Advantages	Disadvantages	Sensitivity, specificity	Ref.	Comments
Molecular diagnostic tests							
Polymerase chain reaction (PCR)	Serum, urine, blood, sputum, saliva, lung biopsy samples, BALF, pleural fluid	4–5 h	High specificity, quickness, and informativeness	High cost, limited availability, low sensitivity for non-respiratory samples; detection of DNA only	Sensitivity: 47% (42–51%) Specificity: 95% (93–97%) CrI: 95%	[10]	The sensitivity and specificity are averaged results of 9 studies in 709 subjects
Xpert MTB/RIF Ultra	Raw sputum or concentrated sediment	1.5 h	Detection of *rpoB* gene mutations associated with rifampicin resistance	High cost	Sensitivity: 89% (85–92%) Specificity: 99% (98–99%) CrI: 95%	[11]	The sensitivity and specificity are averaged result of 22 studies in 8998 subjects, including 2953 confirmed TB cases and 6045 cases without TB
Truenat	Raw sputum	1 h	Use of a portable, chip-based and battery-operated device. Suitability for laboratories with technical equipment	Lower accuracy compared to Xpert MTB/RIF Ultra	Sensitivity: 80% (70.2–88.4%) Specificity: 98% (94.5–99.6%)	[12]	The sensitivity and specificity have been estimated in tests in 250 subjects
LF-LAM	Urine	0.5 h	High efficiency, ease of use, low cost, simple	Lower sensitivity compared to Xpert MTB/RIF (though	Sensitivity: 45% (29–63%)	[13]	The sensitivity and

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			technology, no special equipment. Detection of TB in patients for whom other diagnostic methods cannot be used (e.g., HIV patients)	higher compared to microscopy methods). Suitability for a limited group of patients only. Impossibility to distinguish *M. tb.* from other mycobacteria, which requires using other diagnostic methods after the test	Specificity: 92% (80–97%) CrI: 95%		specificity are averaged results of 5 studies in 2313 subjects, including 35% of TB cases
TB-XT HEMA EXPRESS	Blood, serum	0.5 h	Quickness, relatively low cost	Low sensitivity, suboptimal performance in the case of high TB prevalence	Sensitivity: 31% (3.9–78%) Specificity: 85% так (52–93%)	[14]	The sensitivity and specificity have been estimated in tests in 1386 subjects, including 290 TB cases
TB tests based on T-cells analysis							
IGRA, (T-SPOT.TB, QuantiFERON-TB Gold (QFT))	Blood, serum	up to 2 days	Insensitivity to BCG vaccination or contact with atypical mycobacteria; one-time tests; high efficiency. T-SPOT.TB is less susceptible to immunosuppression than other TB tests and is preferable for the examination of HIV-infected or autoimmunity patients during treatment with immunosuppressants; can be used before starting therapy with biological drugs	Low specificity and sensitivity; high cost; impossibility to distinguish between the active and latent forms of TB and unsuitability as a primary diagnostic test for LTBI or active TB. The bacterium itself is not determined; the result depends on the state of the patient's immune system	**QFT** Sensitivity: 66% (47–81%) Specificity: 87% (68–94%) **T-SPOT** Sensitivity: 60% (48–72%) Specificity: 86% (65–95%)	[15]	The sample consisted of 6,525 HIV-positive patients, including 3,467 TB cases, 806 of them with LTBI and 2,661 with active TB
Cultural methods							
BBL Septi-Chek AFB	Sputum	Up to 23 days	Higher *M.tb* growth rate	Low sensitivity, long time of analysis	Sensitivity: 73%	[16]	The sensitivity

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			compared to methods using an isolated dense medium		Specificity: 93%		and specificity have been estimated in studies on 274 specimens
BACTEC (with different parameters of MGIT 460, 960)	Sputum	Up to 14 days	Rapid identification of *M.tb* and its drug sensitivity; accelerated culture testing of all first-line drugs	High cost, justified only for large laboratories. Semi-automatic monitoring of bacterium growth; many labor-intensive operations; use of radioisotopes and the need for disposal of radioactive waste; long time of analysis	**MGIT 960** Sensitivity: 81.5% Specificity: 99.6% **MGIT 460** Sensitivity: 85.8% Specificity: 99.9%	[17]	The sensitivity and specificity have been estimated in studies on ~8,000 clinical specimens per year. The number after MGIT is the number of wells in the plate.
BacT/ALERT 3D	Sputum	24–72 h	Detection of *M.tb* growth; detection of *M.tb* and fungi in blood cultures. Full automation; no radioactive waste	Long time, high cost	Sensitivity: 87.80% Specificity: 99.21%	[18]	The sensitivity and specificity have been estimated in studies on 2659 clinical specimens
Skin tests							
Tuberculin skin tests, Mantoux tests, and Diaskintest (*in vivo*)	Skin tests	72 h	Availability; low cost; ease of use	Low specificity and sensitivity; unsuitability for diagnosing active TB forms; false positive results in those who have been infected with *M.tb* in the past because their memory T cells still secrete interferon; impossibility to distinguish the active and latent forms of TB	Sensitivity: 59% Specificity: 95%	[19]	The sensitivity and specificity have been estimated in tests in 643,694 U.S. Navy recruits
Tests based on mycobacterium staining							
Gabbett's stain, Ziehl–Neelsen stain, modified cold stain (MCS)	Sputum	~24 h	Simplicity, quickness, ease of use, low cost	Low sensitivity and specificity; suitability for pulmonary tuberculosis only; inaccuracy in children	**Gabbett's stain** Sensitivity: 77%	[20]	The sensitivity and specificity have been

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				and HIV-infected persons; multistage and complex procedure. Impossibility to distinguish between different mycobacteria	Specificity: 98% **Ziehl–Neelsen stain** Sensitivity: 70% Specificity: 97% **MCS** Sensitivity: 60% Specificity: 96%		estimated in tests in 100 patients
Fluorescence microscopy	Sputum	~24 h	Quickness, ease of use, specificity	High cost, frequent burn-out of expensive mercury vapor lamps, need for continuous power supply, need for a dark room	Sensitivity: 72% Specificity: 81%	[21]	The sensitivity and specificity have been estimated in tests in 426 patients
Other methods							
X-ray	Radiographic test	1 h	Quickness	High cost; low specificity	Sensitivity: 96% Specificity: 46%	[22]	The sensitivity and specificity are averaged results of 13 studies
MALDI-TOF MS	BALF, sputum	2.5 h	Rapid, reliable, cost-effective method	Method requires sample preprocessing to generate high-quality proteomic profiles, especially for proteins/peptides or other low-abundance analytes in which MS spectra are obscured by more abundant/high-molecular-weight species. This method is not highly specific because of the matrix proteins and noise issues	Sensitivity: 83%; Specificity: 93%; CrI: 95%	[23]	The sensitivity and specificity have been estimated in tests in 214 patients
LC-MS/MS	Urine, blood	1 h	Proteomic analysis of urine; identification of proteins characteristic of TB with high	Changes in ionization efficiency in the presence of not only proteins, phospholipids, and salts, abut also reagents and contaminants	Sensitivity: 94% Specificity: 100%	[24]	The sensitivity and specificity have been estimated in

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	molecular specificity and sensitivity; simultaneous diagnosis of HIV-1 and TB using a blood sample. Structural identity of individual components	tests in 57 patients

* Abbreviations: LF-LAM, lipoarabinomannan lateral shift test; LTBI, latent tuberculosis infection; MGIT, mycobacteria growth indicator tube; IGRA, interferon-gamma release assay; CrI, credible interval; BALF, bronchoalveolar lavage fluid; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; LC-MS, liquid chromatography tandem mass spectrometry; MS, mass spectrometry.

2.1. Molecular Diagnostic Tests

Polymerase chain reaction (PCR) is a molecular biology technique based on amplification and further analysis of specific DNA fragments. The amplification involves several cycles of heating and cooling, causing a DNA fragment to be duplicated many times to reach a detectable amount. PCR can detect the presence of a DNA fragment specific for *M.tb* [10]. This method is effective for early TB diagnosis when the number of the microorganisms is insufficient for detection by classical methods. PCR tests also allow analyzing drug resistance of specific *M.tb* strains. The PCR tests have strict requirements for laboratory room purity and personnel skills because their high sensitivity entails their downside related to the high risk of engaging contaminants in the reaction. PCR tests are suitable for detecting TB sepsis and disseminated TB but not for population screening for TB, where the results could be false negative.

LF-LAM is a lateral flow urine test for diagnosis of TB through the detection of lipoarabinomannan, a mycobacterial cell wall lipoglycan. A disadvantage of the LF-LAM method is its low sensitivity [13]. Because lateral flow tests are cheap and easy to perform, they are often used in diagnosis of TB by detecting IgG antibodies against TB-specific proteins in blood and serum samples [14]. Simultaneous detection of IgG and IgM antibodies has also been reported [25]. In this case, the test band contained a proprietary mixture of recombinant TB antigens that ensured a diagnostic sensitivity of 94.4% in a sample of 125 microbiologically or clinically diagnosed TB patients and a diagnostic specificity of 98.3% in a sample of 400 subjects who were healthy or had respiratory conditions other than TB.

Loop-mediated isothermal amplification (LAMP) uses special primers to amplify DNAs forming loop-shaped intermediates of different sizes, which can be detected using fluorescence measurements or agarose gel electrophoresis [26]. WHO recommends the use of TB-LAMP as a replacement for microscopy for the diagnosis of pulmonary TB [42].

Xpert MTB/RIF Ultra is an improved version of the Xpert MTB/RIF test [28,29]. Xpert MTB/RIF Ultra (*in vitro*) and Truenat can also identify mutations of the *rpoB* gene associated with rifampicin resistance [12,28–30]. Xpert MTB/RIF Ultra and Truenat have a higher sensitivity and a shorter time of analysis than conventional PCR tests.

Serological tests detecting antibodies against specific protein antigens of the recombinant *M.tb* complex have a high specificity but variable sensitivity [14]. The control of multiple *M.tb* complex antigens increases the sensitivity [14]. It should be pointed out that these methods may give false positive results because specific antibodies can occur in the human body for a long time after recovery from TB.

2.2. Tuberculosis Tests Based on T-Cell Analysis

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Interferon-gamma release assay (IGRA) is a group of laboratory tests that evaluate the release of interferon-gamma (INF-γ) by human immune blood cells (T cells) and are performed *in vitro* [15]. There are two different types of blood tests based on this principle approved by FDA: QuantiFERON-TB Gold Plus (QFT) and T-SPOT.TB (T-SPOT). The QFT test is a whole-blood-based enzyme-linked immunosorbent assay (ELISA) measuring the amount of IFN-γ produced in response to two *M.tb* antigens (ESAT-6 and CFP-10). The T-SPOT test measures the number of T cells that produce INF-γ after stimulation with ESAT-6 and CFP-10. These methods may give false positive results because T cells have a long memory of an *M.tb* invasion that might have occurred a long time ago.

2.3. Cultural Methods

Cultural method remains the gold standard of TB diagnosis confirmation. They consist in inoculation of biological material on solid or liquid differential diagnostic nutrient media for growing mycobacterial colonies. In practice, several cultural methods are used: acid-fast mycobacteria (AFB) [16], BACTEC (with different parameters of MGIT, usually 460 and 960) [31], and BacT/ALERT 3D [18].

The AFB method involves examining human sputum or other samples stained to detect acid-fast bacteria. The latest experimental study using this method dates back to 1997 [31,32], which may be due to the extremely long time of analysis. The sample to be tested is inoculated into one or more vials with a specific growth medium and inserted into the instrument for incubation and periodic fluorescent reading. Each vial contains a chemical sensor detecting an increase in the amount of carbon dioxide produced by the growth of microorganisms. The instrument monitors the sensor every 10 min for an increase in its fluorescence, which is proportional to the amount of CO_2, a positive reading indicating the presence of viable microorganisms in the vial. BACTEC is a fully automated system not only for *M.tb* detection, but also for the analysis of *M.tb* sensitivity to all the first-line drugs, including pyrazinamide. BACTEC is a reference method with high sensitivity and specificity, but it takes about 10 days to obtain the result [33]. BacT/ALERT 3D allows automated monitoring the culture medium to detect the growth of microorganisms via monitoring CO_2 release by an increase in reflectance. It has a high sensitivity with a shorter culturing time. BacT and BACTEC have similar operating principles but differ in the details of technology and design.

The key limitation of these methods is a too long time of analysis.

2.4. Skin Tests

In vivo tuberculin skin tests are based on provocation of the body immune response to TB-associated molecules [19]. For example, the Mantoux test uses a tuberculin solution administered via intradermal injection. All these tests suffer from frequent false positive and false negative results. The point is that the immune system responds to tuberculin if there are mycobacteria in the body, and the majority of people receive the bacteria in the form of the BCG vaccine back in the maternity hospital. Recently, WHO included Diaskintest, which is an advanced and more accurate variant of the Mantoux tuberculin test [34], into the list of recommended TB skin tests.

2.5. Tests Based on Mycobacterium Staining

Staining methods identify acid-fast mycobacteria, actinomycetes, and other acid-fast microorganisms by means of their staining and then analyzing using optical microscopy. These methods differ in the staining solution, which determines the sensitivity and specificity of analysis. The weak point of this group of TB tests is a complex procedure of analysis that requires considerable time and highly skilled personnel.

2.6. Other Methods

Chest X-rays are commonly used in the TB diagnosis. They can help identify abnormalities in the lungs that may be indicative of TB infection, such as nodules, cavities, or infiltrates. However, it should be noted that chest X-rays alone cannot definitively diagnose TB.

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is based on an ionization technique that allows the ionization of biological macromolecules, such as peptides, proteins, DNA, oligonucleotides, and lipopolysaccharides, in the presence of a special matrix under laser irradiation [35]. Wang et al. [36] have evaluated MALDI-TOF MS as a means of *M.tb* nucleic acid detection for rapid diagnosis of TB and drug resistance. The effectiveness of the MALDI-TOF MS can be improved by using the protocol of destruction of mycobacterium cells and protein extraction [37].

Liquid chromatography–tandem mass spectropmetry (LC-MS/MS) is based on coupling mass spectrometers in series to analyze complex mixtures [38]. For example, liquid–liquid extraction and LC-MS analysis were used to determine the pretomanid concentrations in 40 mL of human plasma [39]. The method was proven to be reliable and reproducible for pharmacokinetic analysis of samples in a clinical trial involving TB patients. Another study used the LC-MS technique to detect specific *M.tb* peptides in mouse blood serum. Sixty-five peptides from four recombinant *M.tb* proteins were identified in the mouse blood [40]. This method is not used to directly detect *M.tb*, but it is useful in the monitoring of TB treatment [41].

Figure 1 graphically illustrates the key data from Table 1.

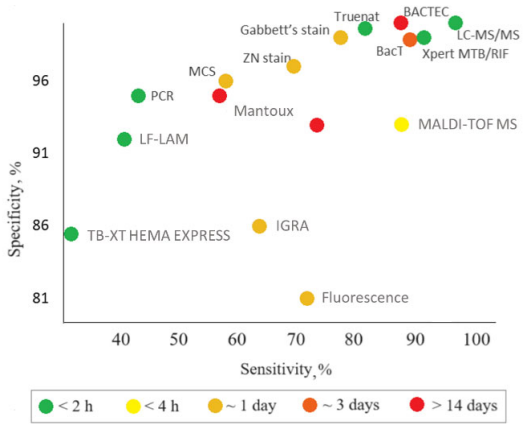

Figure 1. Sensitivity and specificity of tuberculosis diagnostic methods shown in Table 1.

Scrutiny of the above TB diagnostic methods shows that all of them have disadvantages and limitations. Therefore, development of new simple and effective methods of TB diagnosis is an urgent task.

3. Quantum Dot–Based Nanosensors for *M. Tuberculosis* Detection and Tuberculosis Diagnosis

Most of the detection methods discussed above are in some way or another related to the detection of an optical signal, be it molecular detection methods based on PCR with fluorescent probes, lateral flow tests that use colloidal gold nanoparticles or colored latex microparticles, methods based on ELISA and ELISPOT tests, tests for CO_2 accumulation, or specific *M.tb* staining. Traditionally, all commercial products for MTB detection and TB diagnosis use organic fluorescent or colorimetric dyes, which have recently been increasingly replaced with fluorescent quantum dots (QDs) [42].

QDs are inorganic semiconductor nanocrystals 2–10 nm in size with a high fluorescence quantum yield due to a high molar absorption coefficient and a high efficiency of internal conversion of the absorbed photon energy into fluorescence [43]. Another benefit of QDs is their extremely long luminescence lifetime compared to fluorescent biomolecules. This allows time-resolved detection with an increased useful signal-to-noise ratio, which enhances the detection sensitivity [44,45]. The narrow emission peak and wide absorption spectrum make it possible to excite QDs of different colors with a single broad-spectrum source and perform multiplexed measurements. QDs usually have a semiconductor core (CdSe, CdS, CdTe, InP, InAs, AgInS2, CuInS2, PbSe, etc.), often coated

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with a shell to passivate the surface trap states and protect the core from aggressive environment and photo-oxidative degradation, as well as to meet biosafety requirements [46–48]. The typical structure of QD-based nanosensors is shown in Figure 2.

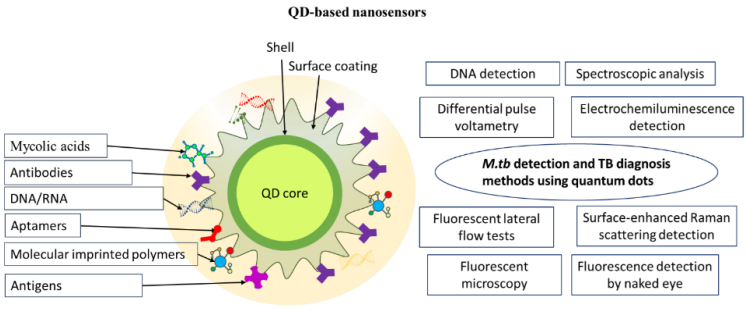

Figure 2. Schematics of a quantum dot–based nanosensor. Abbreviations: *M.tb*, *M. tuberculosis*; TB, tuberculosis.

An ideal QD-based fluorescence nanosensor should combine a bright fluorescent label and a highly specific recognition ligand or capture molecule [49]. This capture molecule can be a protein (e.g., an antibody or recombinant antigen), peptide, oligonucleotide, etc. [43,48,49]. After the QD-based nanosensor has bound the target biomolecule, the QD fluorescence signal can be detected and quantified [52–54]. Numerous methods for covalent and noncovalent conjugation of ligands to the QD surface (e.g., electrostatic interaction and metal ion chelation) have been developed [42,55,56]. The possibility of using multiple QDs with different emission spectra enables simultaneous detection of several biomarkers, which increases the diagnostic accuracy [57–59].

A total of 28 articles retrieved by the keywords *quantum dot*, *tuberculosis*, and *Mycobacterium tuberculosis* and 43 articles retrieved by the keywords *quantum dot* and *tuberculosis* have been found in the PubMed database. Of these publications, 37 dealt with TB diagnosis using QD-based nanosensors, 18 of them published in the past five years (including six reviews published in the past four years [7–9,60–63]). The number of these publications by year is shown in Figure 3. In total, 116 articles are cited in this review, 95 of them published in the past 10 years.

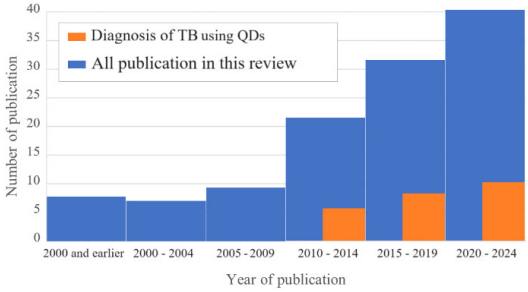

Figure 3. Numbers of analyzed publications by year. Abbreviations: TB, tuberculosis; QDs, quantum dots.

The methods of *M.tb* detection and TB diagnosis using QD-based nanosensors are shown in Table 2.

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Table 2. Quantum dot–based nanosensors for *M. tuberculosis* detection and tuberculosis diagnosis.

No.	Biomaterial analyzed	Biomarker	Capture molecule	Nanosensor	Method of detection	Wavelength, nm (where relevant)	LOD	Ref.
1	Blood	TMCC1, GBP6	Oligonucleotides specific for *M.tb* mRNA biomarkers	QD655 and QD525 conjugated with the capture molecules	Toehold-mediated strand displacement with fluorescence quenching by FRET	Emission: 525 Emission: 655 Excitation: 480	GBP6: 1.6 nM TMCC1: 6.4 nM	[64]
2	Blood	IFN-γ	Anti-human IFN-γ antibodies	CdS QDs coupled to magnetic beads conjugated with the capture molecules. Sandwich-type sensor is fabricated on a glassy carbon electrode covered with a well-ordered gold nanoparticle monolayer, which offers a solid support to immobilize the capture molecules	Square wave anodic stripping voltammetry to quantify the metal cadmium, which indirectly reflects the amount of the analyte	N/A	0.34 pg/mL	[65]
3	Serum	IFN-γ	IFN-γ aptamer	Gold electrode covered with L-cysteine-SnTeSe QDs functionalized with the capture molecules	Electrochemical impedance spectroscopy detection of the change in the electron transfer resistance upon IFN-γ binding	N/A	0.151 pg/mL	[66]
4	Serum	IFN-γ, TNF-α, IL-2	Antibody pairs for IFN-γ-, TNF-α and IL-2	Sandwich immunoassay sensor consisting of luminol and carbon and CdS QDs integrated with gold nanoparticles and magnetic beads functionalized with the capture molecules, as well as the same capture molecules separately immobilized on three spatially resolved areas of a patterned indium tin oxide electrode to capture the corresponding triple latent TB biomarkers	Electrochemilumi nescence detection	N/A	1.6 pg/mL	[67]
5	Sputum	DNA IS1081	Specific DNA nanobeacon	QD-based nanobeacon fluorescence probes containing QDs and black hole quenchers. After the target DNA hybridizes with the nanobeacon, the nanobeacon is cleaved into two DNA fragments, and the QDs fluoresce upon moving away from the black hole quenchers	Fluorescence detection by naked eye	Excitation: 280 Emission: 330	3.3 amol/L (2 copies/μL)	[68]
6	N/A	Anti-MA antibodies	MAs	Graphene QDs covalently functionalized with MAs as detection tags for anti-MA antibodies	Fluorescent lateral flow assay	Excitation: 360 Emission: 470	N/A	[69]

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7	N/A	Anti-MA antibodies	Mas	CdSe/ZnS QDs covalently functionalized with MAs as detection tags for anti-MA antibodies	Fluorescent lateral flow assay	Excitation: 390 Emission: 474	N/A	[70]
8	Pure CFP-10 solution	CFP-10	Pair of anti-CFP-10 antibodies (G2 and G3)	Glass slide coated with magnetoplasmonic core/shell nanoparticles (Fe_3O_4/Au) functionalized with G2. Graphene QDs functionalized with conjugate of gold-binding protein with G3. Upon binding of CFP-10 by a G2–G3 sandwich, immunoassay is formed	Dual metal-enhanced fluorescence and surface-enhanced Raman scattering detection	Excitation: 320 Emission: 436, 516	0.0511 pg/mL	[71]
9	Pure DNA	rpoB531, katG315	ssDNA specific for target DNA	QD535 and QD648 functionalized with specific ssDNA. When the target DNA is absent, the nanosensor is attached to a quencher. Binding with the target DNA leads to detachment of the nanosensor and recovery of fluorescence	Fluorescence measurement	Excitation: 380 Emission (rpoB531): 535 Emission (katG315): 648	rpoB531: 24 pM; katG315: 20 pM	[72]
10	Blood	IFN-γ, IP-10	Aptamers specific for IFN-γ and IP-10	Cytosine–Ag^+–cytosine and thymine-Hg^{2+}–thymine hairpin structures releasing the metal ions upon specific interaction with different biomarker–aptamer complexes. Ag^+ and Hg^{2+} are bound by CdTe and carbon QDs, which are detected by fluorescence	Fluorescence measurement	-	IP-10: 0.3×10^{-6} pg/mL; IFN-γ: 0.5×10^{-6} pg/mL	[73]
11	Sputum	*M.tb* cell	*M.tb*-binding peptide H8, anti-*M.tb* polyclonal antibodies, and anti-HSP65 monoclonal antibodies	QDs conjugated with H8 or anti-HSP65 antibodies and MMS conjugated with H8 or anti-*M.tb* polyclonal antibodies. Magnetic separation of the QD–*M.tb*–MMS complex	Fluorescence microscopy	Excitation: 405 Emission: 610	103 CFU/mL	[74]
12	*M.tb* suspension and sputum	*M.tb* cell	*M.tb*-binding peptide H8	Magnetic beads and QDs conjugated with H8. Magnetic separation of the QD–*M.tb*–magnetic bead complex	Fluorescence microscopy	N/A	103 CFU/mL	[75]
13	Sputum	ESAT-6	Oligonucleotides specific for ESAT-6	FRET-based sandwich biosensor containing CdTe QDs and gold nanoparticles (quencher) conjugated with the capture molecules (obtained by PCR). When the marker id bound, QD fluorescence is quenched via FRET to gold nanoparticles	Fluorescence detection	Excitation: 370 Emission: 400–680	10 fg	[76]
14	Sputum	*IS6110* DNA	ssDNA complementary to the *IS6110* gene fragment	FRET-based biosensor in which CdTe QDs conjugated with the capture molecule serves as a donor and Cu-TCPP, which is more affine for ssDNA than	Fluorescence detection	Excitation: 365 Emission: 586	35 pM	[77]

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				double-stranded DNA, serves as an acceptor. In the absence of the marker, the QD fluorescence is quenched. Interaction of the ssDNA. Hybridization of the ssDNA with the marker results in fluorescence , whose intensity depends on the marker concentration				
15	Urine	Secretory antigen Ag85B	Anti-Ag85B antibodies (GBP-50B14 and SiBP-8B3)	FRET based biosensor in which gold nanorods conjugated with GBP-50B14 serve as acceptors and silica-coated CdTe QDs conjugated with SiBP-8B3 serve as donors. When both tags bind Ag85B, FRET between the QDs and nanorods quenches the QD fluorescence	Fluorescence detection	Excitation: 350 Emission: 630	13 pg/mL	[78]
16	Urine	LAM	Pair of anti-LAM recombinant monoclonal antibodies	Lateral flow test using CdSe/ZnS QDs encapsulated in polymeric bead conjugated with the capture molecules; test strip with the immobilized capture molecules	Portable fluorescence detector	Excitation: 375 Emission: 620	50 pg/mL	[79]
17	Urine	CFP-10	Pair of anti-CFP-10 antibodies	Glassy carbon electrode modified with graphene quantum dot–coated Fe_3O_4@Ag nanoparticles and gold nanoparticles conjugated to the capture antibody. Binding of CFP-10 to the electrode results in an immune sandwich, gold nanoparticles conjugated with the detection antibody serving as signal-amplification labels	Differential pulse voltammetry	N/A	330 pg/mL	[80]
18	Exhaled air	TB related volatile organic biomarkers	No	Suspension of CdSe or carbon QDs. The biomarker causes changes in the absorbance and fluorescence spectra.	Spectroscopic analysis	Excitation: 360–650 Emission: 300–800	N/A	[81]
19	Exhaled air	MN	Co ion	CoTCPP nanosheets with attached CdTe QDs. The QD fluorescence is quenched in the absence of MN and is recovered upon MN binding to CoTCPP causing QD release	Fluorescence detection	Excitation: 370 Emission: 658	0.59 μM	[82]
20	BALS, feces, paraffin-embedded tissues	IS6110 and IS900 DNA	Mycobacterium-specific oligonucleotides	CdSe QDs conjugated with streptavidin and species-specific probes and magnetic beads conjugated with streptavidin and genus-specific probes. Sandwich hybridization is used to bind the biomarkers and subsequent magnet separation, to concentrate the biomarker	Fluorescence detection	Excitation: 260 Emission: 655	12.5 ng	[83]

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21	Pure fprA	fprA	Anti-fprA antibodies	Direct and double antibody sandwich lateral flow tests with CdSe/ZnS QDs conjugated with the capture molecule	Fluorescence detection	Emission: 565	12.5 pg/mL	[84]
22	*M.tb* strains	*M.tb* DNA	ssDNA specific for *M.tb*	FRET-based sensor composed of water-stable $CsPbBr_3$ perovskite QDs conjugated to DNA probe serving as a donor and MoS2 nanosheets serving as acceptor	Fluorescence detection	N/A	51.9 pM	[85]
23	Pure antigens	CFP10-ESAT6	Anti-CFP10–ESAT6 monoclonal antibody	Electrochemical immunosensor consisting of SPCE functionalized with Si nanoparticles and CdSe/ZnS QDs. The target biomarker is adsorbed on the electrode and then captured by the primary antibody, the secondary antibody being labeled with catalase, whose activity is detected electrochemically	Differential pulse voltammetry	N/A	15 pg/mL	[86]

* Abbreviations: TMCC1, transmembrane and coiled-coil domain family 1; GBP6, guanylate binding protein family member 6; QD, quantum dot; IFN-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; IL-2, interleukin-2; MAs, mycolic acids; CFP-10, culture filtrate protein 10; ssDNA, single-strand DNA; IP-10, IFN-γ-induced protein 10; MMS, magnetic microsphere; HSP65, heat shock protein 65; ESAT-6, early secretory antigenic target 6; FRET, Förster resonance energy transfer; Cu-TCPP, two-dimensional metal-organic framework; LAM, lipoarabinomannan; MN, methyl nicotinate; CoTCPP, cobalt-metalized tetrakis (4-carboxyphenyl) porphyrin; BALS, bronchoalveolar lavage specimens; fprA, flavoprotein reductase; SPCE, screen-printed carbon electrode; DPV, differential pulse voltammetry.

Not all of the biomarkers described above are completely specific, because their occurrence may be related to concomitant diseases, body conditions, etc. Currently, there is no biomarker or combination of biomarkers that allows diagnosing active forms of TB with an accuracy close to 100%. Thus, the search for a combination of biomarkers with a high specificity is an urgent task. New potential *M.tb* biomarkers that can be detected by new QD-based fluorescent nanosensors are listed in Table 3.

Table 3. Potential *M. tuberculosis* biomarkers.

Biomarker	Already detected with QD-based nanosensors	Comment	Ref.
Host RNA transcript/DNA signatures			
GBP2, GBP5, GBP6, TMCC1	+	Oligonucleotides (RNA, DNA)	[64,87]
PRDM1	-	PR domain zinc finger protein 1 gene	[87]
ARG1	-	Arginase 1 gene (encoding the arginase enzyme)	[87]
IS6110	+	IS6110 gene	[77]
IS1081	-	IS1081 gene	[88]
rpoB531	+	rpoB531 gene	[72]
katG315	+	katG315gene	[72]
Acids and their derivatives			
MN	+	Menthyl nicotinate	[81]
MAs	+	Mycolic acids	[69,70]
Enzymes			

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MNAzymes	+	Multicomponent nucleic acid enzyme	[68]
ADA	-	Adenosine deaminase (enzyme of purine metabolism)	[89]
KatGs	-	Catalase-peroxidase enzymes (responsible for the activation of the antituberculosis drug isoniazid)	[90]
Cytokines			
IL-1ra		Interleukin-1 receptor antagonist	[91]
IL-2	+	Interleukin-2	[91,92]
IL-10	+	Interleukin-10	[91,93]
IL-13		Interleukin-13	[91]
INF-y	+	Interferon gamma	[65,92,93]
TNF-α	+	Tumor necrosis factor alpha	[92]
INF-y IP-10	+	Interferon-gamma-inducible protein 10	[25]
MIP-1β	-	Macrophage inflammatory protein	[91]
Specific surface proteins			
CFP-10	+	10-kDa culture-filtered protein	[86,94,95]
Mtb Rv1468c (PE_PGRS29)	-	*M.tb* surface protein	[96]
Rv1509	-	*M.tb*-specific protein	[97]
ESAT-6	-	6-kDa early secreted antigenic target	[94,98–100]
MPT-64	-	*M.tb* protein 64	[101]
Ag85B	+	Secreted protein antigen 85 complex B	[78,102]
PPE-68	-	Proline-proline-glutamic acid	[103,104]
Rv2536	-	Potential membrane protein	[105]
Rv2341		Probable conserved lipoprotein LppQ	[106]
Mycobacterial antigens			
14-kDa antigen	-	14-kDa protein antigen	[107]
16-kDa antigen	-	*M.tb*-specific antigens	[108]
19-kDa antigen	-	19-kDa lipoprotein	[107]
30-kDa antigen	-	Immunodominant phosphate-binding protein	[109]
38-kDa antigen	-	Immunodominant lipoprotein antigen	[110]
55-kDa antigen	-	*M.tb*-specific antigens	[111]
LAM	-	A glycolipid and a virulence factor associated with *M.tb*	[112]
A60	-	Tuberculosis antigen	[113]
Mtb81	-	Recombinant protein	[114]
ESAT-6	+	*M.tb*-specific antigens	[86,115]

Host transcript RNA/DNA signatures is a group of biomarkers associated with the host gene expression in response to *M.tb* infection. For some markers listed in Table 3, there are suitable QD-based nanosensors presented in Table 2 (GBP2 [64,87], GBP5 [64,87], GBP6 [64,87], IS6110 gene [77], rpoB531 gene [72], and katG315gene [72]). Regarding PRDM1, it is also associated with lymphoma [116]. To date, there is no QD-based nanosensor for arginase 1 detection. The group of acids and their derivatives consists of two important TB biomarkers: MN [81] and MAs [69,70]. For both markers, sets of QDs and conjugates that can be used for TB diagnosis are shown in Table 2. Three most common enzymes can be used for TB diagnosis: MNAzymes, ADA, and KatG. To date, QDs functionalized with MNAzymes [68] have been proposed as TB diagnostic agents. Regarding KatGs, there are methods for detecting the encoding genes, but there are no biosensors for detecting the enzymes themselves. No nanosensors for ADA detection have been reported to date.

The groups of specific surface protein and mycobacterial antigen biomarkers can be pooled because both include specific proteins and other antigens associated with *M.tb*. At the moment, three main protein antigens from this group have been studied for TB diagnosis using nanosensors: CFP-10, ESAT-6, and Ag85B [71,78,80,86].

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4. Summary and Outlook

TB remains a major global health problem, with millions of new cases and significant mortality every year. Early and accurate diagnosis is crucial for effective treatment and control of this disease. Traditional methods of TB diagnosis, such as PCR tests, immunofluorescence and immunochemical analyses, flow cytometry, cell culture tests, and microscopic analysis can be improved by the use of optical tags based on fluorescent QDs. Some alternative nanomaterials, such as other nanoparticles [114–116], graphene [88], and graphene-like 2D-materials (trans-graphenes) [113,117–122], can also be used for the development of nanosensors solving similar tasks and based on the same physical principles. QDs have already established themselves as promising constituent elements of biosensors providing increased sensitivity and specificity of detection than routinely used assays and allowing the development of multiplexed assays for early, more detailed detection of *M.tb* and diagnosis of TB. Despite the undoubtedly high potential, several challenges need to be addressed for enabling widespread use of QD-based nanosensors for TB diagnosis, such as the search for new suitable conjugates and available highly specific biomarkers, standardization and validation of diagnostic protocols, and advanced cost- and time-reducing solutions. However, the data reviewed here show that the unique properties of QDs make the QD-based nanosensors promising candidates for biosensing applications, including *in vitro M.tb* diagnosis. The use of QDs makes it possible to increase the sensitivity and speed of analysis, which is important for point-of-care diagnosis of TB and wider coverage of diagnostic procedures. The possibility of excitation of QD fluorescence in a wide range of wavelengths and a long fluorescence lifetime allow reducing the requirements for fluorescence detectors and, hence, the cost of their manufacture, as well as designing more compact devices for reading the fluorescent signal. This could ensure their wider use of these tools in diagnostic practice, thus decreasing the morbidity and mortality from TB.

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