Tuberculosis remains one of the most serious infectious diseases worldwide requiring new tools to circumvent current molecular diagnostics limitations. Nanodiagnostics, i.e. nanotechnology based diagnostics, may do just that by decreasing the time needed for the molecular characterisation of the infecting agent, and allowing for miniaturisation and portability for point-of-need adapted to remote regions without suitable lab equipment.

**Introduction**

Tuberculosis (TB) remains one of the most serious infectious diseases in the world demanding new, fast, reliable and effective diagnostic tools (World Health Organisation [WHO], 2011). Several technological strategies have been proposed that rely on decreasing the time needed for unequivocal molecular identification and characterisation of the infecting agent. Nanotechnology has triggered the development of new, fast and cheap approaches for biomolecular recognition that may circumvent some of the current limitations of molecular methods for laboratory diagnostics of TB.

Nanodiagnostics can be defined as the use of nano-sized materials, devices or systems for diagnostics purposes. Here, we shall provide an overview of nanodiagnostics for *Mycobacterium tuberculosis* (Mtb) detection and characterisation, and how it can contribute for the early and accurate detection of TB. Current advances in nanofabrication may enable the construction of cheap and full-automated devices, which may allow for point-of-need diagnostics.

**Tuberculosis**

By the end of the 1970’s, a global agreement reasoned that tuberculosis was at the edge of being eliminated worldwide due to the idealistic “full effectiveness” of the anti-TB therapy, especially in view of the promising results with therapy combining the two most effective anti-TB drugs, isoniazid (INH) and rifampicin (RIF). The lack of surveillance of patient adherence to this long-term anti-TB therapy and the reduced investment on laboratory diagnosis and drug susceptibility tests for TB, created the appropriate environment for the appearance and spread of Mtb strains resistant to INH and RIF (multi-drug resistant TB, MDRTB) (WHO-IUTALD 2004; WHO 2006; Frieden et al, 1995; Barr et al, 2001). Control programmes had to be created that focused on the implementation of aggressive second line short-course directly observed therapy (DOTplus), as well as on implementation of rapid methods for laboratory identification and antibiotic susceptibility of Mtb strains isolated from these patients (Paolo et al, 2004). New cases of pulmonary TB and MDRTB infections dramatically increased in Africa, South America and Asia, where the average incidence of TB ranges from 100 to more than 300 cases per 100,000 inhabitants and where only a few countries provide full laboratory diagnosis and the rate of drug resistant tuberculosis is known (WHO-IUTALD, 2004, WHO 2006).

In Portugal, the incidence of TB remained the highest for Western Europe during the XX\textsuperscript{th} century, although a small gradual decline was evident. In Lisbon, the rates of MDRTB were as high as 28% in 2003 and declined to less than 3% by 2011 (new cases plus re-treatments) (Perdigão et al 2011, DGS 2012). This
A decrease was due to a concerted action involving health authorities on DOTs implementation and applied research programmes for new rapid molecular based direct identification of Mtb in smear-positive respiratory specimens, our team was successful at establishing a TB control protocol involving 12 hospitals from Lisbon Health Region, capable of detecting the bacilli within 24-28 hrs upon sample arrival using the commercially available INNO-LiPA Rif.TB assay, now adapted for direct detection (Viveiros et al, 2005). This approach was a major breakthrough in TB control because, besides the early detection of *M. tuberculosis*, it allowed a presumptive detection of MDTB via identification of mutations in the *rpoB* gene as indicators of rifampin resistance (Viveiros et al, 2010).

More recently, MDRTB has evolved into extensively-drug resistant TB (XDRTB) – *M. tuberculosis* strains resistant to INH, RIF, any of the fluoroquinolones, and to at least one of the three injectable anti-TB drugs (capreomycin, kanamycin and amikacin) (WHO 2006). Despite the gradual decrease in Mtb infected patients notified in the Lisbon Health Region in the last years, the numbers are still well above the EU average and the increasing number of XDRTB isolates is a major threat in terms of Public Health, with a prevalence above 50% of all MDRTB isolated in this region (Perdigão et al 2011; DGS 2012). Considering that this is the current situation in one European country where the health care system provides conditions for complete laboratorial TB diagnosis and the DOT program is enforced and monitored for every diagnostic tools directed to the early detection of MDRTB cases (Viveiros et al, 2005; 2010). By putting together hospital laboratories and state-of-the-art molecular methods that allow TB patient, the forecast for countries where these structures are poorly implemented is gloomy.

In Africa, South America and Asia, TB laboratory diagnosis remain a major barrier for TB control, particularly in HIV-infected persons, where the laboratory diagnosis still relies on the poorly sensitive smear microscopy, and culture or drug susceptibility testing are not available. The real dimension of drug-resistant tuberculosis in these areas is unknown and this lack of information has pushed forward the implementation of new molecular based techniques for direct detection of TB and drug resistance (WHO-Stop-TB Program 2006), such as the Gene Xpert MTB/RIF Real-Time PCR platform from Cepheid, a new rapid molecular diagnostic test for direct detection of both TB and rifampicin resistance recently endorsed by WHO (2011). Nevertheless, this technology involves elaborate technology and logistics for reagents and maintenance, with prohibitive running costs for low-income countries. Nowadays, new, cheap, reliable and easy to implement nanotechnology based approaches are being developed for the direct detection of *M. tuberculosis* and mutations related with drug resistance, not only RIF but also other 1st and 2nd line drugs, as we believe it can be a major contribution for WHO-STOP TB, in particular of XDRTB, in low-income countries (Veigas et al. 2010; 2012).

**Table 1. Nanotechnology systems for TB diagnostics (proof-of-concept)**

<table>
<thead>
<tr>
<th>Technology</th>
<th>Description</th>
<th>Application(s)</th>
<th>Reference(s)</th>
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<tr>
<td>Noble metal NPs</td>
<td>SPR change leading to colorimetric changes.</td>
<td>&gt; <em>M. tuberculosis</em> complex, <em>M. avium</em> complex, <em>M. avium subsp. paratuberculosis</em>, <em>M. bovis</em> and <em>M. tuberculosis</em>. &gt; <em>rpoB</em> mutations associated with drug resistance.</td>
<td>Baptista et al., 2006; Costa et al., 2010; Liandris et al., 2009; Silva et al., 2008, 2010; Soo et al., 2009; Veigas et al., 2010</td>
</tr>
<tr>
<td>Magnetic NPs</td>
<td>Measurement of spin-spin relaxation time. Minimal sample preparation.</td>
<td>&gt; <em>M. bovis</em> BCG (<em>bacillus Calmette-Guérin</em>)</td>
<td>Kaittanis et al., 2007; Lee et al., 2009</td>
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(continued)
Nanotechnology for TB diagnostics

The most promising nanotechnology based approaches for detection of proteins and nucleic acids include nanoparticle (NPs), nanotubes, nanopores and nanocantilever technologies (Azzazy et al., 2006; Veigas et al., 2012). Their potential arises from recognition events occurring at one-to-one interactions between analytes and signal-generating nanostructures, allowing for increased sensitivity and specificity at lower costs. Amongst the range of nanoscale systems proposed for biomolecular assays, nanoparticle based systems, such as gold, silver, silica and quantum dots (QDs), have shown high potential for TB diagnostics – see Table 1.

The first application of AuNPs functionalised with thiol-modified oligonucleotides capable of targeting the rpoB for Mtb diagnostics was introduced by Baptista et al. (Baptista et al., 2006) using the differential stabilisation of gold nanoprobes (Au-nanoprobes) in the presence of the specific target: presence of a complementary target prevents nanoprobe aggregation and the solution remains red, while non-complementary/mismatched targets or their absence do not prevent aggregation, resulting in colour change from red to blue (Baptista et al., 2006) – see Figure 1.

This strategy was then applied to the rapid detection of M. tuberculosis complex (MTBC) strains and simultaneous characterisation of mutations associated with rifampicin resistance (Veigas et al., 2010) – a two-step approach based on PCR amplification of a fragment of rpoB gene and subsequent hybridisation with specific MTBC nanoprobe. This approach allowed for detection of MTBC in 83.3% of all samples, when compared to the

![Fig. 1. Non-cross-linking detection of MTBC members. A DNA sample is extracted from a patient and amplified by PCR. The resulting PCR product is characterised with the specific gold nanoprobes following the non-cross-linking approach involving the visual comparison between solutions before and after salt induced nanoprobe aggregation: ‘Blank’, nanoprobe alone; ‘MycONEG’, nanoprobe in the presence of a non-complementary DNA sequence; and ‘MycPOS’, nanoprobe in the presence of a complementary DNA sequence.](image-url)

### Table 1 (continued)

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<tr>
<th>Technology</th>
<th>Description</th>
<th>Application(s)</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td>Quantum Dots</td>
<td>Fluorescence detection with size-dependent optical properties. Optimal for multiplex assays.</td>
<td>&gt; Conjugation of streptavidin-coated QDs. Integration with magnetic NPs for M. tuberculosis and M. avium subsp. paratuberculosis.</td>
<td>Rotem et al., 2006; Gazouli et al., 2010</td>
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<tr>
<td>Silica NPs</td>
<td>Fluorescence detection using NPs with large quantities of fluorophore inside matrix (e.g. polymer or silica). Multiplex assay.</td>
<td>&gt; Improved two-colour flow-cytometry assay.</td>
<td>Qin et al., 2008</td>
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<tr>
<td>Electrochemical devices</td>
<td>Electrochemical nanofabricated sensors. Portable microfluidic nuclear magnetic resonance biosensor.</td>
<td>&gt; M. tuberculosis complex members.</td>
<td>Das et al., 2010; Lee et al., 2010; Wang et al., 1997; Prabhakar et al., 2008.</td>
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INNO-LiPA Rif.TB assay. By means of a set of probes for each mutation associated to RIF resistance to be screened (mutations in codons 516, 526 and 531 of the rpoB gene), it was possible to correctly score the presence of at least one of the mutations in 81% of all samples also screened via the INNO-LiPA Rif.TB assay.

Conclusions
The advantages provided by molecular based diagnostic tools directed to the early detection of the MDRTB are significant for the management and selection of therapy of these patients. Consequently, reduction or elimination of the patient non-compliance directly reduces rates of new MDRTB infections and serial transmission is rapidly prevented. The molecular based diagnostic tools currently available rely on costly and demanding technology, difficult to implement and maintain in countries with limited resources and deficient health care systems. Nanodiagnostics have the ability to provide results within hours, with increased sensitivities at a fraction of the cost of conventional microbiological and molecular biology methodologies. Nevertheless, these nanodiagnostics platforms still have to make their way to the clinics. Future trends in TB nanodiagnostics will continue through miniaturisation and portability for point-of-need with a sample-in answer-out approach for use in more remote regions without the proper laboratory equipment.

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References


