Introduction

It is estimated that approximately one-third of the human population are carriers of *Mycobacterium tuberculosis*, i.e. (Mtbt), are latently infected with tuberculosis (TB) but do not show any of its symptoms [1]. In 2012, 8.6 million people were diagnosed with active clinical TB, and 1.3 million died from the disease. However, only approximately 30% of people suffering from active TB have been diagnosed [2]. This is largely due to a lack of diagnostic capacity in low-resource settings. Not only is there an unmet demand for cost-effective diagnostic kits for use in low-resource settings with a high TB burden, but there is also a need to address the caveats of existing diagnostics for childhood TB, drug-resistant TB, smear-negative TB, extrapulmonary TB, HIV-TB, and latent tuberculosis infection (LTBI). The management of infectious diseases, such as malaria and HIV, has been significantly improved by the development of low-cost point-of-care (POC) tests [3]. This has particularly benefitted resource-limited settings where the healthcare infrastructure is less developed. POC tests are simple and fast, and enable extensive use and timely therapeutic responses and/or healthcare management [4].

Current diagnosis of TB and its caveats

When TB symptoms such as coughing and blood in the sputum are observed in humans at risk of TB infection, the clinical diagnosis includes lung imaging (e.g., chest X-rays), sputum smear microscopy, isolation of Mtbt from the sputum, growth of Mtbt in a liquid culture, molecular identification of Mtbt using the nucleic acid amplification test (NAAT), and histopathological assessment of specimens. In cases where treatment of TB with antibiotics has failed, recently developed drug susceptibility tests (DSTs) are employed [5].

The tuberculin skin test (TST) and the interferon-gamma release assay (IGRA) are also often implemented for the initial assessment of suspected TB cases. However, these tests are
currently not recommended for the diagnosis of active TB because they lack sensitivity and specificity while solely relying on an immune response against mycobacterial antigens not necessarily linked to clinical symptoms of active TB, i.e., they do not currently differentiate between exposure to environmental mycobacteria, bacillus Calmette–Guérin (BCG) vaccination, and latent or active TB [6, 7]. Owing to this low specificity and lack of sensitivity—as demonstrated by randomized control trials—TST and IGRA are not recommended for the diagnosis of active TB by the WHO [8, 9]. There is an unmet demand for an advanced TST and an IGRA showing enhanced performance with respect to sensitivity and specificity. A recently developed and very versatile polyester bead technology has been demonstrated in an animal model that exhibits properties suitable for use as an advanced TST [10-12]. This new protein display technology and its potential impact on TB diagnostics will be discussed below in some more detail.

Another diagnostic challenge is childhood TB because the pathogen detection-based diagnostic tests (e.g., sputum smear microscopy, culture, NAAT) are significantly impaired owing to a reduced pathogen load and the difficulty of obtaining sputum samples. A gold standard diagnostic test is required.

Latent TB: diagnosis and caveats

In developed non-endemic countries, post-primary re-activation of TB in humans with LTBI significantly contributes to new TB cases [13]. Therefore, the management of TB in these countries needs to include diagnosis of LTBI. Once LTBI has been diagnosed, re-activation of TB can be reduced by up to 90% if treated with isoniazid for 6 months, or by other regimens [14]. In high-income countries, LTBI risk groups have been identified and screened using physical examination, TST, IGRA, and chest X-rays to exclude pulmonary TB (PTB) [15].

The diagnosis of LTBI lacks a gold standard test because both TST and IGRA only detect lasting immune responses to Mtbc antigens, and are unable to determine the presence or absence of dormant Mtbc. Therefore, TST/IGRA-positive tests do not predict the risk that a patient will progress to active TB [16-18]. These tests perform particularly badly in children (less than 5 years old) and immune-compromised patients, so a significantly improved test is needed [13, 18].

The tuberculin skin test for TB diagnosis: new developments towards advanced TB diagnostics

As indicated above, the current TST has limitations with regard to specificity and sensitivity. The antigens—the purified protein derivatives (PPDs)—used for the TST are derived from the
culture filtrate of Mtb grown in vitro. The PPD represents a complex mixture of antigens, some of which also show cross-reactivity with other mycobacteria including the currently used vaccine strain BCG, i.e., they are conserved and produced by other mycobacterial species. The complexity of antigens results in a lack in specificity, and the development of a TB-specific skin test would necessitate the exclusion of antigens other than those specific to Mtb.

Recently, a novel recombinant polyester bead technology has been developed that allows the cost-effective and scalable microbial production of selected antigens at the surface of bacterial polyester inclusions [10, 19-22]. Polyester beads were designed as a skin test reagent for the detection of Mycobacterium bovis-infected cattle. M. bovis, which is a pathogenic mycobacterium, causes bovine tuberculosis and infects various hosts such as domestic livestock and wildlife; it also causes TB in humans. Bovine TB potentially affects approximately 50 million cattle worldwide, and represents a particular public health risk in regions where the pasteurization of milk is not routinely carried out. Because the majority of the human population lives in such regions, M. bovis contributes to up to 10% of TB cases in humans [23]. Therefore, monitoring and control of bovine TB will significantly assist the management of human TB. Bovine TB severely affects the agricultural industry because infected cattle are lost to dairy farming and human consumption.

Similar to the TST for human TB, the current TST for diagnosing TB in cattle (comprising a PPD prepared from M. bovis (PPD-B)) lacks specificity because it contains antigens that are also present in environmental non-pathogenic mycobacteria.

Three immunodominant TB antigens, ESAT6 (Rv3875), CFP10 (Rv3874), and Rv3615c, are present in members of the pathogenic Mtb complex, but are absent from the BCG strain and the majority of environmental mycobacteria. These antigens showed high performance in the IGRA blood assay for the diagnosis of TB in cattle [24-26]. Chen et al. (2014) recombined these antigens in Escherichia coli as a single fusion protein, and additionally fused a polyhydroxyalkanoate (PHA) synthase (from Ralstonia eutropha), which catalyzes the synthesis of natural biopolymers (that serve as carbon and energy storage materials in the original bacterial host) deposited as spherical inclusions inside the bacterial cell [22] (Figure 1). Interestingly, the PHA synthase fused to these antigens remains tightly attached to the surface of the biopolyester beads displaying the antigens. After the accumulation of polyester beads inside the producing cells, the cells are disrupted and the beads are purified by a series of washing steps to remove cell debris (Figure 1). These polyester beads show adjuvant properties and mediate specific and protective immune responses (Th1 and Th2) when displaying selected antigens that are relevant to TB or hepatitis C [19-21]. The simultaneous display of ESAT6 (Rv3875), CFP10 (Rv3874), and Rv3615c on the polyester beads was confirmed using
antigen-specific monoclonal antibodies [10]. The beads were used for skin testing in cattle. All the experimentally infected cattle were detected, and the cattle exposed to environmental mycobacteria did not give false positive reactions. Hence, TB antigen-displaying polyester beads offer the potential to serve as diagnostic reagents for distinguishing TB-infected from non-infected animals.

Figure 1. Schematic representation of the bioengineering of bacterial cells for the production of polyester beads displaying tuberculosis (TB) antigens suitable for diagnostic applications (modified according to Parlane et al., 2016) [27].

Moreover, there is a need for a specific diagnostic reagent that can differentiate between TB-infected and environmental strain-sensitized animals and BCG-vaccinated animals. To differentiate between environmentally sensitized animals, a PPD (PPD-A) derived from environmental mycobacteria is directly compared with PPD-B. A strong response to PPD-B combined with a weak or absent response to PPD-A indicates infection with M. bovis. When comparing the three-antigen-displaying polyester beads with PPD-A and PPD-B responses in cattle skin tests, only the bead reagent accurately detected non-infected cattle naturally exposed to environmental mycobacteria, whereas PPD-A and PPD-B showed false-positive skin test responses [10].
A mixture of purified CFP10, ESAT6, and Rv3615c was used to distinguish TB-infected from non-infected and BCG-vaccinated cattle [28]. Immunodominant antigen Rv3615c can also detect ESAT6- and/or CFP10-unresponsive TB-infected cattle [29]. Rv3615c added to ESAT6 and CFP10 in the reagent significantly improves the sensitivity of skin tests [28, 30]. Hence, the combination of these three antigens has potential as a sensitive and specific TB diagnostic reagent. Recently, it has been demonstrated that the display of antigens B5A and ESAT6 on polyester beads stimulates stronger cellular immune responses than the soluble antigens [19]. Hence, it is anticipated that the display of CFP10-Rv3615c-ESAT6 on the surface of polyester beads will result in a more sensitive diagnostic reagent compared with the respective free antigens. Moreover, the antigen-displaying beads can be produced cost-effectively, suggesting an attractive new approach to the development of new diagnostic reagents.

The three-TB-antigen-displaying polyester bead reagent also has potential for use in the diagnosis of human TB caused by Mtb because CFP10, ESAT6, and Rv3615c orthologous proteins can be found in M. bovis, and these antigens are immunodominant in both active and latent human TB infection [29, 31].

This three-antigen polyester bead skin test reagent, which is currently being manufactured and commercialized by New Zealand-based PolyBatics Ltd, will be tested in field trials over the next few months.

Overall, polyester bead technology offers the opportunity to quickly develop a custom-made diagnostic reagent by bioengineering E. coli to display selected antigens on the surface of polyester beads (Figure 1). In addition to a short development timeline and enormous design potential, the beads can be produced cost-effectively for POC applications.

References


