Tuberculosis 4

Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice

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Human infection with *Mycobacterium tuberculosis* can progress to active disease, be contained as latent infection, or be eradicated by the host response. Tuberculosis diagnostics classify a patient into one of these categories. These are not fixed distinct states, but rather are continua along which patients can move, and are affected by HIV infection, immunosuppressive therapies, antituberculosis treatments, and other poorly understood factors. Tuberculosis biomarkers—host or pathogen-specific—provide prognostic information, either for individual patients or study cohorts, about these outcomes. Tuberculosis case detection remains difficult, partly because of inaccurate diagnostic methods. Investments have yielded some progress in development of new diagnostics, although the existing pipeline is limited for tests for sputum-smear-negative cases, childhood tuberculosis, and accurate prediction of reactivation of latent tuberculosis. Despite new, sensitive, automated molecular platforms for detection of tuberculosis and drug resistance, a simple, inexpensive point-of-care test is still not available. The effect of any new tests will depend on the method and extent of their introduction, the strength of the laboratories, and the degree to which access to appropriate therapy follows access to diagnosis. Translation of scientific progress in biomarkers and diagnostics into clinical and public health programmes is possible—with political commitment, increased funding, and engagement of all stakeholders.

Introduction

Tuberculosis, although a curable disease, continues to be one of the most important infectious causes of death worldwide. Despite substantial investments and progress made in expansion of the directly observed therapy, short course (DOTS) strategy and improved treatment completion rates, inadequate case detection remains a major obstacle to global control of tuberculosis. Efforts during the past decade to consistently diagnose and treat the most infectious cases have slowed the rate of disease incidence, but have not yielded substantial progress towards elimination. This experience has refocused attention on research and development for improved diagnostics, therapeutics, and vaccines—areas in which progress has historically been slow. Human *Mycobacterium tuberculosis* infection is almost always acquired by inhalation of infected aerosol droplets, which are generated by people with active pulmonary disease

Search strategy and selection criteria

For the tuberculosis biomarker section, we searched publications in PubMed and Google Scholar (1995–2009), the Cochrane library (2001–09), and Embase (2001–06) with the terms "tuberculosis", "*Mycobacterium tuberculosis*", "biomarkers", "diagnostics", and "clinical trials". For the section on tuberculosis diagnostics, the search strategy was a 10-year review of diagnostic studies in PubMed and Embase. This search was supplemented by searching the website Evidence-based TB Diagnosis by the Stop TB Partnership's New Diagnostics Working Group. We searched with the terms "tuberculosis", "*Mycobacterium tuberculosis*", "diagnosis", "diagnostics tests", and "accuracy". For both sections, we mainly selected publications in the past 10 years, but did not exclude commonly referenced and highly regarded older publications. We also reviewed studies cited by articles identified by this search strategy, and selected those that we regarded as relevant. Review articles are preferentially cited to provide readers with more details and references than this overview can accommodate.

Key messages

- Diagnostics classify patients at one point in time, whereas biomarkers can provide prognostic information about future health status and can advance knowledge of disease pathogenesis.
- Qualified tuberculosis biomarkers are most urgently needed as predictors of reactivation and cure, and indicators of vaccine-induced protection. The biomarker most closely approaching qualification is 2-month culture conversion as a predictor of relapse risk.
- The tuberculosis diagnostics pipeline has rapidly grown, with development of several promising technologies.
- The existing tuberculosis diagnostics pipeline still does not have a simple, rapid, inexpensive point-of-care test. Accurate, rapid tests are also needed for smear-negative and childhood tuberculosis, as are tests for latent tuberculosis with increased predictive value for reactivation.
- Several diagnostics and diagnostic strategies have been endorsed by WHO and are being introduced into clinical use and national tuberculosis control programmes.
- Governments in all countries, especially industrialised countries, have to increase funding for tuberculosis research and control.

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coughing (figure 1). However, the infection infrequently progresses directly to active disease, and is more often contained—at least initially—by the host immune response. The resulting latent infection can be eradicated, or can persist and reactivate many years later. Tuberculosis chemotherapy can also contain the disease, but leave a latent infection that is capable of causing relapse. This dynamic process can be started anew at any time by exogenous reinfection.

Tuberculosis diagnostics form the basis of classification of patients in this system. As diagnostic accuracy has increased, it has become apparent that these are not entirely distinct states, but instead represent gradations along which patients might move. Even within individual patients, foci of latent infection can coexist alongside sites of active *M tuberculosis* replication. Medical treatment, vaccine and immune status, and concomitant illness all affect this balance between host and pathogen, favouring one or another clinical outcome and thus representing the interface between prognostics and diagnostics. In this overview, we describe the development of tuberculosis biomarkers and diagnostics, knowledge gaps and scientific obstacles, and limitations of the existing pipeline of biomarkers and diagnostics, and summarise the major challenges in translation of scientific progress into action.

Biomarkers for tuberculosis

Biomarkers provide prognostic information about future health status, either for individual patients or cohorts in clinical trials. Biomarkers can thus indicate normal or pathogenic processes, or pharmacological responses to therapeutic intervention.[1] In clinical trials, biomarkers can form the basis of surrogate endpoints, which can substitute for a clinical endpoint based on epidemiological, therapeutic, pathophysiological, or other scientific evidence, thereby assisting candidate selection during drug discovery, accelerating dose selection in early clinical research, and shortening the time to licensing of new drugs and vaccines. In routine clinical care, biomarkers can allow stratification of individual patients according to outcome risks, thus easing targeted interventions that might not otherwise produce overall benefit. Biomarkers can also help to advance basic knowledge of disease pathogenesis.

The need for biomarkers in tuberculosis is most crucial in three areas: in patients with active disease, to predict durable (non-relapsing) treatment success; in patients with latent *M tuberculosis* infection, to indicate reactivation risk and predict treatment success; and in people other than those with active disease, to indicate protection from tuberculosis by new vaccines (panel 1).

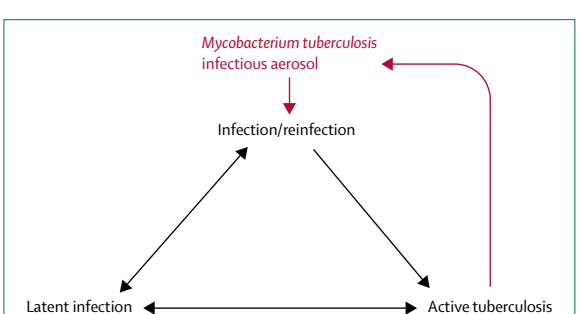


Figure 1: **Clinical stages or states of *Mycobacterium tuberculosis* infection**
Bidirectional movement between states can occur as a result of exogenous or endogenous effects, including inhalation of infected aerosol droplets, vaccination, antituberculosis chemotherapy, or concomitant illness such as HIV.

Biomarkers predicting durable cure

The marker with which there is greatest experience as a predictor of non-relapsing cure is sputum culture status after 2 months of therapy. Wallis and colleagues[4] used meta-regression to examine these parameters in 30 paired study groups of 5500 patients in four regions worldwide. The analysis found that an incremental effect of a new treatment on relapse is highly likely to be captured as a corresponding change in culture conversion (figure 2; $p<0·0001$). This finding supports a role for 2-month sputum culture conversion in the accelerated approval of new tuberculosis drugs, potentially shortening the time needed for licensing of new drugs for multidrug-resistant (MDR) tuberculosis by many years. No other tuberculosis biomarker approaches this level of qualification. However, despite this compelling performance as a surrogate endpoint in clinical trials, sputum culture conversion is a poor prognostic marker for individual patients. One study noted, for example, that although 2-month culture positivity was an independent predictor of relapse for individuals (hazard ratio 2·8, 95% CI 1·7–4·7), its positive predictive value (18%) and sensitivity (50%) were low.[3] This apparent discordance between trial surrogacy and patient prognostication could arise from the practice of collecting sputum cultures only once per month, thus obscuring within-patient variability. Some relapses could also arise from bacterial subpopulations that are not readily detected in sputum by culture on solid medium.

Efforts to improve on these characteristics convert the binary endpoint of culture conversion to a continuous variable by measuring the rate of decline of viable *M tuberculosis* in sputum at several timepoints during the first 1–2 months of therapy, either as colony counts on agar or time to positivity in liquid culture.[5–8] One small trial[9] using serial counts identified moxifloxacin and gatifloxacin as superior to ofloxacin and ethambutol despite similar rates of 2-month culture conversion. However, three of four adequately powered trials of moxifloxacin did not show an effect on 2-month status, including the regimen that was indicated in mice as most likely to accelerate sterilisation.[102–105] A very large clinical trial (Rapid Evaluation of Moxifloxacin in the Treatment of Sputum Smear Positive Tuberculosis [REMOX-TB]) in progress with relapse as its primary endpoint will probably help to resolve these contradictory findings.

Small studies have examined levels of *M tuberculosis* antigen 85 and 85B RNA in sputum during treatment. In one study,[8] the magnitude and duration of increases in this protein during the first week of treatment predicted relapse or failure in four of 42 patients. A second study[12] noted that 85B RNA was cleared more rapidly from sputum during therapy than viable colony counts, but did not predict subsequent relapse in one patient. Other factors associated with mycobacterial dormancy (including proteins, RNA species, or lipids) could have greater predictive value.[13,14]

An important shortcoming of all sputum biomarkers is their limited role in paucibacillary and paediatric tuberculosis, and their lack of usefulness in latent *M tuberculosis* infection. One study has reported the presence of small fragments of *M tuberculosis* IS6110 DNA in urine of 34 of 43 patients with tuberculosis but not in healthy controls.[15] None of the patients had overt renal tuberculosis. The DNA fragments, termed transrenal DNA (tr-DNA), are thought to arise because of apoptosis of host cells. The investigators have reported that none of the 20 patients who were positive at diagnosis remained positive after 2 months of therapy.[16] Other studies of urinary mycobacterial DNA using different methods have generally shown lower sensitivity.[106–109] None has examined paediatric samples. A urinary test that could serve as both diagnostic and prognostic would be an important advance in paediatric tuberculosis. Other studies have examined lipoarabinomannan and other mycobacterial markers in urine, also with varying degrees of sensitivity.[17–26] Detection of volatile organic compounds in the breath of patients with pulmonary tuberculosis has been reported.[28] No studies have reported the changes in these markers during treatment or established a relation to clinical outcome or to another surrogate endpoint. Further study of non-sputum microbial markers is an area of priority for tuberculosis research.

Measurement of bactericidal activity in whole blood culture after oral dosing of new tuberculosis drugs can help with selection of dose and dosing interval, and can identify compounds which, owing to their mechanism of action and pharmacokinetic profiles, can show additive or synergistic effects when combined. Such effects might not be predicted well from animal models because of differences in absorption and metabolism.[110,111] Whole blood bactericidal activity during tuberculosis treatment correlates with the rate of decline in sputum colony counts, is superior in patients whose sputum cultures convert by the second month of treatment, and is superior during the intensive (four-drug) phase of treatment.[31] Two studies[29,30] reported that regimens for drug-sensitive tuberculosis were better than were those for MDR tuberculosis. These findings suggest that the whole blood model could also help in the identification of efficacious multidrug regimens.

Macrophages are activated by *M tuberculosis* via interactions with toll-like receptors. Several blood markers of this activation might have roles as tuberculosis biomarkers. Neopterin, for example, is increased at diagnosis of disease in proportion to extent of disease; it decreases during and after treatment.[41–45] In a small sample of HIV-uninfected patients matched for extent of disease at baseline, increased neopterin concentrations after completion of treatment were associated with relapse.[44] Several other markers are also increased at baseline in proportion to disease extent and to decline with treatment, including soluble intercellular adhesion molecule (sICAM) 1,[60–63] C-reactive protein,[55,57–59] soluble urokinase plasminogen activator receptor,[65] and procalcitonin.[46–53] In one study, a

***Panel 1:* Candidate *Mycobacterium tuberculosis* and host tuberculosis biomarkers**

Predication of durable (non-relapsing) tuberculosis cure

Microbial markers in sputum
- 2-month culture conversion[2–4]
- Serial colony counts or time to culture positivity[5–9]
- Other microbial markers[8,10–14]

Other microbial markers
- Urine *M tuberculosis* DNA,[15,16] lipoarabinomannan[17–26]
- Volatile organic compounds[27,28]

Mycobactericidal activity
- Whole blood culture[29–31]

Tuberculosis-specific T-cell function
- Interferon γ,[32–37] interleukin 4δ2 splice variant[38–40]

Macrophage activation markers
- Neopterin,[41–45] procalcitonin,[46–53] C-reactive protein,[54–59] soluble intercellular adhesion molecule 1,[60–64] soluble urokinase plasminogen activator receptor,[64,65] monocyte CDllc[66]

Multiple host markers
- Proteomics[67,68]
- Transcriptomics[69,70]

Indication of reactivation risk and prediction of eradication of latent infection

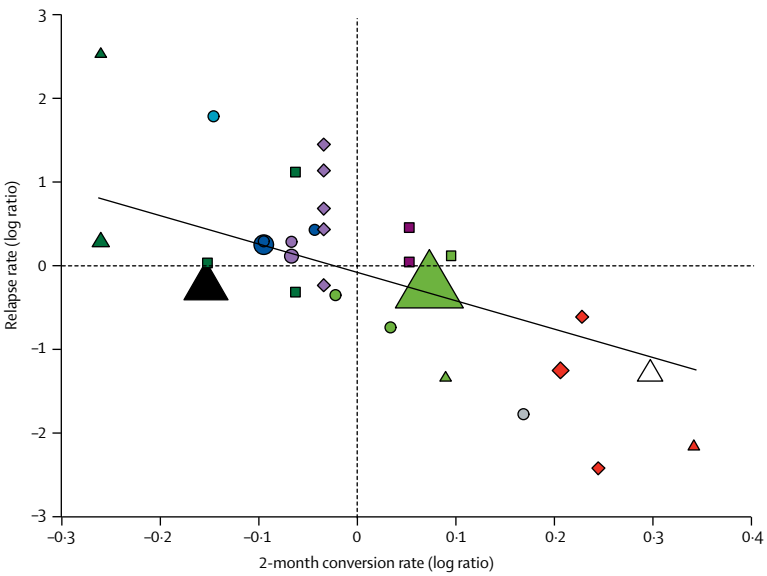
Tuberculosis-specific T-cell function
- Interferon γ[71–77]
- Interferon-induced protein 10[78–80]
- Interleukin 4δ2 splice variant[38–40,81,82]
- Skin test[83,84]

Macrophage activation
- Neopterin[85]
- Procalcitonin[51]

Prediction of vaccine efficacy

Tuberculosis-specific T-cell function
- Interferon γ[86]
- Polyfunctional T cells[87–90]

Mycobactericidal activity
- Whole blood culture[91–99]
- Mononuclear cells[86,98,100,101]



Africa	Hong Kong	Singapore	India	Treatment modification
▲				Thiacetazone added
△				Pyrazinamide added
▲			◆	Rifampicin added
▲	●	■		Streptomycin added
▲		■		Intensive phase shortened
	●			Three times weekly
	●		◆	Twice weekly
	●			Pyrazinamide removed
	●			Ethambutol changed to pyrazinamide
		■		Streptomycin removed, intensive phase lengthened

Figure 2: **Relation between effects of new tuberculosis treatments on rates of relapse and 2-month sputum culture conversion in randomised controlled trials**

Axes indicate natural log rate ratios (experimental/control), with dotted lines indicating equality (no effect). Symbols indicate pairs of study groups, differing only in the intensive phase (n=16) or throughout treatment (n=14). Symbol sizes vary according to precision. The solid line indicates the results of meta-regression analysis (p<0·0001). Reproduced from reference 4, with permission from author and publisher.

mathematical model including change in sICAM 1 during the first week of therapy predicted 2-month sputum culture conversion.[64] As a group, these assays are simple, inexpensive, widely available, and can be done on frozen plasma samples; as a result, they can be readily incorporated into clinical trials or treatment protocols. They seem to have greatest prognostic value when measured at or near the completion of therapy. Further research is needed to establish the sensitivity of these tests to predict tuberculosis reactivation or relapse, and the extent to which their lack of specificity for *M tuberculosis* infection confounds their interpretation.

Multiple biomarkers, when combined, can do substantially better than can any one marker. For example, a panel consisting of leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor, and CA-125 yielded 95·3% sensitivity and 99·4% specificity for diagnosis of ovarian cancer, for which measurement of CA-125 alone detects only 30–40% of early cases.[112] Increased specificity and high predictive value may similarly be achieved for otherwise non-specific tuberculosis biomarkers by measuring multiple parameters by proteomics, transcriptomics, and metabolomics.[10,68] Tuberculosis can be differentiated from other infectious and inflammatory diseases on the basis of proteomic fingerprinting study of serum by surface-enhanced laser desorption/ionisation time-of-flight (SELDI-ToF) mass spectrometry.[67] Serum amyloid A and transthyretin were among the candidate biomarkers identified. This analytical method can detect a very large number of peptides, although it is fairly insensitive. A related approach reduces the potential number of candidate molecules to a small set of small molecules termed the metabolome, representing metabolic intermediates, hormones, other signalling molecules, and secondary metabolites. Its main disadvantage is that several analytical methods are necessary to complete their characterisation.

Two reports suggest the feasibility of distinguishing various stages of *M tuberculosis* infection by gene expression microarray. One study[70] recorded many candidate genes that were differentially expressed by mononuclear cells of patients with tuberculosis, people with latent infection, and uninfected people. However, a small subset of these genes—lactoferrin, CD64, and the Ras-associated GTPase 33A—was sufficient for classification of the three groups. A second report identified signature profiles of nine genes in blood that could distinguish four groups: patients with active tuberculosis, those with latent infection, cured patients, and cured patients with several previous episodes of tuberculosis.[69] Similar studies undertaken during or at completion of therapy could identify profiles associated with durable cure or relapse. However, the genomics and proteomics platforms might be susceptible to biases indicating regional differences in host and microbial genetics. These findings have yet to be verified across several clinical populations.

T-cell-based assays of interferon-γ release for diagnosis of latent tuberculosis infection tend to show high levels in people with active disease at diagnosis that decrease after completion of treatment, but this pattern does not occur consistently.[32] A small study using a non-commercial assay to monitor responses at earlier timepoints noted that of 18 active cases of tuberculosis with positive T-cell responses at baseline, only five who did not show a microbiological or clinical response remained positive after 3 months of treatment.[33] Subsequent studies using commercial assays have not yielded such definitive results. Although most studies have concurred in finding sustained positive T-cell responses in tuberculosis treatment failures, most have found reversion of T-cell responses in responders to be too incomplete and delayed to be useful as a biomarker.[34–37,113] The diagnosis of active tuberculosis can also be established on the basis of T-cell frequencies at the site of disease rather than in blood.[114–116] However, the requirement for an

invasive procedure restricts the feasibility of this approach for diagnosis and treatment monitoring. Lastly, antibody concentrations to some mycobacterial antigens are raised at diagnosis and might be modulated by treatment; however, performance characteristics seem inadequate for a prognostic role.[117,118] Immunological memory seems to hamper the ability to quickly detect treatment effects, as is the case with T-cell assays.

Biomarkers indicating reactivation risk

Several natural history studies of household contacts of active cases of tuberculosis suggest that in HIV-uninfected people, particularly high or increasing concentrations of tuberculosis-specific interferon-γ production might predict overt tuberculosis, although the numbers of tuberculosis cases in these studies are small.[75–77] Positive responses to interferon-γ release assays (IGRAs) otherwise seem to confer only a small risk of reactivation (10–20 per 1000 person-years), which is similar to that of a positive tuberculin skin test. Some studies have suggested that relative mRNA concentrations of interferon γ, interleukin 4, and interleukin 4δ2 (a splice variant of interleukin 4) might be better predictors than interferon γ alone, since ratios of interferon γ or interleukins 4 and 4δ2 fall as healthy contacts develop tuberculosis, and increase as patients with tuberculosis are cured.[38–40,81] The ratio of interleukin 4 to 4δ2 is also increased in longstanding latent tuberculosis infection, presumably suggesting low risk of reactivation.[82] One study[85] reported finding intermediate concentrations of neopterin in health-care workers who were heavily exposed to tuberculosis, potentially indicating risk of reactivation of latent *M tuberculosis* infection. No studies have yet examined macrophage and T-cell markers together in this context.

Findings from studies in experimentally infected guineapigs suggest that prognostication of tuberculosis with the tuberculosis-specific antigens ESAT 6 and CFP 10 as skin tests might also be possible.[83] A similar though less pronounced occurrence had been described in patients with responses to a tuberculin skin test.[84] Such a skin test might show better specificity for latent *M tuberculosis* infection and increased positive predictive value for tuberculosis than might the tuberculin skin test. Confirmation of these findings in human beings and validating their prognostic significance are priority areas of research.

How the loss of CD4 T cells due to HIV infection will affect prognostication of tuberculosis with use of T-cell-based assays is unclear. One study of acute HIV infection noted that tuberculosis-specific T-cell interferon-γ responses were lost rapidly in four of five patients, all of whom remained well.[119] In the fifth, tuberculosis-specific responses increased progressively after HIV infection was acquired, culminating in the diagnosis of active tuberculosis. Increasing counts might correlate with antigen burden and presage tuberculosis reactivation in HIV-positive and HIV-negative people, but studies in HIV-infected patients with specific ranges of CD4 T cells will be needed to confirm this observation.

Several studies of people recently exposed to tuberculosis showed that T-cell frequencies decrease after completion of isoniazid preventive therapy, although they infrequently reverted to negative.[120–123] However, in other studies,[124–126] responses were unaffected. Factors affecting the likelihood of IGRA reversion due to isoniazid preventive therapy could include the duration of infection, the type of assay, the magnitude of the response, or the risk of reinfection.[127] No studies have specifically examined the prognostic significance of reversion. IGRAs are unlikely to be adequate indicators of successful isoniazid preventive therapy. Multiplex assays assessing both T-cell and macrophage factors could prove useful.

Biomarkers predicting vaccine efficacy

There are no qualified biomarkers to indicate protection by new vaccines against tuberculosis. Although both natural infection and vaccination with *Mycobacterium bovis* BCG result in the acquisition of delayed-type hypersensitivity and expansion of antigen-specific interferon-γ-producing T-cell populations, the link between these responses and protection from disease is weak. In the case of BCG, for example, interferon-γ-producing T-cell frequencies poorly predict the protective efficacy of various BCG strains in animals.[128] Protection might instead correlate better with the presence of polyfunctional antigen-specific T cells that secrete several cytokines, as has been described in leishmaniasis.[129] However, data for the use of this biomarker for vaccine-induced protection in tuberculosis are scarce. The potential effect of this insufficient knowledge is shown in two studies of the effect of route of BCG administration on its efficacy. The first study[89] noted superior immunogenicity in infants (interferon-γ, tumour necrosis factor, and interleukin-2 responses in both CD4 and CD8 T cells) when the vaccine was administered percutaneously rather than intradermally. However, a subsequent study[130] in this population showed the two methods of administration did not differ in protection against tuberculosis. These findings suggest that assessment of T-cell responses alone might be insufficient to predict protection from tuberculosis by vaccination.

For all other licensed vaccines, bactericidal or viral neutralisation assays have supplemented standard measurements of immunogenicity during development. Bactericidal assays have been described for *M tuberculosis* with mononuclear cell or whole blood culture.[86,91,92,101] Immune control of growth in these assays is inferior in people with negative tuberculin skin test and in young children; improved by BCG vaccination or vitamin D; impaired by chemokine receptor blockade, T-cell depletion, or HIV infection; restored by antiretroviral treatment; and might be strain specific.[86,91–99,131] Their predictive role for new tuberculosis vaccines has yet to be studied.

Effect of biomarkers on development timelines

The potential effect of biomarkers on the time and costs of development of new tuberculosis drugs and vaccines can be substantial. In the USA, Federal regulations (subpart H of 21CFR314) allow accelerated approval of new drugs for serious or life-threatening illnesses on the basis of a surrogate endpoint that is "reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict clinical benefit".[132] In 2009, an Advisory Committee convened by the US Food and Drug Administration (FDA) recommended nearly unanimously in favour of accelerated approval of new drugs for MDR tuberculosis on the basis of sputum culture conversion. Such approval will shorten the time needed for licensing of new, more effective treatments to patients with MDR tuberculosis by as much as 3 years. This strategy might also be used in development of new regimens, rather than single compounds. Here, measurement of serial sputum colony-forming unit counts and whole blood bactericidal activity in trials of 1–4 weeks' duration can provide a seamless progression from preclinical studies through trials resulting in licensing with culture conversion. Such a development plan might reduce by as much as a decade the time required to have new regimens for MDR tuberculosis without cross-resistance to any existing tuberculosis drug.

Strategies for biomarker qualification

Although biomarkers have historically been widely used in drug development and medical practice, only recently have pathways been created to include them in the regulatory review process. In the USA, the impetus for this change came from the National Institutes of Health Road Map and the FDA Critical Path Initiative, both of which sought to introduce greater efficiency in drug research and development.[133,134] In this context, the term validation refers to assay performance characteristics (eg, how accurately urinary albumin is measured), whereas qualification refers to linkage to biological processes (eg, to what extent do increases in urine albumin predict aminoglycoside nephrotoxicity).[135] Biomarker review at the FDA includes a voluntary data submission that is examined by a biomarker qualification review team. Three categories of certainty are described: biological plausibility; prognosis of clinical outcomes in disease; and capturing differences in efficacy in clinical trials.[136] The first category could be described as appropriate only for exploratory purposes, whereas the third might be needed for registration of a new therapy or vaccine. Of all the markers described in this review, only 2-month sputum culture conversion falls into the third category. Reaching this level of certainty is particularly challenging in the case of new vaccines for tuberculosis, since there is no effective modern vaccine to which BCG might be compared for the purposes of biomarker qualification, and since innate genetic factors not amenable to modulation by vaccination might account for and be detected by biomarkers that predict risk of disease in natural history studies.

Tuberculosis diagnostics

Progress towards elimination of tuberculosis has remained elusive despite intensified standard measures of control. After a period of global acceleration in 2001–05, the case detection rate worldwide decelerated in 2006 and 2007, reaching 63% in 2007.[137] Thus, the target of a case detection rate of at least 70% by 2005 has not yet been achieved, and is unlikely to be met until 2014.[137]

Insufficient access to advanced diagnostic tests has contributed to this suboptimum performance. Even in 2010, national tuberculosis programmes in disease-endemic countries continue to rely largely on antiquated and inaccurate methods such as direct smear microscopy, solid culture, chest radiography, and tuberculin skin testing. There is no rapid, point-of-care test that allows early detection of active tuberculosis at health clinics. Diagnosis of smear-negative tuberculosis in adults infected with HIV and in children continues to pose substantial clinical challenges. Even existing diagnostics are not used to their full potential because of poor access to health care and failures in health-care delivery systems, including poor synergy between national HIV/AIDS and tuberculosis programmes. Diagnostic delays, misdiagnosis, and inadequate implementation of existing tests result in increased morbidity and mortality in patients, and allow continued transmission of tuberculosis.[138] These restrictions of present case detection approaches are starkly visible in countries with a high prevalence of HIV infection or MDR tuberculosis, or both.[139–141]

Barriers to development of new tuberculosis diagnostics

Market failure has been an important factor hindering the development of new diagnostics for tuberculosis. Industry tends to avoid developing and marketing products that will be mainly used for poor patients in resource-limited countries because such products will not generate profits.[142,143] When products are available, neither pricing nor performance is adapted for developing countries, and their potential benefits are effectively unavailable for patients and health-care providers who need them most.

Furthermore, health systems in developing countries are generally weak, making them unable to take advantage of tuberculosis diagnostics to achieve best possible performance, and to introduce new advances in diagnostic technologies. This situation is the result of poor management, insufficient financial resources, inadequate human resources, and poor laboratory capacity.[144] For example, rapid tests for malaria are a model of the type of assay widely needed for tuberculosis, but only a small proportion of patients receiving malaria treatment are tested.[145] Rapid tests for HIV infection are highly accurate, but undiagnosed HIV infection is very common, and a large proportion of HIV-infected individuals do not

present for HIV testing until late in infection.[146] Only about 10–20% of people infected with HIV in Africa are aware of their status.[144] Furthermore, less than 3% of people with HIV infection are screened for tuberculosis, and globally, only about 20% of notified tuberculosis patients are aware of their HIV status.[147] These estimates suggest that even existing diagnostic strategies are poorly implemented in many settings.

Knowledge gaps and scientific obstacles impeding progress

Our understanding of the biology of *M tuberculosis* and interactions with the human host is incomplete, and these knowledge gaps impede the development of biomarkers that can distinguish between latent and active tuberculosis, and distinguish active tuberculosis from other diseases, especially in HIV-infected adults and children.[148–150] Present tests for latent *M tuberculosis* infection do not adequately distinguish resolved from persistent infection, and are unable to efficiently identify individuals who are at highest risk of reactivation.[151–155] Studies into predictive value of IGRAs show only modest predictive ability, and several studies show similar (and rather low) rates of progression in people with positive tuberculin skin test and IGRA results.[156–160]

Other important knowledge gaps pertain to diagnosis of smear-negative tuberculosis in children and HIV-infected individuals, and rapid and accurate identification of resistance to second-line antituberculosis drugs. Although molecular markers have been identified and successfully used as rapid and accurate tests for isoniazid and rifampicin resistance, testing for the resistance that characterises extensively drug-resistant tuberculosis is on a less robust scientific footing than is testing for MDR tuberculosis.[161]

The diagnostics pipeline and new WHO policies

Over the past decade, tangible progress has been made in the development of new tuberculosis diagnostics. The increase in investments has resulted in an expanded pipeline of new diagnostic tests.[162,163] The private sector, led by funding from the Bill & Melinda Gates Foundation, is increasingly engaged in public-private partnerships such as the Foundation for Innovative New Diagnostics (FIND) to develop and deliver a pipeline of tests that are appropriate for disease-endemic countries. Furthermore, under the umbrella of the Global Laboratory Initiative—one of the Working Groups of the Stop TB Partnership—plans are underway for a large scale-up of laboratory services for tuberculosis. For example, UNITAID is providing US$81 million funding for a programme called EXPAND-TB that will supply rapid diagnostics for MDR tuberculosis to 27 high-burden countries.[164] Another example is the allocation of substantial resources to laboratory strengthening by the US President's Emergency Plan for AIDS Relief (PEPFAR).[165]

For the first time in many years, progress is being made in developing a range of diagnostic options for laboratories in disease-endemic countries. The Stop TB Partnership's Retooling Task Force and New Diagnostics Working Group produced a summary on the diagnostics pipeline.[166] Figure 3 shows an updated version of the pipeline,[167] which displays the tests that have been endorsed by WHO between 2007 and 2009. A complete description of existing and novel tuberculosis diagnostics is available elsewhere.[139,168]

Since 2007, several tuberculosis diagnostics have been endorsed by WHO for use in disease-endemic countries (panel 2). In 2007, WHO endorsed the use of liquid culture systems and rapid tests for species confirmation through antigen detection.[169] This WHO policy, along with FIND's negotiations with industry, made implementation of liquid culture systems affordable and feasible for the first time, especially in countries with high HIV prevalence.

Line-probe assays, which are based on reverse hybridisation technology, have consistently shown excellent accuracy for rapid detection of MDR tuberculosis.[175] As a result, in 2008, WHO endorsed the use of these assays for rapid detection of MDR tuberculosis in smear-positive patients.[170] Several non-commercial and less expensive options have been explored for MDR screening of clinical specimens with a variety of culture methods within centralised reference laboratories, including microscopic observation drug susceptibility, thin-layer agar, direct nitrate reductase, and colorimetric redox indicator assays. WHO considered evidence for their accuracy and role, and recommended that selected non-commercial culture and drug-susceptibility testing methods be used as an interim solution in resource-constrained settings, in reference laboratories, or in other laboratories with sufficient culture

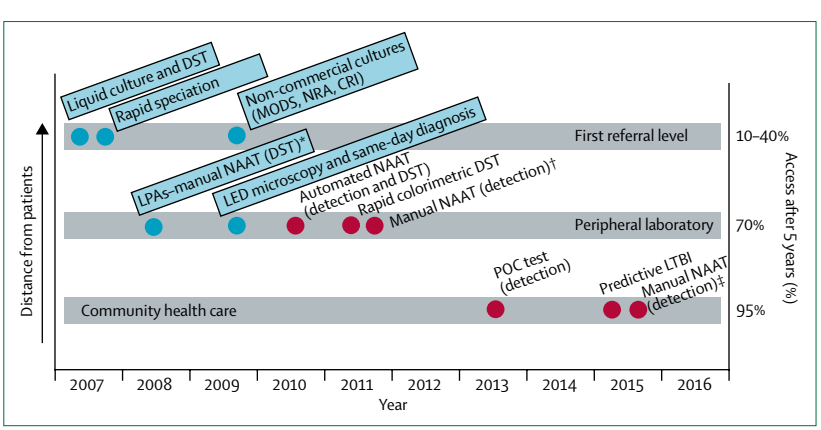


Figure 3: **The tuberculosis diagnostics pipeline**

Technologies in boxes have been endorsed by WHO. DST=drug-susceptibility test. MODS=microscopic observation drug susceptibility. NRA=nitrate reductase assay. CRI=colorimetric redox indicator assay. LPA=line-probe assay. NAAT=nucleic acid amplification test. LED=light-emitting diode. POC=point of care. LTBI=latent tuberculosis infection. *Manual NAAT: technology for *Mycobacterium tuberculosis* drug-susceptibility testing. †Manual NAAT: technology for *M tuberculosis* detection at the peripheral laboratory. ‡Manual NAAT: technology for *M tuberculosis* detection at the community health-care level. Source: adapted from Stop TB Partnership. Global Plan to Stop TB, 2006–2015,[167] and reproduced with permission from author and publisher.

capacity, while capacity for genotypic or automated liquid culture and drug-susceptibility testing is being developed.[171] Although non-commercial assays have similar accuracy as do commercial liquid culture systems and cost less, these tests are not standardised and need extensive training, optimisation, and quality assurance before clinical use.

Panel 2: Summary of WHO policies and statements on tuberculosis diagnostics

Liquid media for culture and DST (introduced in 2007)
WHO recommends, as a step-wise approach:
- The use of liquid medium for culture and DST in middle-income and low-income countries.
- Rapid species identification to address the needs for culture and DST, taking into consideration that implementation of liquid systems will be phased, will be integrated into a country-specific comprehensive plan for laboratory-capacity strengthening, and will address several issues including biosafety and training.

Definition of a new sputum-smear-positive tuberculosis case (introduced in 2007)
The revised definition of a new sputum-smear-positive case of pulmonary tuberculosis is based on the presence of at least one acid fast bacilli in at least one sputum sample in countries with a well functioning external quality-assurance system.

Reduction of number of smears for diagnosis of pulmonary tuberculosis (introduced in 2007)
WHO recommends the number of specimens to be examined for screening of tuberculosis cases can be reduced from three to two, in places where a well functioning external quality-assurance system exists, where the workload is very high, and human resources are scarce.

Molecular line-probe assays for rapid screening of patients at risk of MDR tuberculosis (introduced in 2008)
The use of line-probe assays is recommended by WHO, with the following guiding principles:
- Adoption of line-probe assays for rapid detection of MDR tuberculosis should be decided by ministries of health within the context of country plans for appropriate management of patients with MDR tuberculosis, including the development of country-specific screening algorithms and timely access to quality-assured second-line antituberculosis drugs.
- Direct use of line-probe assays on smear-negative clinical specimens is not recommended.
- The use of commercial line-probe assays, rather than in-house assays, is recommended to ensure reliability and reproducibility of results.
- Adoption of line-probe assays does not eliminate the need for conventional culture and DST capability; culture remains necessary for definitive diagnosis of tuberculosis in smear-negative patients, whereas conventional DST is needed to diagnose XDR tuberculosis.

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LED-based microscopy (introduced in 2009–10)
- WHO recommends that conventional fluorescence microscopy be replaced by LED microscopy using auramine staining in all settings where fluorescence microscopy is currently used, and that LED microscopy be phased in as an alternative for conventional Ziehl-Neelsen light microscopy in both high-volume and low-volume laboratories.
- The switch to LED microscopy should be undertaken through a carefully phased implementation plan, with use of LED technologies that meet WHO specifications.

Non-commercial culture DST methods (introduced in 2009–10)
WHO recommends that selected non-commercial culture and DST methods be used as an interim solution in resource-constrained settings, in reference laboratories, or in those with sufficient culture capacity, while capacity for genotypic and/or automated liquid culture and DST are being developed. With due consideration of the above issues, WHO endorses the selective use of one or more of the following non-commercial culture and DST methods:
- Microscopically observed drug susceptibility as direct or indirect tests, for rapid screening of patients suspected of having MDR tuberculosis.
- Nitrate reductase assay, as direct or indirect tests, for screening of patients suspected of having MDR tuberculosis, and acknowledging that time to detection of MDR tuberculosis in indirect application would not be faster than conventional DST methods using solid culture.
- Colorimetric redox indicator methods, as indirect tests on *Mycobacterium tuberculosis* isolates from patients suspected of having MDR tuberculosis, and acknowledging that time to detection of MDR tuberculosis would not be faster (but would be less expensive) than conventional DST methods using commercial liquid culture or molecular line-probe assays.

Same-day diagnosis by microscopy (introduced in 2009–10):
- WHO recommends that countries that have successfully implemented the current WHO policy for a two-specimen case-finding strategy consider a switch to the same-day-diagnosis approach, especially in settings where patients are likely to default from the diagnostic process.
- Countries that are still using the three-specimen case-finding strategy consider a gradual change to the same-day-diagnosis approach, once WHO-recommended external microscopy quality-assurance systems are in place and good quality microscopy results have been documented.
- Changes to a same-day-diagnosis strategy be preceded by a detailed situation assessment of the programmatic, logistical, and operational implications within countries, and supported by a carefully phased implementation plan.

Source: WHO.[169-174] DST=drug-susceptibility testing. MDR=multidrug resistant. XDR=extensively drug resistant. LED=light-emitting diode.

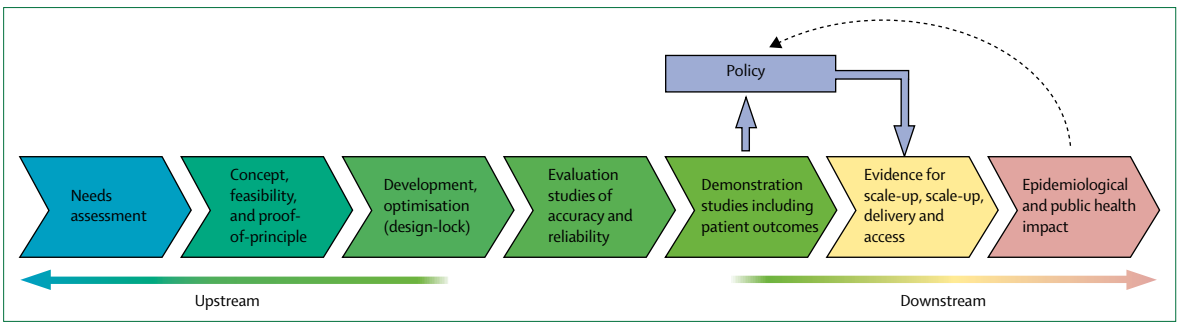

Figure 4: **Schematic showing the pathway to tuberculosis diagnostics, from concept to delivery**
Source: Stop TB Partnership's New Diagnostics Working Group. Pathways to better diagnostics for tuberculosis: a blueprint for the development of TB diagnostics (2009),[180] and reproduced with permission from author and publisher.

Fluorescence microscopy is widely used in high-income countries since it offers increased sensitivity, and has logistical advantages such as less technician time,[176] but is rarely used in resource-limited countries. Several light-emitting diode (LED) microscopes that can be used in fluorescence microscopy have been developed in the past few years.[177] They are inexpensive, robust, consume little electricity, are highly sensitive, and need less technician time than does Ziehl-Neelsen microscopy. WHO recommended that conventional fluorescence microscopy be replaced by LED microscopy in all settings and that LED microscopy be phased in as an alternative for conventional Ziehl-Neelsen microscopy in both high-volume and low-volume laboratories.[171] Efforts are also underway to minimise diagnostic delays and to improve system efficiency by optimising the number of specimens that are needed and the way in which they are collected (eg, so-called same-day diagnosis, using two sputum smears collected on the same day).[178] In fact, WHO recently endorsed the use of the same-day microscopy approach.[171]

The growing evidence base for tuberculosis diagnostics

Evidence presented to WHO expert committees over the past few years that has informed the endorsement of new technologies (panel 2) included feasibility studies assessing the technology aspect, evaluation studies of the final manufactured product, and large-scale demonstration projects focused on cost, effect, and practicability of use in real-world settings.[179] This extensive and robust platform of evidence is time consuming and expensive to generate, but necessary to lend support to evidence-based policies for tuberculosis diagnosis.[172] An outline for the development of tuberculosis diagnostics, published by the Stop TB Partnership's New Diagnostics Working Group, formalised this evidence platform by describing the development pathway for new tuberculosis diagnostics in detail (figure 4), from initial concept to development, evaluation, delivery, scale-up, and impact assessment.[180]

WHO has assumed a leadership role in ensuring that new tuberculosis diagnostic policies are evidence based,[179] and in line with the grading of recommendations assessment, development and evaluation (GRADE) approach to guideline development.[172] To enable and help with this process, existing systematic reviews on tuberculosis diagnostics, policies, guidelines, and research agendas for diagnosis have been compiled by the Stop TB Partnership's New Diagnostics Working Group.[181] Panel 3 summarises the findings of systematic reviews of various tuberculosis diagnostics.

Optimism for the future

The product pipeline for the future looks promising. In 2009, data were published on the first automated molecular test for tuberculosis, the Xpert MTB/RIF, which was co-developed by the Foundation for Innovative New Diagnostics, Cepheid (Sunnyvale, CA, USA), and the University of Medicine and Dentistry of New Jersey, NJ, USA.[182] This assay, which was CE (Conformité Européenne) marked in 2009, avoids most of the pitfalls of conventional nucleic acid amplification tests (safety, contamination, ease of use, etc), can be done by staff with little training, and can be used for case detection or MDR screening. Data from evaluation trials showed excellent performance in both smear-positive and smear-negative patients, and high accuracy for determination of rifampicin resistance. Thus, this highly sensitive and simple-to-use system can detect *M tuberculosis* directly from sputum in less than 2 h.[182] Data from ongoing demonstration projects are likely to be reviewed by WHO in 2010.

For the diagnosis of latent *M tuberculosis* infection, commercially available IGRAs have emerged as a strong alternative to the tuberculin skin test. These assays have very high specificity and have specific logistical advantages compared with the tuberculin skin test[183] for diagnosis. IGRAs, however, have no role as rule-in tests for active tuberculosis diagnosis for adults in endemic settings.[183] The use of IGRAs is steadily increasing, with several countries with low and intermediate incidence opting to use them, mostly as follow-up tests in people with positive results from tuberculin skin tests, especially in BCG-vaccinated populations.[184] A survey of IGRA guidelines showed much diversity in how various countries recommend and use

Panel 3: Summary of findings from systematic reviews on tuberculosis diagnostic tests

Diagnosis of active tuberculosis

Sputum-smear microscopy for pulmonary tuberculosis

- FM is on average 10% more sensitive than is conventional microscopy. Specificity of both FM and conventional microscopy is similar. FM is associated with improved time efficiency.
- LED FM performs equivalently to conventional FM, with added benefits of low cost, durability, and ability to use without a darkroom.
- Centrifugation and overnight sedimentation, preceded with any of several chemical methods (including bleach), is slightly more sensitive (6–9%) than is direct microscopy; specificity might be slightly decreased (1–3%) by sputum processing methods.
- When serial sputum specimens are examined, the mean incremental yield and/or increase in sensitivity from examination of third sputum specimen ranges between 2% and 5%.
- A same-day-diagnosis approach (microscopy of two consecutive spot-spot sputum specimens) is equivalent, in terms of diagnostic accuracy, to conventional case-finding strategies by microscopy.

NAATs for pulmonary and extrapulmonary tuberculosis

- NAATs have high specificity and positive predictive value. NAATs, however, have relatively lower (and highly variable) sensitivity and negative predictive value for all forms of tuberculosis, especially in smear-negative and extrapulmonary disease.
- In-house (so-called home brew) NAATs produce highly inconsistent results compared with commercial, standardised NAATs.

Serological antibody detection tests for pulmonary and extrapulmonary tuberculosis

- Commercial serological tests for both pulmonary and extrapulmonary tuberculosis produce highly inconsistent estimates of sensitivity and specificity; none of the assays do well enough to replace microscopy.
- Several potential candidate antigens for inclusion in an antibody detection-based diagnostic test for pulmonary tuberculosis in HIV-infected and uninfected individuals were identified.
- Combinations of select antigens provide higher sensitivities than do single antigens.

ADA for tuberculosis pleuritis, pericarditis, peritonitis

- Measurement of ADA concentrations in pleural, pericardial, and ascitic fluid has high sensitivity and specificity for extrapulmonary tuberculosis.

Interferon γ for tuberculosis pleuritis

- Pleural fluid interferon-γ determination is a sensitive and specific test for the diagnosis of tuberculosis pleuritis.

Phage amplification assays for pulmonary tuberculosis

- Phage-based assays have high specificity but lower and variable sensitivity. Current commercial phage-based assays are limited by high rates of indeterminate results.

Automated liquid cultures for pulmonary tuberculosis

- Automated liquid cultures are more sensitive than are solid cultures; time to detection is more rapid than for solid cultures.

Diagnosis of latent tuberculosis

TST for latent tuberculosis infection

- Individuals who had received BCG vaccination are more likely to have a positive TST; the effect of BCG on TST results is less after 15 years; positive TST with indurations of >15 mm are more likely to be the result of tuberculosis infection than of BCG vaccination.

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IGRAs.[184] The two-step approach (initial tuberculin skin test followed by confirmatory IGRA testing) seems to be the most common strategy, partly because of economic considerations. The optimum strategy for IGRA use is yet to be established.

Although targeted testing and preventive therapy for latent *M tuberculosis* infection is well established in low-incidence countries, the exact role of testing and treatment in disease-endemic countries remains controversial. However, testing for latent *M tuberculosis* infection is receiving increased attention in vulnerable subgroups, such as HIV-infected people and childhood contacts of active tuberculosis cases.[185,186] A WHO policy for IGRAs is under consideration for 2010.

Limitations of the existing diagnostics pipeline

A simple, rapid, inexpensive point-of-care test for active tuberculosis that can perform as well or better than conventional smear microscopy, and can deliver results within minutes without sophisticated equipment or laboratory requirements, is still missing from the development pipeline. Point-of-care diagnostic tests offer important potential advantages for control of diseases such as tuberculosis that need lengthy standardised, decentralised therapy.[142,187,188] Patient, community, and activist groups have urged for increased funding and resources to develop point-of-care tests, and specifications for an ideal point-of-care test have been proposed.[189]

The existing tuberculosis diagnostics pipeline is also restricted with respect to tests that address important diagnostic challenges, especially in HIV-infected people, and children and adults with smear-negative tuberculosis. Unfortunately, most existing tests have shown disappointing performance in smear-negative tuberculosis. Conventional nucleic acid amplification tests have inadequate sensitivity in patients with smear-negative tuberculosis. Improved tests such as Xpert MTB/RIF might have improved sensitivity in these patients, but further validation is needed.[182]

Childhood tuberculosis is a well known diagnostic challenge, and all available tests do poorly in cases of paucibacillary tuberculosis.[190] Furthermore, since young children are unable to produce sputum, alternative specimens such as urine, saliva, or breath condensate would be helpful to use. The absence of a gold standard for childhood tuberculosis and smear-negative tuberculosis is an important impediment to rapid assessment of new diagnostic methods in these high-risk subgroups. One potential solution to the problem of an inadequate gold standard would be to follow up well characterised cohorts of patients after initial testing until tuberculosis is definitely ruled in or out. This type of study could also assess whether use of a new test actually improved patient-important outcomes, rather than examining sensitivity and specificity only.

Although serological antibody tests for tuberculosis have potential as point-of-care tests, their performance

thus far has been disappointing.[191,192] Urine mycobacterial antigen (eg, lipoarabinomannan) detection tests are attractive options for point-of-care testing. Results of studies of detection of urinary lipoarabinomannan have been variable but generally suboptimum, although somewhat better in patients with advanced HIV infection.[193–197] Alternative detection targets are being sought, such as *M tuberculosis* tr-DNA.[198]

Several options are being explored for simpler, less expensive point-of-care and multiplexed assay formats in the future, including manual molecular testing that can be done in peripheral settings, lab-on-chip approaches that can be used to detect several infections simultaneously, antigen detection on highly sensitive platforms, and antibody detection with panels of recently identified antigens of diagnostic value. Owing to growing interest and funding for new methods and biomarkers, several agencies, industries, and groups are working on developing point-of-care platforms for tuberculosis, including novel serological assays, detection of volatile organic compounds in breath, handheld molecular devices, microchip technologies, and tests that exploit approaches such as microfluidics, nanotechnology, proteomics, and metabolomics.[139,162,168,187]

Overcoming barriers for implementation in tuberculosis control programmes

What will be the outcome of all this technology development for tuberculosis diagnostics, and how can this progress be translated into concrete gains in control of tuberculosis? The effect of new tests will depend largely on the extent of their introduction into the global public sector, which will itself depend partly on policy decisions made by international technical agencies such as WHO, and by donors, and ultimately by national tuberculosis programmes in countries of low and middle income. So far, most evaluations of diagnostic methods have reported only sensitivity and specificity; many of these studies were poorly designed and incompletely reported.[172,199] Some have assessed time-to-test result, and a few have reported unit costs. However, to lend support to the introduction of new diagnostic technologies, broader evidence is needed, including implementation issues.[180] For example, the performance of new tests in programmatic conditions should be studied; tests done by experts in carefully controlled research settings are not likely to be indicative of future field performance. In addition to unit costs, costing studies should include costs for labour, equipment depreciation, initial and ongoing training, supervision, and quality control.[200]

Future studies of completed diagnostic products have to go beyond test accuracy and aim to generate evidence for the incremental value of new tests, their effect on patient outcomes, and their use for diagnostic decision making and cost-effectiveness.[172,199] Operational research is also essential to improve service delivery and to understand why diagnosis is delayed or missed, and to guide optimum implementation of new methods. To help with these types

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- The effect on TST of BCG received in infancy is small, especially 10 years after vaccination. BCG received after infancy produces more frequent, more persistent, and larger TST reactions. NTM infection is not a clinically important cause of false-positive TST, apart from in populations with a high prevalence of NTM sensitisation and a very low prevalence of tuberculosis infection.

T-cell-based IGRAs for latent tuberculosis infection

- IGRAs have excellent specificity (higher than the TST), and are unaffected by previous BCG vaccination.
- IGRAs cannot distinguish between latent tuberculosis infection and active tuberculosis, and have no role for active tuberculosis diagnosis in adults.
- IGRAs correlate well with markers of tuberculosis exposure in low-incidence countries.
- IGRA sensitivity varies across populations and tends to be lower in high-endemic countries and in HIV-infected individuals.

Diagnosis of drug-resistant tuberculosis

Phage amplification assays for rapid detection of rifampicin resistance

- Commercial phage amplification assays produce variable results when used directly on sputum specimens.
- Studies have raised concerns about contamination, false positive results, and technical assay failures.

Line-probe assays: INNO-LiPA Rif and GenoType MTBDR assays for rapid detection of rifampicin resistance

- The INNO-LiPA Rif assay is a highly sensitive and specific test for the detection of rifampicin resistance in culture isolates. The test has lower sensitivity when used directly on clinical specimens.
- GenoType MTBDR assays have excellent sensitivity and specificity for rifampicin resistance, even when directly used on clinical specimens.

CRI methods and NRA for rapid detection of rifampicin and isoniazid resistance

- Colorimetric methods are sensitive and specific for the detection of rifampicin and isoniazid resistance in culture isolates. CRIs use inexpensive non-commercial supplies and equipment and have a rapid turnaround time (7 days).
- NRA has high accuracy when used to detect rifampicin and isoniazid resistance in culture isolates. Data for its use when directly applied to clinical specimens are scarce, but results are promising. NRA is simple, uses inexpensive non-commercial supplies and equipment, and has a rapid turnaround time (7–14 days) compared with conventional methods.

MODS for rapid detection of rifampicin and isoniazid resistance

- MODS has high accuracy when testing for rifampicin resistance, but shows slightly lower sensitivity when detecting isoniazid resistance.
- MODS seems to do equally well with use of direct patient specimens and culture isolates.
- MODS uses non-commercial supplies and equipment, and has a rapid turnaround time (10 days) compared with conventional methods.

TLA for rapid detection of rifampicin and isoniazid resistance

- Data assessing TLA for the detection of drug susceptibility are scarce; however, all studies so far have reported 100% concordance with their reference standards.
- TLA uses inexpensive non-commercial supplies and equipment, and has a rapid turnaround time (11 days) compared with conventional methods.

FM=fluorescence microscopy. LED=light-emitting diode. NAATs=nucleic acid amplification tests. ADA=adenosine deaminase. TST=tuberculin skin test. NTM=non-tuberculous mycobacterial. IGRAs=interferon-γ release assays. CRI=colorimetric redox indicator. NRA=nitrate reductase assays. MODS=microscopically observed drug susceptibility. TLA=thin-layer agar. Adapted from reference 172 and reproduced with permission from the author, under creative commons open access license.

of research, the TB Research Movement—recently initiated by the Stop TB Partnership and WHO—is engaging tuberculosis researchers, tuberculosis programme managers, and affected communities in a collaborative and concerted strategic effort to increase the scope, scale, and speed of tuberculosis research across the continuum, linking together basic research, development of new methods, and operational research.[201]

The GRADE approach,[202] now being used by WHO, was originally designed for interventions such as drugs and vaccines, for which the product is the health intervention itself and the use of the product can be largely judged by its safety and effectiveness alone. Diagnostics, however, are only the start of a health intervention, and their effect will depend on where and how they are used, and what clinical decisions they can lend support to. The GRADE approach has been adapted and applied to diagnostic tests,[203,204] but will need to be further adapted or supplemented by careful considerations of the diversity and challenges of health systems when examining diagnostics aimed at public-sector populations in developing countries. Although GRADE has its limitations and can be improved and adapted for tuberculosis diagnostics, it is a major advance compared with the conventional policy-making process.[205]

Inadequate funding is another major barrier that needs to be overcome. New tuberculosis diagnostics will be of no practical value if they are not readily available at points of care in endemic areas, and if they are not taken seriously by governments of developing countries. Insufficient commitment to tuberculosis control by many developing country governments is largely responsible for poor programme performance. The Global Plan to Stop TB, 2006–2015, estimated that at least US$9 billion ($900 million per year) should be spent on tuberculosis research and development between 2006 and 2015 to develop new drugs, diagnostics, and vaccines.[167] The budget needed for tuberculosis diagnostics was $516 million; yet according to the 2009 Treatment Action Group and Stop TB Partnership reports, development for tuberculosis diagnostics received only $50 million in 2008.[206,207] This amount represented only 10% of the total funding for tuberculosis research and development.[207] Furthermore, philanthropic grants are outstripping government funding for tuberculosis research.[207]

To overcome this worrisome trend in declining public-sector investment, governments in all countries, especially industrialised countries, need to increase their funding for tuberculosis research and development.[167,206,207] Emerging and rapidly growing economies such as China, India, Brazil, and South Africa can and should increase their investments in tuberculosis, especially since these countries account for a large proportion of the global tuberculosis burden. Countries such as China and India can also make a big contribution by producing locally manufactured, low-cost generic tuberculosis drugs, diagnostics, and vaccines. In the long term, these countries have the potential to spearhead the next wave of innovation in tuberculosis research and development.

Conclusions

The need for a more accurate, inexpensive point-of-care tuberculosis diagnostic test that is applicable in tuberculosis and HIV endemic areas is greater nowadays than ever before, and will be crucial for achieving global tuberculosis control. Several modelling studies[208–215] suggest that new diagnostics for tuberculosis disease and MDR tuberculosis could have an important effect within populations, especially in disease-endemic countries, although improving population health and health services, and economic growth, might be as important.[216,217] Clinical and field studies are needed to assess whether programmatic introduction of new diagnostics contributes to improved individual patient outcomes and a measurable beneficial public health effect. After nearly a century of neglect and underinvestment, the tuberculosis diagnostics pipeline has rapidly grown, with several technologies showing great promise. Indeed, several have already been endorsed by WHO and are being introduced into clinical use. This progress needs to be translated into improving the lives of patients with tuberculosis, and reducing the future incidence of tuberculosis. This aim can and must be achieved, but will need strong political commitment, sustained funding, and engagement of public and private stakeholders and civil society. Donors and governments have to synergise their activities to ensure maximum programme performance for optimum care for patients with tuberculosis and with both tuberculosis and HIV infection.

To advance the area of tuberculosis biomarkers to that needed for registration, substantial investments will be required to undertake the necessary studies. Studies of MDR tuberculosis have been advocated by some as a rich source of poor outcomes for biomarkers research. However, whether markers predicting failure due to resistance will necessarily also predict relapse (which seems somewhat paradoxically to occur infrequently in MDR tuberculosis) is uncertain.[218] As studies are undertaken to shorten MDR treatment, we might need to rely on biomarkers for relapse developed in drug-sensitive disease to guide them. Tuberculosis incidence rarely approaches 1% in the general population even in high-prevalence countries, hampering prospective studies. Ethical concerns preclude natural history studies in high-risk patients, such as children or people with HIV infection, meaning that isoniazid preventive therapy should be offered. As a result, studies to validate markers that predict the transition from health to illness (or vice versa) in these populations will necessarily be large and protracted. If the plethora of potential biomarkers described here is to be converted into clinically useful tests, we not only need continuing research, but also

improved funding to synergise and improve multidisciplinary cross-cutting collaborations between scientists working with cohorts of patients and contacts participating in clinical trials of new drug regimens, diagnostics, and vaccines.

Contributors

AZ conceived the article outline and selected and assigned authors' roles. The literature search and the first and subsequent drafts of the report were developed by RSW (biomarkers) and MP (diagnostics). TMD, GW, and AZ contributed to writing of the biomarkers section, and MDP, DM, and AZ contributed to writing of the diagnostics section. AZ merged the two sections as the final article with contributions from all authors. All authors read and approved the final versions of the two sections before submission.

Steering committee

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Conflicts of interests

RSW is employed by Pfizer, USA. AZ is principal investigator of the EuropeAID Active Detection of Active Tuberculosis (ADAT) and European Union Framework 7 Trans-renal DNA (EU-FW7-TrDNA) projects, which are assessing new tuberculosis diagnostics, and serves on the Stop TB Research Movement Task Force. MDP is the Chief Scientific Officer of Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland, a non-profit agency that works with several industry partners in developing and evaluating new diagnostics for neglected infectious diseases. MP serves as an external consultant for FIND; serves as a co-chair of the Stop TB Partnership's New Diagnostics Working Group; and serves as chair of the Task Force of the Stop TB Research Movement. GW receives support from GlaxoSmithKline, Bill & Melinda Gates Foundation, Aeras Foundation, TB Alliance, and EDCTP. TMD receives support from EDCTP and EU-FP7. None of the funding agencies had any role in the development or submission of this report. DM declares that he has no conflicts of interest.

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