Tuberculosis 2

Tuberculosis: advances and challenges in development of new diagnostics and biomarkers

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Tuberculosis remains the leading cause of death from an infectious disease worldwide. Early and accurate diagnosis and detection of drug-sensitive and drug-resistant tuberculosis is essential for achieving global tuberculosis control. Despite the introduction of the Xpert MTB/RIF assay as the first-line rapid tuberculosis diagnostic test, the gap between global estimates of incidence and new case notifications is 4·1 million people. More accurate, rapid, and cost-effective screening tests are needed to improve case detection. Diagnosis of extrapulmonary tuberculosis and tuberculosis in children, people living with HIV, and pregnant women remains particularly problematic. The diagnostic molecular technology landscape has continued to expand, including the development of tests for resistance to several antituberculosis drugs. Biomarkers are urgently needed to indicate progression from latent infection to clinical disease, to predict risk of reactivation after cure, and to provide accurate endpoints for drug and vaccine trials. Sophisticated bioinformatic computational tools and systems biology approaches are being applied to the discovery and validation of biomarkers, with substantial progress taking place. New data have been generated from the study of T-cell responses and T-cell function, serological studies, flow cytometric-based assays, and protein and gene expression studies. Alternative diagnostic strategies under investigation as potential screening and triaging tools include non-sputum-based detection with breath-based tests and automated digital radiography. We review developments and key achievements in the search for new tuberculosis diagnostics and biomarkers. We highlight gaps and challenges in evaluation and rollout of new diagnostics and biomarkers, and prioritise areas needing further investment, including impact assessment and cost–benefit studies.

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Introduction

During 2016, incidence of tuberculosis was estimated to be 10·4 million cases, with 1·7 million deaths.[1] Rapid and accurate detection of tuberculosis is essential for guiding treatment, yet case detection and reporting rates remain low, with 40% of estimated incident cases failing to be identified and reported. Underdiagnosis remains a problem, particularly in countries where patients face substantial geographical and socioeconomic barriers when accessing health care. In most countries with a high burden of tuberculosis, case detection relies on patients reporting symptoms to a health-care facility. Delays in accessing effective treatment provides increased opportunity for transmission and continuation of the epidemic. Detection of extrapulmonary forms of the disease and tuberculosis in children is particularly problematic. Access to tests for drug resistance remains inadequate. In 2016, only 33% of patients with bacteriologically confirmed tuberculosis that was not previously treated were tested for resistance to rifampicin, whereas 60% of patients who had previously received antituberculosis treatment for at least 1 month, and who were considered at higher risk of resistance, were tested.[1] Treatment success rates during 2016 were 83% but outcomes were considerably worse for drug-resistant disease. In 2014, treatment success rates were 54% for multidrug-resistant (MDR) tuberculosis (resistance to at least isoniazid and rifampicin) and 30% for extensively drug-resistant (XDR) tuberculosis (additional resistance to the fluoroquinolones and second line injectable drugs).[1]

The rapid diagnostic test for detection of tuberculosis and rifampicin resistance recommended by WHO is an automated PCR assay, with an integrated semiautomated device for sample extraction. The GeneXpert MTB/RIF assay (Cepheid Inc, Sunnyvale, CA, USA) was endorsed by WHO in 2010. Rollout of the new technology has been

Key messages

- Tuberculosis is the world's major cause of death from a single infectious disease.
- Rapid and accurate detection of tuberculosis is essential for guiding treatment, yet cases being diagnosed, treated, and reported remain low, with 40% of estimated incident cases being missed from diagnosis and reported.
- A range of technologies are being developed and most rapid diagnostic products close to introduction are based on the detection of mycobacterial nucleic acids.
- Roll-out of the GeneXpert MTB/RIF assay has not improved global case detection rates. Alternative rapid screening and diagnostic methods that are affordable and easy to use in resource-poor settings with a high prevalence of tuberculosis are needed to find missed active disease cases.
- Next-generation sequencing is improving knowledge of drug resistance mutations.
- Biomarkers that can be used to differentiate tuberculous disease from latent infection, to predict the risk of progression to clinical disease, response to treatment, and relapse are urgently required to provide accurate endpoints for clinical trials of new drugs and vaccines.
- Progress is being made in the search for biomarkers of latent infection, active disease, cure, and relapse.
- Obstacles to the production and marketing of new detection platforms are considerable, the greatest challenge being inadequate access to sufficient funding for research and development.

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See Online for appendix

facilitated by preferential pricing deals for public sector use in countries with a high tuberculosis burden. However, access remains restricted and long-term sustainability in countries dependent on donor support is of concern. New tools for screening and diagnosing tuberculosis are required that are affordable and suitable for use in poorly resourced communities.

Biomarkers are urgently required to detect tuberculosis and differentiate it from latent infection. Additionally, markers that predict the risk of progression to clinical tuberculosis would greatly aid efforts to eradicate the disease, and indicators of probable treatment failure and relapse would be beneficial for patient monitoring. Biomarkers are also required to provide accurate endpoints for clinical trials of new drugs and vaccines. The pathology of *Mycobacterium tuberculosis* infection and host response is highly complex and not fully understood. Holistic systems biology approaches are being applied with sophisticated computational and mathematical methods. New data have been generated from the study of T-cell responses and T-cell function, serological studies, flow cytometric-based assays, and protein and gene expression studies. We present an overview of developments and key scientific achievements in the search for new tuberculosis diagnostics and biomarkers, and summarise rapid diagnostics tests in development and scientific literature pertaining to potential biomarkers as of Feb 15, 2018, The scientific, operational, and resource challenges are also reviewed. Further resources and links to relevant WHO polices are listed in the appendix.

Status of tuberculosis diagnostics

Sputum microscopy to identify *M tuberculosis* acid-fast bacilli remains the most commonly used test for tuberculosis. It is a low-cost test with low sensitivity that can be done in basic laboratories attached to primary health-care clinics; examination of multiple samples is recommended. Sensitive tests for tuberculosis, such as culture, and tests for drug resistance have historically been based either in specialist centres or reference laboratories, which are not accessible to most of the population.

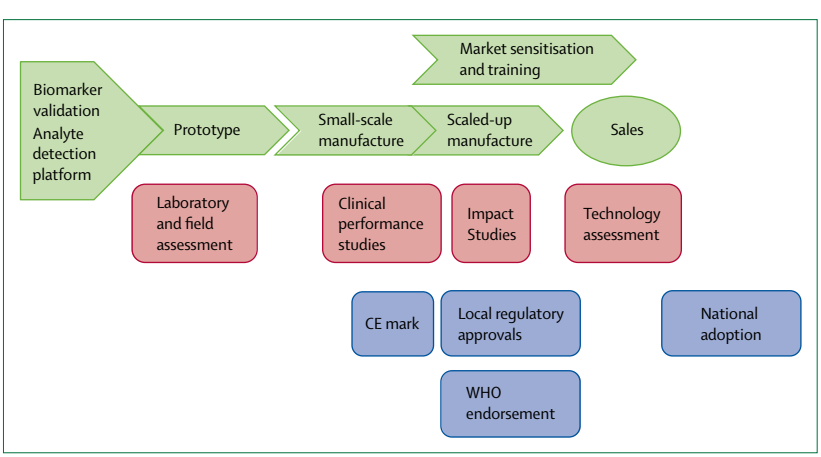

Figure: **Key stages in the development, production, and adoption of a new test for tuberculosis**

New tuberculosis tests are being developed, produced, and adopted, but concern regarding the sale of substandard in-vitro diagnostic assays has led the WHO tuberculosis programme to initiate an endorsement process (figure). Published performance data and information provided by the manufacturers have been reviewed by a committee of experts and recommendations have been made as to how to use these tests. Liquid culture systems and line probe assays (LPAs) for detection of drug resistance were endorsed, but a negative endorsement was declared for serological tests because of their poor sensitivity and specificity.[2]

The Xpert MTB/RIF WHO recommendations[3] provide information about the use of chest radiography in tuberculosis triaging, screening, and diagnosis. For the first time, Xpert MTB/RIF offers rapid access to testing for resistance to rifampicin, a marker for MDR tuberculosis. The assay was initially endorsed for use in the detection of pulmonary disease in populations with a high prevalence of HIV or multidrug-resistant tuberculosis, but the recommendation has since been broadened for the assay to replace microscopy as a first-line diagnostic and for the detection of some forms of extrapulmonary disease.[4] Although easy to use, the assay technology is sophisticated and expensive. Reduced pricing is available to the public sector of 145 low-income and middle-income countries, with a high burden of tuberculosis. A GeneXpert machine costs between US$12 000 and $71 000 depending on the number of test modules incorporated, and a single-use test cartridge is $9·98.[5] The test requires a constant source of electricity and is vulnerable to heat and dust; high rates of instrument failure have been reported in some settings.[6,7] Initial introduction of the technology was via centralised or reference laboratories.

To catalyse uptake at lower levels of the health system, from 2013 to 2016, Unitaid led a project in collaboration with TB REACH, the African Society for Laboratory Medicine, Interactive Research and Development, EXPAND-TB, and the Global Laboratory Initiative to make the test more widely available in 21 countries. 237 machines and 1·46 million cartridges were provided and 201 748 cases of tuberculosis were detected, including 45 278 that were resistant to rifampicin. Unfortunately, the project did not monitor time to treatment initiation, and the effect of the initiative on treatment outcomes (morbidity and mortality) is not known; however, the enhanced capacity to detect drug resistance was noted by some countries to have increased awareness of MDR tuberculosis.[7]

A new version of the test (Xpert MTB/RIF Ultra) has been launched, which is claimed to have increased sensitivity for diagnosis and improved accuracy for detection of rifampicin resistance.[8] A multicentre study[9] reported increased sensitivity particularly among cases of

paucibacillary disease, but with a concurrent loss of specificity from 98% to 96%. Detection of rifampicin resistance was the same for both versions of the test (95%). The new Xpert MTB/RIF Ultra cartridge was endorsed by WHO as a replacement for the Xpert MTB/RIF cartridge in March, 2017.[10] Particularly encouraging is the reported increased capacity to detect tuberculous meningitis, in which sensitivity for probable or definite tuberculous meningitis was 70% (95% CI 47–87) for Xpert Ultra, compared with 43% (23–66) for Xpert MTB/RIF, and 43% (23–66) for culture.[11]

The Alere Determine LAM TB (Alere, Waltham, MA, USA) test is a low-cost, rapid, lateral flow device for use at the bedside or in a clinic. It improved survival of patients who were admitted to hospital and had low CD4 counts when other bacteriological tests were not readily available.[12] The test has been endorsed by WHO.[13]

Screening for latent tuberculosis infection offers an opportunity to prevent progression to active disease, with the use of interferon-γ release assays (IGRAs) in low tuberculosis prevalence settings.[14] However, the inability of IGRAs to differentiate latent tuberculosis infection from active disease led WHO not to endorse their use in countries with a high burden of tuberculosis.[2] No accurate tests are available for predicting progression to active disease, relapse after treatment, or protection after vaccination.

Detecting drug resistance

Reports of MDR tuberculosis and XDR tuberculosis are increasing worldwide. Prompt access to effective treatment is vital to prevent onward transmission and inhibit the emergence of resistance to further drugs during inadequate therapy. Phenotypic culture-based methods remain the mainstay of drug susceptibility testing at reference laboratories but they take weeks and require stringent microbiology safety precautions. Methods vary across laboratories, and for some drugs the link between microbiological breakpoint and clinical efficacy remains uncertain.[15] In 2017, WHO initiated a systematic process to reassess critical concentrations of some second-line drugs and to revise critical concentrations for new and repurposed drugs.[16] WHO is also reviewing the accuracy of sequencing drug resistance genes to inform guidelines for the use of genotypic drug susceptibility testing methods.

Drug resistance in *M tuberculosis* is caused by mutations in the bacterial genome that affect drug targets or enabling enzymes. Single nucleotide polymorphisms (SNPs) are the most frequently observed type of mutation. These small changes in the DNA sequence can easily be detected after amplification, and they provide a rapid and accurate means for assessing resistance to rifampicin. LPAs, in which after PCR of target regions the amplicons are interrogated by membrane-bound probes, are available from several manufacturers. LPAs are available for rifampicin and some of the other first-line and second-line drugs.[17] Several other molecular tests for resistance are in development (table 1). Three technologies (Cepheid Xpert Ultra [Cepheid, Sunnyvale, CA, USA], Genedrive MTB/RIF [Epistem, Manchester, UK], and Truenat MTB [Bigtec Labs, Bangalore, India]) have been designed for use in a clinic, with microscopy facilities; the remainder of the technologies are expected to be used in referral laboratories. Gene sequencing is being increasingly used to detect *M tuberculosis* drug resistance because it gives greater accuracy than other drug resistance detecting technologies.[15]

The Xpert Ultra and LPAs have reported high sensitivities when testing for rifampicin resistance directly from smear-positive sputum. Sensitivities are lower for other tuberculosis drugs than rifampicin due in part to the large number of loci potentially involved in resistance, which exceed the testing capacity of these simple molecular devices. A further technology-related problem is the recording of false positives when the test is unable to distinguish silent mutations—eg, the substitution of TTC for TTT in codon 514 of the

	Intended use	Mechanism of action
Abbott RealTime MTB RIF/INH	Rifampicin, isoniazid	Automated multiplex PCR assay with probe-based fluorescence detection
Autoimmun Diagnostika TB Resistance Module	Rifampicin, isoniazid	Line probe assay
CapitalBio Technology Tuberculosis Drug Resistance Detection Array Kit	Rifampicin, isoniazid	PCR and microarrays
Cepheid Xpert Ultra Cartridge	Rifampicin resistance (a cartridge for additional drugs is in development)	Automated PCR assay with melt curve analysis
Epistem Genedrive MTB/RIF ID Kit	Rifampicin	PCR amplification with melt curve analysis
Hain Lifescience FluoroType MTBDR version 1.0	Rifampicin, isoniazid	PCR with fluorescence-based probe detection
Hain Lifesciences GenoType MTBDRplus version 2.0, GenoType MTBDRsl VER 1.0/2.0	Rifampicin, isoniazid, aminoglycosides or cyclic peptides, ethambutol	Line probe assay
Molbio Diagnostics Truenat MTB	Rifampicin	Chip-based PCR test
Nipro Genoscholar	Rifampicin, isoniazid, or pyrazinamide, or fluoroquinolones, kanamycin	Line probe assay
QuanDx MTB Drug-Resistant Mutation Test Kits	Rifampicin, isoniazid, ethambutol, streptomycin, fluoroquinolones (for research use)	PCR and probe-based melt curve analysis
Seegene Anyplex Assays for MDR Detection	Rifampicin, isoniazid	Multiplex real-time quantitative PCR
Seegene Anyplex II MTB/XDR Detection	Fluoroquinolones, second-line injectables	Multiplex real-time quantitative PCR
YD Diagnostic MolecuTech REBA MDR and XDR	Rifampicin, isoniazid, fluoroquinolones, kanamycin, streptomycin	Line probe assay
Zeesan Biotech MeltPro MTB (MDR-TB, XDR-TB) Kits	Rifampicin, isoniazid, ethambutol, fluoroquinolones, second-line injectables	PCR and probe-based melting curve analysis

MTB=*Mycobacterium tuberculosis*. RIF=rifampicin. INH=isoniazid. TB=tuberculosis. ID=identification. XDR=extensively drug-resistant. REBA=reverse blot hybridisation assay. MDR=multidrug-resistant.

Table 1: **Commercial molecular tests to detect resistance to antituberculosis drugs**

***Panel 1*: Tuberculosis diagnostic pipeline, February, 2018**[1,24]

Technologies in development

Molecular detection of tuberculosis and drug resistance

- Genedrive MTB/RIF ID (Epistem, Manchester, UK)
- Xpert XDR-TB cartridge (Cepheid, Sunnydale, CA, USA)
- TruArray MDR-TB (Akkoni, Frederick, MD, USA)
- INFINITIMTB Assay (AutoGenomics, Carlsbad, CA, USA)
- FluoroType XDR-TB Assay (Hain Lifescience, Nehren, Germany)
- MeltPro TB Assay (Zeesan Biotech, Xiamen, China)
- QuantuMDx (POC, Newcastle upon Tyne, UK)

On the market*

Molecular detection of tuberculosis and drug resistance

- iCubate System (iCubate, Huntsville, AL, USA)
- Genechip, TB Drug Resistance Array (Capital Bio, Beijing, China)
- EasyNAT TB Diagnostic Kit (Ustar Biotechnologies, Hangzhou, China)
- Truelab/Truenat MTB (Molbio/Bigtec Diagnostics, Bangalore, India)

Technologies endorsed by WHO

Molecular detection of tuberculosis and drug resistance

- Xpert MTB/RIF Ultra for detection of tuberculosis and rifampicin resistance in pulmonary, extrapulmonary, and paediatric samples (Cepheid, Sunnyvale, CA, USA)
- Line probe assays for the detection of *Mycobacterium tuberculosis*, isoniazid, and rifampicin resistance in acid-fast bacilli smear-positive sputum or *M tuberculosis* cultures (FL-LPA) (Hain Lifescience, Nehren, Germany and Nipro, Osaka, Japan)
- Line probe assays for the detection of resistance to fluoroquinolones and second-line injectable agents (SL-LPA) (Hain Lifescience, Nehren, Germany)
- TB LAMP for detection of tuberculosis (Eiken, Tokyo, Japan)

Non-molecular technologies

- Alere Determine TB-LAM (Alere, Waltham, MA, USA) (tuberculosis detection in people who are seriously ill and HIV positive)
- Inteferon-γ release assay for the diagnosis of latent tuberculosis infection (Immunotec, Oxford, UK, and Qiagen, Germantown, MD, USA)

Culture-based technologies:

- Commercial liquid culture systems and rapid speciation
- Culture-based phenotypic drug-susceptibility testing using 1% critical proportion in Löwenstein-Jensen (LJ) and Middlebrook 7H10/7H11 agar media, and mycobacteria growth indicator tube media

Microscopy

- Light and light-emitting diode microscopy (diagnosis and treatment monitoring)
- Microscopic observation drug susceptibility (MODS) test (Hardy Diagnostics, Santa Maria, CA, USA)

Scheduled for WHO assessment in 2018–19

Molecular detection of tuberculosis and drug resistance

- FluoroType MTBDR (Hain Lifescience, Nehren, Germany)
- m2000 RealTime MTB System (Abbott, Lake Bluff, IL, USA)
- BD Max MDR-TB (Becton Dickinson, Franklin Lakes, NJ, USA)
- GeneXpert Omni (Cepheid, Sunnyvale, CA, USA)

Imaging

- Chest radiography
- Computer-aided imaging (CT, PET, PET-CT, MRI)

MTB=*Mycobacterium tuberculosis*. RIF=rifampicin. XDR=extensively-drug resistant. TB=tuberculosis. MDR=multidrug resistant. POC=point of care . LAMP=loop-mediated isothermal amplification. LAM=lipoarabinomannan. *Evidence for use not submitted to WHO for assessment.

rpoB gene, which does not result in resistance to rifampicin.[18] Resistance mutations are not fully characterised for all tuberculosis drugs, and they are particularly deficient for the second-line and newer drugs used to treat MDR and XDR tuberculosis. To accelerate progress in this area, an international consortium of researchers and international agencies, the ReSeqTB Initiative, has been established to create an open access platform. Consensus has been published[19] of a method that interprets the association between mutations and phenotypic drug resistance.

A new cartridge for the Cepheid GeneXpert is being developed that tests for resistance to isoniazid and some second-line drugs.[20] When compared with phenotypic tests at sites in China and South Korea, a prototype cartridge had sensitivities, when detecting resistance, of 83·3% (95% CI 77·1–88·5) for isoniazid, 88·4% (80·2–94·1) for ofloxacin, 87·6% (9·0–93·7) for moxifloxacin at a critical concentration of 0·5 µg/mL, 96·2% (7·0–99·5) for moxifloxacin at a critical concentration of 2·0 µg/mL, 71·4% (6·7–83·4) for kanamycin, and 70·7% (54·5–83·9) for amikacin. The specificity of the assay was 94·3% or greater for all drugs except moxifloxacin, which had a specificity of 84·0% (95% CI 78·9–88·3) at a critical concentration of 2·0 µg/mL.[20]

A large genome-wide association study[21] of MDR and XDR tuberculosis found that the capacity to detect resistance to ethionamide, pyrazinamide, capreomycin, cycloserine, and para-aminosalicylic acid was enhanced by the inclusion of insertions and deletions. This increased capacity suggests simple SNP detection might not be adequate for these drugs and more sophisticated molecular devices might be required. Next-generation sequencing (NGS) and analysis of the whole genome might eventually become the reference standard for drug resistance identification. Reduced costs and the establishment of high-throughput sequencing centres and easy-to-use analytical tools have greatly increased access to NGS, which is being implemented as a routine service in several countries. However, in most high-burden countries it remains a means for research with the analysis undertaken overseas. Proof of principle for NGS directly from sputum has been shown, but for comprehensive analysis the need to first isolate and culture the organism to obtain sufficient bacterial DNA hinders its application to patient management.[22,23]

Tuberculosis diagnostics pipeline

The current diagnostic pipeline is shown in panel 1 and the online appendix. Several online resources track progress in the development of new diagnostics for tuberculosis, including the Treatment Action Group,[24] Unitaid,[14] and a dynamic website established by the Foundation for Innovative New Diagnostics.[25] These resources reveal that most novel technologies reported as in development are not yet close to market, being either studies of feasibility or early validation of prototype

devices.[17] Panel 2 shows the top ten priorities for diagnostics development. Test developers face considerable technical challenges arising from the pathology of tuberculosis. Traditional methods of detecting infectious pathogens via unique biomarkers have been unsuccessful for tuberculosis because of the complex and variable host immune response and the paucity of bacteria in clinical samples. Novel approaches being investigated include testing breath for tuberculosis metabolites and the application of nanotechnology and microfluidics to increase detection capacity and reduce hands-on sample manipulation. Promising results have been reported on the use of new, more sensitive technology to detect lipoarabinomannan[26] and circulating antigen peptides.[27] Technologies favouring measurement of host-response biomarkers will probably require novel platforms that detect multiple proteins. Similarly, antigen detection might require technology capable of assessing molecules of variable size and composition, while remaining both easy to operate and affordable.

New tests are under development that are not based on the detection of nucleic acids (table 2). Competitors for the Xpert MTB/RIF assay are undergoing assessment for regulatory approval and are closest to launch. These competitors are devices designed for low-technology microscopy centres or high-throughput instruments with extended drug resistance detection intended for the reference laboratory. Most tests are sputum-based but some aim to use blood or urine, which might detect extrapulmonary tuberculosis and tuberculosis in children who are unable to expectorate sputum. Devices are now being designed to run on batteries and they aim for robustness that obviates the problems caused by dust and temperature extremes—eg, a portable version of the Xpert MTB/RIF assay, the Xpert Omni. However, unforeseen technical challenges have delayed the development of the product and performance data are awaited. To aid prospective test developers and manufacturers, a web-based compendium of available resources has been compiled. The TB Diagnostics Pathway outlines the steps needed for product development from market research and the development of a business plan through to product launch. The pathway includes target technical profiles for tuberculosis diagnostic tools prioritised by WHO, and it gives details of specimen banks that can provide samples for validation studies.

Advances in communication technologies and cloud-based data management systems have permitted the incorporation of wireless-based and mobile phone-based reporting within diagnostic devices. This connectivity allows automated reporting of results to the health system and enables monitoring to aid quality assurance measures and stock control.[28] The importance of integration across devices and diseases, and the need to maintain confidentiality, has spurred the development of national guidelines in many countries, which test developers need to be cognisant of when designing connectivity software. Although still in its infancy, the new technology is anticipated to revolutionise data collection for monitoring tuberculosis incidence and the emergence of drug resistance.

***Panel 2*: Top ten priorities for tuberculosis diagnostics development**

1. Affordable, accurate, and rapid diagnostic devices for use at the point of care
2. Screening devices for community-based case finding or triaging patients in the clinic
3. Non-sputum-based tests for patients with sputum-scarce samples and extrapulmonary tuberculosis
4. Validated diagnostic tests for tuberculosis in children
5. Rapid tests for drug resistance to guide the treatment of drug-resistant tuberculosis
6. Tests to predict progression from latent tuberculosis infection to active tuberculosis, treatment failure, or reactivation
7. Tests to predict vaccine efficacy, to shorten and reduce costs of assessing new vaccines
8. Independent clinical performance studies to produce evidence for national regulatory approval and WHO endorsement
9. Independent studies to assess effect on health outcomes and on the local health system
10. Technology assessment programmes to assess cost benefits, compare technologies, and assess diagnostic algorithms

	Technology	Target market
Delft Imaging SystemsCAD4TB	Software for reading digital chest images	Community-based screening and health clinics
Otsuka Pharmaceutical LAM ELISA (seeking regulatory approval; not released for sale)	ELISA for LAM detection in sputum	To measure treatment response in drug trials
The eNose Company Aeonose for TB (evaluation studies ongoing, not released for sale)	Breath test using hand-held device for pulmonary and extrapulmonary tuberculosis	Community-based screening and health clinics
Signature Mapping Medical Sciences TbDX	Automated smear microscopy slide reader	Microscopy clinics
Colorimetric Delamanid REMA	Solid culture Delamanid resistance	Reference laboratories
Becton Dickinson MGIT Bedaquiline-Delamanid	Semiautomated liquid culture	Reference laboratories
Salubris Mycolor Tk platform	Colorimetric culture media	Reference laboratories
Thermo Fisher VersaTREK	Liquid culture for detection and susceptibility testing for rifampicin, isoniazid, ethambutol, and pyrazinamide	Reference laboratories
Thermo Fisher Sensititre MYCOTB MIC plate	Culture system to determine MICs for rifampicin, isoniazid, ethambutol, ofloxacin, moxifloxacin, amikacin, streptomycin, rifabutin, para-aminosalicylic acid, cycloserine, kanamycin, and ethionamide	Reference laboratories
Chiang Rai TB/HIV Research Foundation (THRF), Auto-MODS	Automated microscopic observation drug susceptibility assay	Reference laboratories

LAM=lipoarabinomannan. MGIT=mycobacterial growth indicator tube. MICs=minimal inhibitory concentrations.

***Table 2:* New diagnostic tests for tuberculosis not based on detection of nucleic acids**

Host biomarker updates

The measurement of host immune-response molecule concentrations is a complementary strategy to the

For more on the **ReSeqTB Initiative** see https://platform.reseqtb.org/

For more on the **TB Diagnostics Pathway** see www.tbdxpathway.org

detection of intact *M tuberculosis* or its products. The presence of host markers in accessible samples, such as peripheral blood, saliva, or urine, would be of great advantage in detecting paucibacillary disease or extrapulmonary disease, and for patients in whom sputum expectoration is problematic, such as young children. A feature of the adaptive immune response is antigen specificity, in which accelerated and enhanced responses follow previous antigen sensitisation, allowing association of measured responses with specific *M tuberculosis* antigens. Circulating antibodies against pathogen products are readily measurable in ex-vivo blood samples by ELISA or similar assays to reflect humoral immune responses, but performance of serological commercial assays has been poor, leading to a WHO advisory against the use of the available tests.[29]

Cell-mediated immune responses require stimulation of lymphocytes before measurement of changes in the expression of activation markers or effector molecules, such as co-stimulatory cell surface molecules or secreted molecules, including cytokines. Such stimulation assays require substantial laboratory infrastructure and expertise, and they take at least several hours to days to produce results. Examples of such stimulation assays include IGRAs and a range of host marker signatures—eg, host gene expression, protein, metabolic, and other host markers. Although no validated diagnostic tests exist based on these host markers, promising host marker biosignatures have been identified and are under clinical investigation. Measurement of circulating soluble markers other than antibodies from either the innate (antigen non-specific, rapid response group of the immune system) or adaptive immune system is also possible in accessible sample types and has the additional advantage of allowing testing without time-consuming stimulation assays. However, the specificity of such measurements could be an obstacle because responses to many subacute or chronic insults to the immune system considerably overlap. Interpretation of such measurements will have to be within a well defined clinical context. One approach to increase disease relatedness is the use of host marker signatures, rather than single markers.[30] Several host signatures are at early stages of development or trial phase as possible new tools for tuberculosis diagnosis (table 3).

Host gene expression

Several studies have explored the use of host transcriptional biosignatures as diagnostic candidates and biomarkers for prediction of the risk of future development of tuberculosis. Genome-wide transcriptional biosignatures detected in whole blood have been the most promising. Some groups have combined transcriptomic and proteomic approaches to explore progression to active tuberculosis.[48] An 86-gene whole-blood transcriptional *M tuberculosis* signature—predominantly neutrophil-driven type 1 interferon—was reported by Berry and colleagues.[31] The *FCGR1B* gene was highly expressed in patients with tuberculosis, and when in combination with four other genes (*CD64*, [also known as *FCGR1A*] *LTF*, *GBP5*, and *GZMA*), allowed discrimination between tuberculosis and latent tuberculosis infection, with high sensitivity (94%) and specificity (97%) in smaller case-control studies.[49]

Kaforou and colleagues[33] investigated blood transcriptional biosignatures in individuals with active tuberculosis, latent tuberculosis infection, or other diseases, and identified a 44-transcript signature, which distinguished culture-confirmed tuberculosis from other diseases, with a sensitivity of 100% and specificity of 96% in a case-control study. Laux da Costa and colleagues,[32] building on the findings of earlier work,[49] compared the expression profiles of *GZMA*, *GBP5*, and *FCGR1A* (*CD64*) genes in blood samples from patients with tuberculosis, asthma, or non-tuberculosis pneumonia. A combination of the three genes discriminated between active tuberculosis and the other conditions, with a sensitivity of 93% and specificity of 95%.[32] Sutherland and colleagues[34] took the work further, assessing the biomarkers previously identified by Maertzdorf and colleagues[49] and other mRNA transcript signatures in 523 study participants from four different African countries, using the reverse transcriptase multiplex ligation-dependent probe amplification technique. *CD64* was confirmed as a useful marker for tuberculosis irrespective of HIV infection and study site. A four-gene signature, comprising *GBP1*, *IFITM3*, *P2RY14*, and *ID3*, diagnosed tuberculosis with a sensitivity of 88% and specificity of 75%.[35] Bloom and colleagues[50] compared the blood genome-wide transcriptional profiles of patients with tuberculosis to those of patients with sarcoidosis, pneumonia, lung cancer, and healthy controls in a small study. Although transcriptional signatures from patients with tuberculosis and sarcoidosis were significantly more similar to each other than any of the signatures for the other diseases, 144 transcripts distinguished tuberculosis from other diseases with sensitivity of over 80% and specificity of more than 90%.[50]

Anderson and colleagues[51] assessed mRNA transcript signatures in children with suspected tuberculosis from South Africa, Kenya, and Malawi, and compared them with the profiles of children with latent tuberculosis infection and other diseases. A 51-transcript biosignature diagnosed culture-confirmed tuberculosis in the validation sample set, with a sensitivity of 82·9% (95% CI 68·6–94·3) and specificity of 83·6% (95% CI 74·6–92·7). Multiple studies describe mRNA transcript candidate markers for tuberculosis, including in-silico studies on published datasets, and most of the signatures identified in these studies seem promising (accuracy >80%). However, a common limitation in these studies is that most of them, even those undertaken at multiple sites, still used a case-control design and are at most phase 2 diagnostic studies.[52] Most of these studies also have small

	Platform (sample type)	Sensitivity	Specificity	Diagnosis
Host gene expression				
Neutrophil-driven type 1 interferon, interferon-γ (393 genes), and type 1 interferon-α or interferon-β (86 genes)[31] gene signatures	Illumina microarray, RT-qPCR validation (blood)	61·7%*, 94·1%†, 92%	93·7%*, 96.6%†, 83%	Tuberculosis, LTBI, healthy controls
FCGR1B, *CD64* (*FCGR1A*), *LTF*, *GBP5*, and *GZMA*[32]	Agilent microarray (blood)	94%	97%	Tuberculosis, LTBI
Gene signature (44 genes)[33]	Illumina microarray (blood)	100%	96%	Tuberculosis, LTBI, other disease
GZMA, *GBP5*, and *CD64* (*FCGR1A*)[30]	RT-qPCR (blood)	93%	95%	Tuberculosis, asthma, non-tuberculous pneumonia
CD64 (*FCGR1A*)[34]	MLPA technique	88%	75%	Tuberculosis
Gene signature (144 genes)[35]	Illumina microarray (blood)	>80%	>90%	Tuberculosis, lung cancer, pneumonia, healthy controls
Gene signature (51 genes)[36]	Illumina microarray (blood)	82·9%	83·6%	Tuberculosis, other lung disease
Gene signature (16 genes)[30]	Illumina RNA sequencing (blood)	66·1%*, 53·7%†	80·6%*, 82·8%†	Tuberculosis progression
Host protein markers				
CRP[37]	POC fluorescent scanner	89%	72%	Tuberculosis and in participants who were HIV-positive
CRP, transthyretin, interferon γ, CFH, ApoA1, IP10, SAA[38]	Multiplex cytokine platform (serum)	93·8%	73·3%	Tuberculosis, pulmonary disease
SYWC, kallistatin, CC9, gelsolin, testican-2, aldolase-C[39]	SOMAscan aptamer proteomics (serum)	90%	80%	Tuberculosis
Metabolomic markers				
Ketone bodies, lactate, pyruvate metabolites[40]	NMR spectroscopy (plasma)	NR	NR	Tuberculosis, healthy controls, diabetes, pneumonia
IDO-1, phospholipase, adenosine metabolites[41]	GCMS (serum)	NR	NR	Tuberculosis, LTBI, healthy controls
Fatty acids, mycolic acids, carbohydrates[42]	GCTOFMS (sputum)	NR	NR	Tuberculosis, pulmonary disease
Glycolipids, resolvins, glutamate, choline metabolites[43]	LCMS (plasma)	NR	NR	Tuberculosis, tuberculosis household contacts
Ceramide, cholesterol sulphate, eicosatetraenoic acid, 4α-formyl-4β-methyl-5α-cholesta-8-en-3β-ol[44]	UHPLC–ESIQTOFMS (plasma)	>70%	>80%	Tuberculosis, pneumonia
Host microRNA				
hsa-miR-196b and hsa-miR-376c[45]	Illumina sequencing (serum)	NR	NR	Tuberculosis, LTBI, healthy controls
hsa-miR-150, hsa-miR-21, and hsa-miR-29c[46]	microRNA microarray, RT-qPCR validation (blood)	91%	88%	Tuberculosis, LTBI, healthy controls
miR-769-5p, miR-320a and miR-22-3p[47]	Illumina sequencing, RT-qPCR validation (plasma)	NR	NR	Tuberculosis, healthy control

RT-qPCR=quantitative PCR. LTBI=latent tuberculosis infection. MLPA=multiplex ligation-dependent probe amplification. CRP=C-reactive protein. POC=point-of-care. CFH=complement factor H. ApoA1=apolipoprotein A1. IP10=interferon-γ inducible protein. SAA=serum amyloid A. CC9=complement component 9. NMR=nuclear magnetic resonance. NR=not reported. IDO-1=indoleamine 2,3-dioxygenase 1. GCMS=gas chromatography mass spectrometry. GCTOFMS=gas chromatography time-of-flight mass spectrometry. LCMS=liquid chromatography high-resolution mass spectrometry. UHPLC–ESIQTOFMS=ultra-high-performance liquid chromatography–electrospray ionisation-quadrupole time-of-flight mass spectrometry. *Test cohort. †Validation cohort.

Table 3: **Host biomarkers discriminating individuals with tuberculosis disease**

sample sizes and have been undertaken at single study sites because of the high costs involved in properly designed multisite phase 3 diagnostic studies, including the relatively high costs of RNA sequencing followed by RT-PCR validation. Therefore, validation is needed, in multiple settings, of the candidate transcript signatures identified in prospectively recruited patients with suspected tuberculosis.[52] Such approaches would also have to be done on rapid test platforms that would require minimum training, preferably laboratory-free inexpensive technology, to be considered of value in high-burdened, resource-constrained settings.

A study of South African adolescents by Zak and colleagues[30] identified a 16-gene transcript signature, which discriminated between adolescents who would subsequently progress or not progress to active tuberculosis in a high incidence setting. The signature had a sensitivity of 66·1% (95% CI 63·2–68·9) and specificity of 80·6% (95% CI 79·2–82·0), with enhanced sensitivity closer to the time of tuberculosis diagnosis. When samples from South African and Gambian household contacts of people with active tuberculosis were assessed, the sensitivity of the correlate of risk (CoR) signature was 53·7% (42·6–64·3) with a specificity of 82·8% (76·7–86·0), on samples that were collected 12 months before the development of active tuberculosis. This performance exceeds the predictive performance of IGRAs or the tuberculin skin test by far. The effect of biomarker-driven preventive treatment of CoR-positive individuals is the subject of an ongoing clinical trial (NCT02735590).

Host protein markers

IGRAs are not useful in high-burden settings because of the high prevalence of latent tuberculosis infection and the inability of the assays to discriminate between latent tuberculosis infection and active tuberculosis. Host markers other than interferon-γ that are produced in response to new or alternative *M tuberculosis* antigens have been investigated. Although multiple antigens and host markers showing potential have been identified,[38] the performance of tests based on (often overnight) stimulation assays did not warrant the longer lag time to a result and were not suitable for point-of-care rapid diagnostic tests. Yoon and colleagues[37] reported on a point-of-care C-reactive protein (CRP) finger-prick test as a screening tool for tuberculosis in individuals with HIV, with CD4 counts of up to 340 cells per mL, who were initiating antiretroviral therapy in Uganda. This test diagnosed culture-confirmed tuberculosis with a sensitivity of 89% and specificity of 72%.[37] However, CRP in combination with six other proteins, diagnosed tuberculosis in individuals with suspected pulmonary tuberculosis in field sites situated in five African countries, with a sensitivity of 93·8% and specificity of 73·3%, with CRP as a single marker achieving the best result in individuals infected with HIV.[53] A six-protein biosignature was also discovered through SOMAscan technology as a screening tool for tuberculosis, with a sensitivity of 90% and specificity of 80% in more than 700 samples from various tuberculosis-endemic settings.[39] Diagnostic tests are reported to be in development that are using various combinations of the reported markers,[53] but data are not yet available on their performance.

Metabolomic markers

Various platforms, including nuclear magnetic resonance (NMR) spectroscopy, gas chromatography time-of-flight mass spectrometry (GCTOFMS), liquid chromatography high-resolution mass spectrometry (LCMS), and ultra-high-performance liquid chromatography–electrospray ionisation-quadrupole time-of-flight mass spectrometry (UHPLC–ESIQTOFMS) have been used to detect small metabolites that can differentiate between tuberculosis and other diseases. Although candidate metabolites and pathways have emerged, the work done so far has been in mostly small case-control studies, and the diagnostic potential of these candidate metabolites is yet to be validated in large studies. With NMR spectroscopy, Zhou and colleagues[54] assessed differences in the serum metabolic profiles of patients with tuberculosis and healthy controls, building on earlier findings[41] of several differentiating metabolites identified between tuberculosis, latent tuberculosis infection, and healthy controls. Zhou and colleagues[54] identified 30 metabolites, 17 of which had a higher expression in serum samples from patients with tuberculosis than in controls. In a follow-up study,[40] they identified ketone bodies, lactate, and pyruvate as metabolites with the highest potential for discriminating between tuberculosis and other conditions, including community-acquired pneumonia, diabetes, and various malignancies. In a proof-of-concept study,[42] an untargeted metabolomics approach was used to investigate host metabolites in sputum samples using two-dimensional GCTOFMS. Although variability was high in the metabolites identified in samples from different patients, candidate metabolites, including fatty acids, mycolic acids, and carbohydrates, showed potential as biomarkers. Other metabolites identified using LCMS included *M tuberculosis*-derived glycolipids and resolvins in plasma samples[43] and urinary metabolites, which showed potential as markers for monitoring of tuberculosis treatment.[55] Combinations between four plasma metabolites discriminated between patients with tuberculosis and controls, including patients with pneumonia (identified by UHPLC–ESIQTOFMS) with sensitivities of more than 70% and specificities of more than 80%.

More metabolic biomarkers are continuously being identified in plasma samples—eg, metabolites that are involved in the glucose, lipid, and aminoacid metabolism pathways.[56] Although findings from these studies continue to provide useful information about our understanding of the host metabolic response to infection with *M tuberculosis*, and they ultimately help to shed more light on the intracellular survival of *M tuberculosis*,[57] the potential of these approaches as tuberculosis diagnostics still has to be confirmed. Validated signatures will also have to be translated from mass-spectrometry platforms into tools that are appropriate for resource-constrained, high-burden settings.

microRNA

Many investigators have studied the role of microRNAs (miRNAs) as possible diagnostic biomarkers for tuberculosis. Zhang and colleagues[45] assessed serum

miRNA signatures discriminating between active tuberculosis, latent tuberculosis infection, and healthy controls in a small sample size of 15 patients with tuberculosis and 82 controls. They identified different miRNAs that were upregulated (24) or downregulated (six) in patients with tuberculosis, and although two of these miRNAs (hsa-miR-196b and hsa-miR-376c) showed potential as tuberculosis diagnostic markers after validation by RT-PCR, the small sample size and absence of validation cohort limits the global applicability of the study's findings. In a similar study[46] (ie, blood miRNA signatures in patients with tuberculosis, latent tuberculosis infection, and healthy controls) using microarrays and validated by RT-PCR, a three-marker miRNA signature diagnosed active tuberculosis (tuberculosis *vs* latent tuberculosis infection controls), with a sensitivity of 91% and specificity of 88%. In another study,[47] 29 miRNAs were differentially expressed between patients with tuberculosis and controls, with three of the miRNAs discriminating between patients with tuberculosis and control patients, with areas under the curve between 0·69 and 0·97 after validation by RT-PCR. All these small case-control studies require validation in larger cohort studies.

Medical imaging

Chest radiography can serve as a triaging or screening tool for pulmonary, miliary, pleural, and pericardial tuberculosis and can potentially close the case-detection gap when used in appropriate algorithms. It can identify patients in need for bacteriological examination and can provide important information when bacteriological confirmation is unhelpful. However, chest radiographs are not widely available in resource-constraint settings. Radiography, which made a major contribution to the eradication of tuberculosis in well resourced countries of Europe and North America, has become a more feasible option in settings with poor infrastructure, with the introduction of digital systems. Software for automated reading is available, which provides high throughput with reduced reliance on radiologists, and the technology is being used to triage patients during case finding and to assist with prevalence surveys.[17] More sophisticated imaging methods, such as CT, offer enhanced sensitivity, and fluorodeoxyglucose PET-CT can be used for assessing treatment response, but such specialised instrumentation remains beyond reach for most patients with tuberculosis.[58]

Challenges and needs

Improved tools for case finding and detection of drug resistance for tuberculosis remain top priorities of WHO and the Stop TB Partnership. Progress is being made, but some key research questions for the development of priority diagnostic tests remain to be resolved (table 4).

Validation and assessment of new technologies is ongoing, with more tests in the pipeline. The ideal test should provide both high sensitivity and specificity, which is not always possible and compromises might have to be made. The accuracy of a new test should be non-inferior to the tests already available, but lower sensitivity might be acceptable for technologies that increase case finding as a result of their suitability for use in communities with poor access to traditional clinic or laboratory-based health services. High specificity is required if a test is used to initiate antituberculosis therapy, but lower specificity can be tolerated when triaging patients or during community-based screening. Target product profiles published by WHO propose a specificity of 98% for a diagnostic test but a minimum of 70% if follow-up confirmatory testing will be undertaken.[59] The reported biomarker studies suggest that it might be possible to reach the desired performance targets, but for maximum performance multiple markers need to be examined.

Test developers face two major challenges when developing novel biomarker-based tests. First, commercial pressures might prevent selection of the optimum panel of biomarkers. Patenting of biomarkers is common practice and competition to be the first-to-market is a powerful disincentive to cooperation. Second, multiple markers are measured across a broad range of concentrations or in very low concentrations. The detection platforms used are expensive and unsuitable for use at the point of need in countries with a high burden of tuberculosis. When asked how much it would cost to acquire or develop a new detection platform for a novel diagnostic test, industry representatives provided estimates of between $3 million and $20 million.[36] This cost was in addition to estimated research costs of between $3 million and $8 million. Thus, although the long-term prospects for biomarker-based diagnosis of tuberculosis appear positive, these badly needed tests will only be attained if sustained funding is available.

Aside from the biological and technical challenges of creating novel diagnostic devices for tuberculosis, considerable operational, economic, and organisational barriers need to be overcome. Production and adoption of a novel tuberculosis test (figure) might take more than 10 years and cost in excess of $100 million. Preparation of a new test for market entry might cost more than was spent on developing the device. Studies of test performance must be undertaken in populations of intended use to provide evidence for regulatory approval and WHO endorsement. The absence of a credible gold standard for tests that detect extrapulmonary forms of tuberculosis is a considerable hindrance. Reliance on sputum-based tests is not appropriate and capacity for diagnosing extrapulmonary tuberculosis needs to increase to enable accurate estimates of test accuracy.

Previously, few diagnostic options have been available for the beleaguered tuberculosis programmes to choose from, but a range of tests and technologies will soon be available that appear promising. Shelf life and cost will remain key parameters in decision making, but other

	Prospective test	Challenges
What is the minimum biomarker panel for diagnostic use?	Accurate diagnosis of all forms of tuberculosis, including in children	Requires cooperation between research groups and large multicentre studies
What is the minimum biomarker panel for a screening test?	Rapid screening test to facilitate active (community-based) case finding and triaging of patients seeking diagnosis	Requires cooperation between research groups and large multicentre studies
Does *Mycobacterium tuberculosis* lineage affect the predictive value of tuberculosis biomarkers?	Diagnostic tests for global use	Systematic studies are needed
How many mutations should be incorporated in a test to guide treatment for MDR tuberculosis?	A rapid test to guide treatment for MDR tuberculosis	To cover all drugs used to treat MDR tuberculosis
How many mutations should be incorporated in a test to guide treatment for XDR tuberculosis?	A rapid test to guide treatment for XDR tuberculosis	To cover all available antituberculosis drugs
In which settings do the new imaging technologies provide cost-effective screening for active tuberculosis?	Rapid, affordable, non-invasive screening for tuberculosis	Technology assessment programme to include impact studies and cost-benefit modelling

MDR=multidrug-resistant. XDR=extensively drug-resistant.

Table 4: **Research questions for development of priority tuberculosis diagnostic tests**

Search strategy and selection criteria

We searched reports published in English between Jan 1, 2013, and Feb 15, 2018, in PubMed, Google Scholar, Cochrane Library, Embase, and websites of tuberculosis-related international organisations (WHO, FIND, Stop TB Partnership, Treatment Action Group, TB Alliance, Consortium for TB biomarkers [CTB2], National Institute of Health–National Institute of Allergy and Infectious Disease). We use the search terms "tuberculosis", "*Mycobacterium tuberculosis*", "latent TB" plus "diagnostics", or "biomarker", "gene expression", "micro-RNA", "proteomics", "metabolomics", "imaging", "interferon gamma release", or "clinical trial". We reviewed studies cited by articles identified in this search strategy and selected those that were relevant. We collated and synthesised information on the development of new tuberculosis diagnostics and biomarkers through communications with various stakeholders. Some review articles are cited to provide readers with more details and references than this Series paper can accommodate.

considerations include accuracy, reliability, and sample throughput capacity. Test performance, predictive value, and cost-effectiveness might vary by geographical setting or level of health system due to differing local environmental, demographic, and epidemiological factors. Few data have been published that compare test performance and even less that cover the potential effect and cost benefits. What little data there are often come from studies involving the developer of the test, and one of the greatest challenges facing the tuberculosis community is how to fund and organise independent assessment of new technology to maximise the benefits for tuberculosis control.

Conclusions

Reliance on passive case finding of infectious cases has not stopped the pandemic, and improved diagnostic technology will not reduce transmission by itself unless interventions are implemented to enable earlier detection and treatment. To eradicate the disease, active case-finding and screening strategies will be needed and community involvement will be crucial, particularly in less well resourced countries. To implement these new methods, national tuberculosis control programmes and their supporting donor agencies, who have long advocated clinic-based detection, will be required. In their Tuberculosis Control Report of 2017,[1] WHO noted that insufficient gains have been made and the Stop TB Partnership continues to call for increased political commitment and increased funding. Renewed hope for advancing diagnostic and biomarkers research and the clinical development portfolio comes from the first Ministerial Meeting on Tuberculosis held in Moscow, Russia (November, 2017); delegations from 128 countries committed to fundamentally transform the fight against tuberculosis and to pursue a series of actions at a national and international level to create research-enabling environments and to boost tuberculosis research.[60]

Contributors

AZ, RM, and GW initiated the idea and developed the first draft outline. Subsequent drafts were developed by RM, AZ, TDM, MB, GW, NNC, and NdP. All authors contributed to all sections relevant to their experience and helped finalise the text and content.

Declaration of interests

We declare no competing interests.

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Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Appendix

Accompanying Lancet ID article:

Title: Tuberculosis: Advances and challenges in development of new diagnostics and biomarkers

Authors:

Gerhard Walzl PhD, Ruth McNerney* PhD, Nelita du Plessis* PhD, Matthew Bates PhD, Timothy D McHugh PhD, Novel N Chegou* PhD and Alimuddin Zumla FRCP

Links to online WHO policy documents

The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV 2015 Policy update
http://www.who.int/tb/publications/use-of-lf-lam-tb-hiv/en/ -accessed December 18th 2017

Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children.
http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf?ua=1 -accessed December 18th 2017

High-priority target product profiles for new tuberculosis diagnostics
Report of a consensus meeting
[http://www.who.int/tb/publications/tpp_report/en/]. -accessed December 18th 2017

Chest Radiography in Tuberculosis Detection
Summary of current WHO recommendations and guidance on programmatic approaches
http://apps.who.int/iris/bitstream/10665/252424/1/9789241511506-eng.pdf?ua=1

Gli Quick Guideto Tb Diagnostics Connectivity Solutions
http://www.stoptb.org/wg/gli/assets/documents/gli_connectivity_guide.pdf -accessed December 18th 2017

TBXpert: Innovative diagnostics for multi-drug resistant tuberculosis (MDR-TB)
https://unitaid.eu/project/tbxpert/#en -accessed December 18th 2017

Appendix Table 1

Product	Technology		Target market	Further information
Delft Imaging Systems CAD4TB	Software for reading digital chest images		Community based screening and health clinics.	http://www.delft.care/cad4tb
Otsuka Pharmaceutical Co., Ltd LAM ELISA	ELISA for Lipoarabinomannan detection in sputum		To measure treatment response in drug trials	http://www.resisttb.org/wp-content/uploads/2017/06/Otsuka-LAM-test_Resist-TB-Webinar_06-22-2017.pdf Seeking regulatory approval. Not released for sale.
The eNose Company Aeonose for TB	Breath test using hand held device for pulmonary and extrapulmonary tuberculosis		Community based screening and health clinics.	http://www.enose.nl/clinical-results/tuberculosis/ Evaluation studies ongoing, not released for sale
Signature Mapping Medical Sciences Inc. TbDX	Automated smear microscopy slide reader		Microscopy clinics	http://www.appliedvs.com/technology.php?id=infectious
Colorimetric Delamanid resazurin microtitre assay (REMA)	Solid culture Delamanid resistance		Reference laboratories	https://academic.oup.com/jac/article/71/6/1532/1751769/Delamanid-susceptibility-testing-of-Mycobacterium
Becton-Dickinson MGIT Bedaquiline-Delamanid	Semi automated Liquid culture		Reference laboratories	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4468675/pdf/zac4352.pdf
Salubris Mycolor TK platform	Colorimetric culture media		Reference laboratories	http://salubrisinc.com/new/product/tk-slc-growth-medium/
Thermo Fisher VersaTREK	Liquid culture for detection and Susceptibility Testing for RIF INH ETH and PZA		Reference laboratories	http://www.trekds.com/products/versatrek/mdst.asp
Thermo Fisher Sensititre MYCOTB MIC plate	Culture system to determine MICs for OFLOX, Moxi,RIF, AMK, SM, Rifabutin PAS, EMB, CYS, INH, KAN, ETH		Reference laboratories	http://www.trekds.com/products/sensititre/c_pltformats.asp

Appendix Table 2

Product	Intended use	Further information
Abbott Laboratories RealTime MTB RIF/INH	RIF and INH	https://www.molecular.abbott/int/en/products/infectious-disease/realtime-mtb-rif-inh-resistance
Autoimmun Diagnostika (AID)TB Resistance Module	RIF and INH	https://www.aid-diagnostika.com/en/kits/molecular-genetic-assay/infections-diseases/antibiotic-resistences/tb-isoniazid-rifampicin/
CapitalBio Technology Tuberculosis Drug Resistance Detection Array Kit	RIF and INH	http://www.capitalbiotech.com/en/products-content.html?id=67
Cepheid Xpert Ultra Cartridge	RIF resistance (a cartridge for additional drugs is in development)	http://www.cepheid.com/en/cepheid-solutions/clinical-ivd-tests/critical-infectious-diseases/xpert-mtb-rif-ultra
Epistem Ltd. Genedrive MTB/RIF ID Kit	RIF	https://www.genedrive.com/assays/mtb-assay.php
Hain Lifescience GmbH FluoroType MTBDR VER 1.0	RIF and INH	http://www.hain-lifescience.de/en/products/microbiology/mycobacteria/tuberculosis/fluorotype-mtbdr.html
Hain Lifesciences Geno**Type** MTBDR*plus* VER 2.0 Geno**Type** MTBDR*sl* VER 1.0/2.0	Line probe assays for RIF and INH, and aminoglycosides/cyclic peptides and EMB.	http://www.hain-lifescience.de/en/products/microbiology/mycobacteria/tuberculosis.html
Molbio Diagnostics Truenat MTB	RIF	http://www.molbiodiagnostics.com/products_microchips.html
Nipro Co. Genoscholar	Line probe assays for RIF and INH, or PZA or FLQ and KAN	http://www.nipro-europegroupcompanies.com/products/ivd-assays/
QuanDx MTB drug-resistant Mutation Test Kits:	RIF and INH EMB, SM and FLQ . For research use.	http://www.quandx.com/infectious-diseases/tb-drug-resistant-mutation-test-kits/
Seegene Inc.Anyplex assays for MDR detection	RIF and INH	http://www.seegene.com/neo/en/products/tuberculosis/anyplex2_MTB_MDR.php
Seegene Anyplex™ II MTB/XDR Detection	FLQ and SLID	http://www.seegene.com/neo/en/products/tuberculosis/anyplex2_MTB_XDR.php
YD Diagnostic MolecuTech REBA MDR and XDR	Line probe assays RIF INH, FLQ, KAN and SM	http://www.yd-diagnostics.com/2012/eng/channel_02/prt_list.php
Zeesan Biotech MeltPro MTB (MDR-TB, XDR-TB) kits	RIF, INH, ETH, FLQ, and SLID	http://www.zeesandx.com/infectious-disease