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Diagnostics Technology and Market Landscape

3RD EDITION

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Abbreviations

ADA	adenosine deaminase
AFB	acid fast bacteria
AIDS	acquired immune deficiency syndrome
AMG	aminoglycoside
AMTD	amplified Mycobacterium tuberculosis direct
ART	antiretroviral therapy
BED	bedaquiline
BRICS	Brazil, Russian Federation, India, China and South Africa
°C	degrees Celsius
CAD	computer-aided diagnosis
CE-IVD	European conformity (Conformité Européenne)-in vitro diagnostic
CFDA	China Food and Drug Administration
CFX	ciprofloxacin
CHAI	Clinton Health Access Initiative
CMOS	complementary metal oxide semiconductor
CO_2	carbon dioxide
CPA	cross-priming amplification
CPTR	Critical Path to Tuberculosis Drug Regimens
CRI	colorimetric redox indicator assay
cSML	circular Surrogate Marker Locus
CXR	chest X-ray
DNA	deoxyribonucleic acid
DST	drug susceptibility testing
ELISA	enzyme linked immunosorbent assay
EMB	ethambutol
EPTB	extrapulmonary TB
EQA	external quality assurance
FIND	Foundation for Innovative New Diagnostics
FISH	fluorescence in situ hybridization
FM	fluorescence microscopy
FLQ	fluoroquinolone
Global Fund	Global Fund to Fight AIDS, Tuberculosis and Malaria
GHIF	Global Health Investment Fund
HBC	high-burden country
HIV	human immunodeficiency virus
IGRA	interferon-gamma release assay
IQR	interquartile range
IFN-γ	interferon-gamma
INH	isoniazid
IPAQT	Initiative for Promoting Affordable, Quality Tests
IPT	isoniazid preventative therapy
IRISA™-TB	InterGam Ultrasensitive Rapid Immuno-suspension Assay
ISTC	International Standards for TB Care
IVD	in vitro diagnostics
KGI	Keck Graduate Institute
KM	kanamycin
L	litre
LAM	lipoarabinomannan
LAMP	loop-mediated amplification
LATE	linear-after-the-exponential
LED	light emitting diode
L-J	Löwenstein-Jensen
LPA	line probe assay
LTBI	latent TB infection
MDR	multidrug resistant
MGIT™	mycobacterial growth indicator tube
MIC	minimum inhibitory concentration
µL	microlitre
mL	millilitre
ML	mini laboratory
MODS	microscopically observed drug susceptibility

MTB	Mycobacterium tuberculosis
MTBC	Mycobacterium tuberculosis complex
NAAT	nucleic acid amplification test
NaOH	sodium hydroxide
NASBA	nucleic acid sequence-based amplification
NDWG	New Diagnostics Working Group
NEAR	nicking enzyme amplification reaction
NGS	next-generation sequencing
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health (USA)
NRA	nitrate reductase assay
NTM	non-tuberculous mycobacteria
NTP	national tuberculosis programme
NWGHF	North Western Global Health Foundation
O_2	oxygen
PCR	polymerase chain reaction
PEPFAR	US President's Emergency Plan for AIDS Relief
PLHIV	people living with HIV/AIDS
PNA	peptide nucleic acid
POC	point of care
PNB	para-nitrobenzoic acid
PTB	pulmonary TB
PZA	pyrazinamide
RDT	rapid diagnostic test
RIF	rifampicin or rifampin
RNA	ribonucleic acid
rRNA	ribosomal RNA
sCD	soluble cluster of differentiation
SDA	strand displacement amplification
SSM	sputum smear microscopy
STAG-TB	Strategic and Technical Advisory Group for Tuberculosis
STR	streptomycin
TAM-TB	T-cell activation marker–tuberculosis
TB	tuberculosis
TMA	transcription-mediated amplification
TLA	thin layer agar
TPP	target product profile
TRC	transcription-reverse transcription concerted
TST	tuberculin skin test
US	United States
US FDA	United States Food and Drug Administration
USA	United States of America
USAID	United States Agency for International Development
VOC	volatile organic compound
WHO	World Health Organization
XDR	extensively drug resistant
ZN	Ziehl-Neelsen

Foreword

Tuberculosis (TB), despite being a curable disease, continues to be a major public health threat: the World Health Organization (WHO) estimated that 1.3 million people died of the disease in 2012. Rapid, accurate diagnosis is critical for timely initiation of treatment and, ultimately, control of the disease. But of the 8.6 million people who developed TB in 2012, over one third were not diagnosed—an estimated 3 million missed cases. Lack of access to appropriate diagnostic tools is caused, in part, by shortcomings in TB diagnostics markets. For example, currently available diagnostics are often ill-adapted to resource-limited settings or specific patient needs, or may be priced out of reach.

Although TB diagnosis often still relies on basic tools, such as smear microscopy and culture, new diagnostics are changing the TB diagnostics landscape. Several technologies have been endorsed by WHO and incorporated into country policies since 2007. Further change can be expected, with a robust technology pipeline promising new products and continued development to address persisting unmet needs.

This report reviews current and potential future technologies, as well as critical market issues, to identify market-based approaches to alleviate market shortcomings and, ultimately, access issues related to TB diagnostics. For example, potential opportunities may include efforts to accelerate market entry for innovative TB diagnostics that address unmet needs, and to engage private-sector care providers to increase access to appropriate diagnostic tools.

Executive summary

Public health problem and access issues related to diagnostics

The World Health Organization (WHO) estimates that, in 2012 alone, 8.6 million people fell ill with tuberculosis (TB) and 1.3 million died from the disease. Rapid, accurate diagnosis is critical for timely initiation of treatment, but many people with TB do not have access to adequate initial diagnosis. In 2012, only 5.7 million newly diagnosed TB cases were notified to national TB programmes (NTPs). The remaining 3 million cases were either not diagnosed, or not notified to TB programmes. That is, about one third of all TB cases were missed.

Access issues in people with multidrug-resistant (MDR) TB and in children are even more pronounced. Globally, in 2012, WHO estimates that 450 000 people developed MDR TB; of these, 170 000 people died. It is estimated that less than one in four cases of MDR TB was detected in 2012. Also, in 2012, an estimated 530 000 children became ill with TB and 74 000 HIV-negative children died of TB, but the true case burden of paediatric TB is likely higher than estimated. Childhood TB is notoriously difficult to diagnose, and most conventional TB tests perform poorly in this vulnerable population.

TB diagnostics technology and market landscape

This TB diagnostics technology and market landscape highlights current and emerging diagnostic technologies from over 80 manufacturers and developers. Diagnostic products reviewed in this report include those for chest radiology, smear microscopy, culture, tests for latent TB infection (LTBI) and nucleic acid amplification tests (NAATs). In addition, existing and novel biomarker-based technologies are described, along with advances in next-generation sequencing (NGS) methods.

Overall, the TB diagnostics technology landscape is promising, with many product developers and a robust pipeline of technologies. The range of technologies that may replace smear microscopy continues to expand, and smaller, simpler, more robust and portable products expected in the next two to three years herald a more competitive, decentralized market. Several fully integrated tests aim to deliver results in less than one hour, and also incorporate markers of drug resistance; this should improve time to treatment, enable point-of-care (POC) testing programmes and support greater access to drug susceptibility testing (DST). However, lack of sufficient field evidence deters widespread uptake of next-generation NAATs. Current assessments and limited published data have proven inadequate; more performance data are needed to inform NTP policies.

In the medium term, the need for a biomarker-based, low-cost, non-sputum-based test remains a key priority for TB diagnostics beyond the microscopy centre where the majority of people first seek care. Although biomarker discovery is an active area and several potential products (e.g. antigen or antibody detection tests; volatile organic compounds [VOCs]; enzymatic detection) are under development, no test in development is likely to be on the market with policy endorsements within the next three to five years.

With the expected introduction of new TB drug regimens within two to three years, new technologies are also needed for rapid detection of drug resistance to existing as well as new drugs.

Market landscape

Globally, the scale-up of GeneXpert® MTB/RIF (Xpert® MTB/RIF) continues to be the most important, measurable shift in the TB diagnostics market, with over 7.5 million Xpert® MTB/RIF cartridges procured in the public sector in 108 of the 145 countries eligible for concessional pricing. However, data suggest that most NTPs use Xpert® for selected patients at risk of resistance or co-infection with HIV, and not for early case detection in all patients with presumed TB. In fact, most high-TB burden countries still rely on sputum smear microscopy (SSM) as the primary, and often, sole diagnostic test. A recent study showed that 22 high-burden countries (HBCs) performed a total of 77.6 million sputum smears annually at a value of US$ 137 million in 42 827 microscopy centres.

While the Xpert® MTB/RIF assay is a much needed breakthrough, it was not designed to reach lower tiers of the healthcare system, and not intended to meet all needs. High cost is also a hurdle for underfunded NTPs. A recent study of various stakeholders helped establish the most important unmet needs, and helped identify priority target product profiles (TPPs). A rapid, sputum-based, molecular test for microscopy centres (with the option of an add-on DST cartridge) was ranked as highest priority, followed by a rapid biomarker-based, instrument-free test for non-sputum samples. Efforts are ongoing to quantify the potential market value for each priority TPP, and to estimate the current, served available market for TB tests in priority countries.

Market shortcomings related to TB diagnostics

Market shortcomings related to TB diagnostics include issues of availability, affordability, quality, acceptability/adaptability and delivery. For example, there is no true, instrument-free, inexpensive POC TB diagnostic test for use in peripheral settings. While Xpert® MTB/RIF offers rapid diagnosis in decentralized settings, the test is still expensive. Current diagnostics are not adapted for specific patient groups or decentralized health-care settings, and limited (or no) information on the quality of diagnostics is available to guide procurement. Inappropriate tests are commonly used, particularly in the unregulated private sector where WHO-endorsed tests are often very expensive. Also, emerging data suggest that the impact of Xpert® MTB/RIF on TB transmission and mortality may be limited because of widespread empiric therapy, weak health systems and lack of adequate linkages between diagnosis and treatment/follow-up.

Potential opportunities for TB diagnostics market interventions

Potential market-based interventions related to TB diagnostics may include efforts to accelerate market entry for innovative TB diagnostics that address unmet needs. Where critical for access, work may include support for manufacturing scale-up or demonstration of field performance to inform policy. Given the importance of the private sector in many HBCs, potential interventions that engage private-sector care providers could also be critical for addressing global access issues related to TB diagnostics.

1. Introduction

The UNITAID Tuberculosis Diagnostics Technology and Market Landscape is published annually and is prepared as part of a broad and ongoing effort to understand the technology and market landscape for tuberculosis (TB) diagnostics. The first and second editions of the landscape report were published in July 2012 and July 2013, respectively, with semi-annual updates published in December 2012 and December 2013. These documents are available at http://www.unitaid.eu/en/resources/publications/technical-reports.

This document, the third edition of the report, is intended to complement these earlier reports, stimulating discussion and informing potential opportunities for market intervention to improve access to effective TB diagnostics. To serve this purpose, this report:

- reviews the public health problem of TB and critical access issues related to TB diagnostics (sections 3 and 4);
- presents a comprehensive overview of TB diagnostic technologies that are commercially available or close to market (section 5);
- analyses the market landscape, including ongoing efforts to identify and define high priority target product profiles (TPPs) for new TB tests, quantify market potential, and improve access to World Health Organization (WHO)-endorsed TB tests in the private sector (section 6);
- describes major market shortcomings related to TB diagnostics (section 6.4) and presents opportunities for market-based intervention to address these shortcomings (section 6.5).

A dynamic understanding of existing and forthcoming technologies is key for UNITAID in facilitating access to appropriate TB diagnostic tools through market-based interventions. As such, this landscape is intended to be a living document, updated as the TB diagnostics market evolves, to highlight potential opportunities for market-based interventions to improve access to effective TB diagnostic commodities.

2. Methodology

The Tuberculosis Diagnostics Technology and Market Landscape, 3rd edition (2014) was developed by David Boyle (PATH, Seattle) and Madhukar Pai (McGill University, Montreal). Focused technical input was provided by the Foundation for Innovative New Diagnostics (FIND), Geneva. Additional assistance was provided by UNITAID and Carole Jefferson (independent consultant). The material in this landscape report was gathered by the authors from primary sources (e.g. surveys and interviews with technology developers; targeted analyses where needed) and extensive review of secondary sources (e.g. published and unpublished reports; WHO policies and systematic reviews; corporate prospectuses; developer websites).

The technologies described in this document were identified by continued outreach to known diagnostic manufacturers and technology developers with questionnaires addressing their technology, target population(s), intended market, pricing and national or regional regulatory approvals and manufacturing standards. The authors continually review peer-reviewed literature to identify new technologies, assays or validation studies on existing tools to update the landscape reports. The assistance of FIND in the drafting of this document further increased the scope and number of developers approached for product/market information. With the dissemination of UNITAID reports since 2012, diagnostics developers also now approach the authors with product information to be included in the landscape.

While information on cost per test or device and intended markets is provided solely at the discretion of the manufacturer, performance data of any product described in this manuscript are derived only from independent studies that have been published in peer-reviewed literature in an attempt to validate the veracity of claims regarding test accuracy.

All images have been reproduced with permission from the respective companies or agencies. In particular, materials from the following published articles by the authors were adapted, with permission from the authors and copyright holders:

- Kik SV et al.[1] Tuberculosis diagnostics: which target product profiles should be prioritised?
- Kik SV et al.[2] Replacing smear microscopy for the diagnosis of tuberculosis: what is the market potential?
- Kik SV et al.[3] Optimal diagnosis: how early and improved diagnosis can help prevent TB transmission.

2.1. Acknowledgements and conflicts of interest

The authors and UNITAID are grateful to all the industry representatives who shared information (and images) on their products, and also acknowledge technical input from FIND, Geneva, and Carole Jefferson, independent consultant for UNITAID, on sections 5 and 6. Industry and FIND contributions were technical in nature; section 6.5, on potential opportunities for market intervention, was developed independently by authors David Boyle and Madhukar Pai with support from UNITAID.

David Boyle currently holds a grant from FIND to assess the performance of the LoopAMP™ MTBC detection kit in a field setting and is a consultant to the Keck Graduate Institute TBDx System project (R01AI111477). In addition he receives funding from three grants unrelated to TB in which Ustar Biotechnologies (hereafter Ustar; China) and TwistDx Ltd (United Kingdom; a subsidiarity of Alere Inc. [hereafter Alere; USA]) are collaborators. He has no other commercial/financial conflicts pertaining to information described in this document.

Madhukar Pai has no commercial/financial conflicts. He has received grant funding for TB diagnostics market research from the Bill & Melinda Gates Foundation (BMGF). He previously served as co-chair of the New Diagnostics Working Group (NDWG) of the Stop TB Partnership. He serves on the Scientific Advisory Committee of FIND and as a consultant for the BMGF.

3. Public health problem

In 2012, 8.6 million people fell ill with TB and 1.3 million died from TB, according to WHO estimates.[4] A majority of TB cases occur in 22 high-burden countries (HBCs). Brazil, the Russian Federation, India, China and South Africa alone—the BRICS—account for 46% of all incident cases of TB, and 40% of all TB-related mortality. Over 95% of TB deaths occurs in low- and middle-income countries.

TB is also a leading killer of people living with HIV (PLHIV), causing over one quarter of all deaths.[5] For PLHIV, as in children, paucibacillary specimens are very common, and rapid dissemination of disease beyond the lungs makes diagnosis more difficult. In areas of high HIV co-morbidity, up to 50% of cases may be extrapulmonary TB (EPTB),[6] and trying to diagnose EPTB with methods for pulmonary TB (PTB) is often unsuccessful.

Multidrug-resistant (MDR) TB is a form of the disease resistant to isoniazid (INH) and rifampicin (RIF), two of the most important first-line TB drugs. Globally, in 2012, WHO estimates that 450 000 people developed MDR TB, and 170 000 people died of MDR TB.[4] Together, China, India and the Russian Federation account for over half of the global burden of MDR TB, but MDR TB has been reported in virtually all countries surveyed.[4] Most cases of MDR TB never get diagnosed and appropriately treated: it is estimated that less than one in four cases of MDR TB was detected in 2012 (section 4 further details commodity access issues related to diagnostics).[4]

In 2012, an estimated 530 000 children became ill with TB and 74 000 HIV-negative children died of TB, but true case burden of paediatric TB is likely much higher, due to difficulties in diagnosing TB in children.[7] That is, the clinical and radiological features of childhood TB are often non-specific and subject to variable interpretation: very often there is limited microbiologic confirmation of TB infection performed in children from whom specimens are often paucibacillary (fewer *Mycobacterium tuberculosis* [MTB] bacteria) and typically require culture.[8,9]

Recognizing the magnitude and scale of the TB problem, WHO and partners have announced an ambitious Post-2015 Global TB Strategy, approved by the World Health Assembly in May 2014. The new strategy aims to end the global TB epidemic, with targets to reduce TB deaths by 95% and cut new cases by 90% between 2015 and 2035, and to ensure that no family is burdened with catastrophic expenses due to TB. The strategy set interim milestones for 2025 and 2035 (Table 1).

Table 1. Post-2015 Global TB Strategy and targets

Vision	A world free of TB: zero deaths, disease and suffering due to TB
Goal	End the global TB epidemic
Milestones for 2025	75% reduction in TB deaths (compared with 2015) 50% reduction in TB incidence rate (compared with 2015) No affected families facing catastrophic costs due to TB
Targets for 2035	95% reduction in TB deaths (compared with 2015) 90% reduction in TB incidence rate (compared with 2015) No affected families facing catastrophic costs due to TB

Source: WHO.[10]

TB diagnostics are highlighted in two of the pillars of the new TB strategy:

- integrated, patient-centred care and prevention;
- intensified research and innovation.

Under the first pillar, early diagnosis of TB, including universal drug susceptibility testing (DST) and systematic screening of contacts and high-risk groups, are key components. The latter relies on development and rapid uptake of new diagnostics as well as research to optimize implementation and impact of diagnostics.

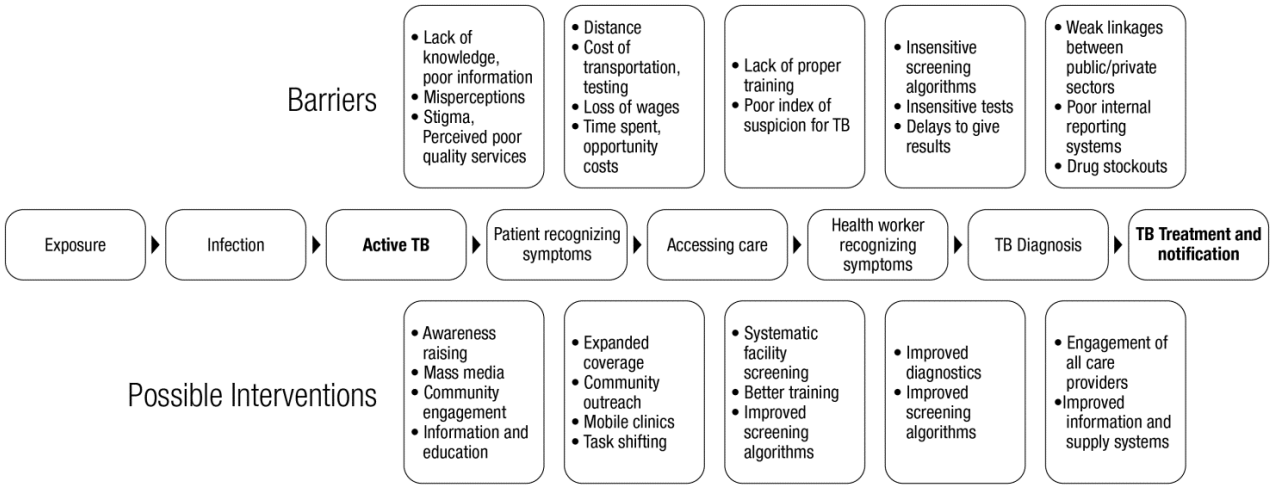
4. Commodity access issues related to diagnostics

Diagnosis is a critical step in effective TB care and control, but many people with TB disease do not have access to adequate initial diagnosis. In 2012, only 5.7 million newly diagnosed TB cases were notified to national TB programmes (NTPs).[4] This suggests that nearly 3 million people with TB were "missed"—either not detected, or detected but not notified.[4]

When appropriately diagnosed, TB is largely curable with currently available medicines. But initiation of appropriate TB drug regimens is impossible without timely access to the right diagnostic tools to diagnose both TB disease and drug resistance. It is estimated that up to one third of patients in high-burden settings does not have access to adequate diagnosis.[4] A variety of barriers can prolong a patient's pathway to TB diagnosis (Figure 1).[11] Systematic reviews suggest that diagnostic delays are common in many settings, and overall diagnostic delay has been attributed to both patients and the health system.[12,13]

Even when patients seek care, and diagnostics are used, lack of a highly sensitive test at the primary care level limits the effectiveness of care. Furthermore, because molecular and culture-based methods are often available only in regional or referral laboratories, few patients are able to access these more sophisticated tests. This is underscored by the fact that only 5% of patients with a new diagnosis of TB and 9% of retreatment cases undergo DST. Without DST to assess drug resistance, a patient with MDR-TB may receive inappropriate treatment—leading to a risk of treatment failure in the individual, and drug resistance in the wider community.

Figure 1. Patient pathway to diagnosis and care, with common barriers and potential interventions



*Adapted from Uplekar et al. 2013[4]

Source: Reproduced with permission from Blok L et al.[11]

The Post-2015 Global TB Strategy calls for early diagnosis of TB, including universal DST. However, while rapid DST tests (e.g. line probe assays [LPAs] and Xpert® MTB/RIF) are available and endorsed by WHO, they are still not widely implemented in many low-income countries. Currently, most NTPs do not offer universal DST, resulting in detection of less than one in four cases of MDR TB. In many countries, the diagnostic infrastructure in the public sector relies primarily on sputum smear microscopy (SSM) that cannot detect drug resistance. Patients often receive MDR TB screening only when they fail to respond to standard first-line TB treatment, or have recurrence of TB; this contributes to morbidity, mortality and continued transmission.

While the Xpert® MTB/RIF assay is a much needed breakthrough, it was not designed to reach lower tiers of the health-care system, and not intended to meet all needs (e.g. it cannot detect resistance against mul-

tiple drugs). Cost is also a hurdle for many NTPs and private sector providers, the latter typically excluded from subsidized pricing agreements. A recent survey of Xpert® MTB/RIF use in 22 HBCs suggested that most NTPs only use the assay for selected patients at risk of MDR or HIV, and not as a tool for early case detection in all patients with presumed TB.[14]

Thus, a majority of the high-TB burden countries still rely on SSM as the primary and, often, sole diagnostic test. SSM has limited sensitivity, and cannot detect drug resistance. Access to accurate diagnosis is also a big challenge in children with suspected TB, and in people with HIV co-infection (who often present with disseminated or extrapulmonary disease).

Diagnostics are also necessary to detect latent TB infection (LTBI), which can be treated to prevent progression to active disease. Although about half of all PLHIV is thought to be eligible for isoniazid preventive therapy (IPT), only 30% of people infected with HIV in 30 countries that reported was started on IPT in 2012.[15,16]

For the many situations described above, access to the right tools to detect TB and guide appropriate treatment is poor. Better access to more appropriate, effective diagnostics tools is, therefore, critical to improve detection and care of TB.

5. Technology landscape

5.1. Overview of the technology landscape and approach

Overview of the technology landscape

In 2014, the TB diagnostics technology landscape looks very promising, with many product developers and a robust pipeline of technologies. The range of technologies that may replace smear microscopy continues to expand. In addition, in the next two to three years, new products on the market will compete with Xpert technology, all deployable in laboratory settings where the Xpert is currently installed and many designed to operate in more decentralized environments by virtue of smaller size, reduced complexity, increased robustness and use of battery power.

Many of these technologies use innovative and proprietary components to target the same needs of rapid diagnosis of TB at decentralized clinics and microscopy centres. Several fully integrated tests aim to deliver results in less than one hour; this should enable point-of-care (POC) testing programmes and reduce time to treatment. Traditional concerns around contamination rates associated with open nucleic acid amplification test (NAAT) platforms have not yet been noted in larger evaluation studies of open or semi-automated NAATs, but more information is still needed to assess use by minimally skilled users in intended settings.[17,18] Several developers have assays for drug resistance incorporated into the detection test or as a reflex test upon confirmation of TB disease, thus facilitating universal DST. Microarrays and next-generation sequencing (NGS) will increasingly become cheaper and easier to perform and will become a critical component of molecular detection of drug resistance as newer drug regimens emerge over the next three to five years.

A significant deterrent to widespread application of NAATs is the need for appropriate field evaluation of newer tests. Currently, there have been limited assessments of the next-generation NAATs, with only two evaluations of LoopAMP™ MTBC Detection Kit and EasyNAT™, and one each for Genedrive®, Truelab™ and FluoroCycler® technologies. For most of these products, on the market for a few years now, more performance data are needed to inform NTP policies. Manufacturing capacity can also be challenging for manufacturers of new technologies, particularly when faced with widespread scale-up.

In the medium term, the need for a biomarker-based, low-cost, non-sputum-based test remains a key priority for TB diagnostics beyond the microscopy centre where the majority of people first seek care. Although biomarker discovery is an active area and several potential products (e.g. antigen or antibody detection tests; volatile organic compound (VOCs); enzymatic detection) are under development, no test under development is likely to be on the market with policy endorsements within the next three to five

years. With the impending introduction of new TB drug regimens within two to three years, there is also a need for new technologies for rapid detection of drug resistance to existing as well as new drugs. New drug regimens will require companion diagnostics to ensure a coherent, seamless approach to "test and treat". In the longer term, breakthrough technologies are necessary to identify those with latent infection who are at the highest risk of progressing to TB disease, so that the vast pool of latently infected individuals can be successfully reduced. The pipeline for such tests is currently weak, with few companies working on such products.

Approach to developing the TB technology landscape

While the 2013 edition of this landscape report focused primarily on molecular tests, this edition presents a comprehensive range of products and their roles in the TB diagnostic spectrum—from reference laboratories to peripheral clinics and microscopy centres. Tools include those used for systematic screening, LTBI diagnosis, TB detection and DST.

For the purposes of this report, TB technologies are grouped into the following eight categories:

1. chest X-rays (CXRs) and computer-aided diagnosis (CAD);
2. sputum collection and sample processing tools;
3. microscopic diagnosis of MTB;
4. culture-based tools for the diagnosis of TB and DST;
5. biomarkers to detect active TB or indicate LTBI;
6. serodiagnostic assays for detection of MTB antigens or immune response to MTB;
7. VOCs;
8. NAATs and sequencing methods.

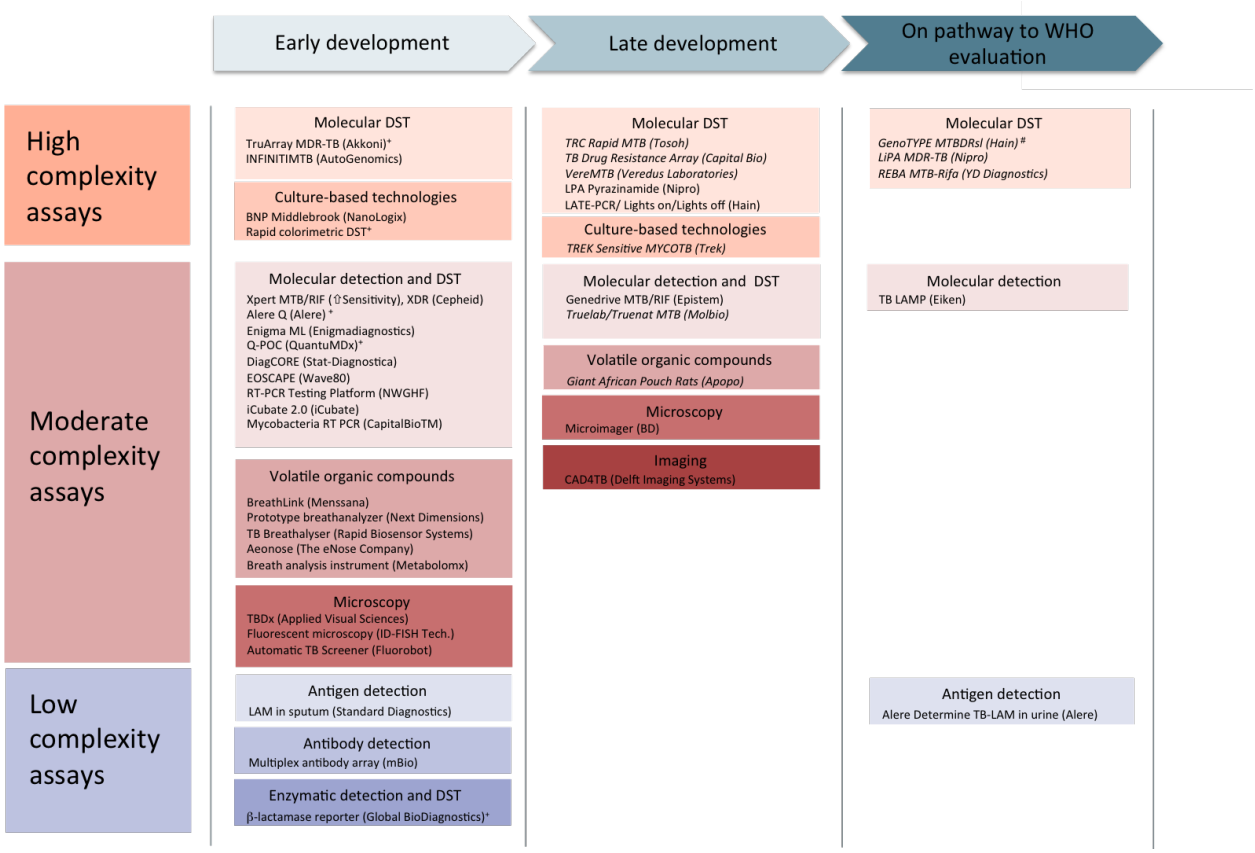
Pipeline analyses in previous WHO TB reports focused on TB diagnostic products and policies endorsed by WHO since 2007, and include automated liquid culture, rapid MTB speciation tests for culture, LPAs, fluorescence microscopy (FM), liquid non-commercial culture and the Xpert® MTB/RIF assay. These, however, reflect only a subset of a much larger set of products on the market or those that are in late development or evaluation.

Our search identified a total of 81 manufacturers and developers. Surveys provided data on 191 products (tests or associated hardware); of which 144 are commercially available and 47 are in late- or mid-stage development. Several academic groups were also included as their technologies were considered sufficiently advanced that a product release date could be estimated. NAATs accounted for 123 products, of which 93 are currently marketed. However, the only WHO-endorsed NAATs are the first-generation LPAs and the more recent Xpert® MTB/RIF assay (Cepheid Inc. [hereafter Cepheid]; California, USA). That is, the emerging NAAT technology pipeline has many new products, but limited evidence to extensively validate products in independent, multicentre evaluations in high-burden settings.

5.2. Role of FIND in product development

For many of the currently available WHO-endorsed TB tests, the evidence presented to a WHO Expert Group was compiled by FIND, an international not-for-profit product development partnership. FIND works with a variety of academic and commercial partners to create a pipeline of new diagnostic tests, from early development to multicentre evaluations, presenting the most promising solutions for review by WHO Expert Groups (Figure 2).

Figure 2. Current FIND TB diagnostics pipeline listing the development phases and the types of technologies in development or evaluation



Notes: Complexity categorization according to criteria that are applied for similar diagnostics by the US Food and Drug Administration. Tests marked in italics are commercialized (not including research use only products). Early development: prototype development post proof-of-concept. Late-stage development: turning prototype into design-locked, manufacturable product.

+ Indicates that the development is cosponsored by direct donor funding to the company (e.g. BMGF; National Institutes of Health [NIH]; National Health Service; Wellcome Trust or other).

CE marked (for both instrumentation and assay).

Source: FIND, Geneva.

5.3. Role of WHO in policy development

WHO issues global policies on TB diagnostics and algorithms based on best evidence. Key steps in the WHO policy process for diagnostics, described in detail by Weyer K et al.,[19] are illustrated in Figure 3.

Figure 3. WHO policy process for review and endorsement of TB diagnostics

Source: Reproduced with permission from Weyer K et al.[19]

The WHO process for endorsement for TB products relies on review conducted by an expert group to inform guidance on use. The expectations for the type and level of evidence required by WHO for expert review is described in Table 2. This is particularly relevant for product developers who will be required to compile data not only on the analytical performance of assays, but also to gather evidence for outcome and health benefit. Adequate evidence on patient-important outcomes is also required to inform WHO guideline development using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) framework (http://www.gradeworkinggroup.org/) and the WHO policy process.

Table 2. Evidence required for the WHO policy process for TB diagnostics

Phase 1: research and development	■ Typically consists of upstream research and development to define and validate a prototype, followed by laboratory validation under international standards that culminate in a design-locked product. ■ WHO interacts with developers, if requested, to discuss end-user requirements such as biosafety, assay robustness and intended setting of use.
Phase 2: evaluation and demonstration	■ The performance of the new diagnostic product should be evaluated in controlled trials at three to five trial sites in high-burden TB and HIV countries, ideally using pre-specified and user product specifications. The data are often used for product registration. ■ Product specifications and performance should be subsequently validated in uncontrolled trials under field conditions in 5–10 trial sites in HBCs and include cost-effectiveness studies.
Phase 3: WHO evidence assessment using GRADE	For new technologies or new indications for use of technologies already endorsed by WHO: ■ submission of dossier with Phase 1 and 2 data to WHO; ■ structured evidence assessment process. For fast-follower or generic versions of already endorsed tools: ■ manufacture of the technology under ISO (International Organization for Standardization) 13:485 standards; equivalent performance demonstrated in two or three independent supranational reference laboratories to the reference technology already approved by WHO.
Phase 4: Phased uptake and collection of evidence for scale-up	■ New diagnostic successfully implemented in routine diagnostic services by early implementers in HBCs; systemic assessment of proposed algorithms, laboratory workload, operational constraints and cost-effectiveness.
Phase 5: Scale-up and policy refinement	■ Scale-up of the new diagnostic, with subsequent data used to inform and refine WHO policy guidance in a dynamic and ongoing process.

Source: Adapted from Weyer K et al.[19]

Since 2007, WHO has used the above process (or a variant thereof) to endorse a variety of TB diagnostics (Table 3).

Table 3. TB diagnostic technologies endorsed by WHO

Year	Technology reviewed by WHO
2007	Commercial liquid culture and DST tools, and rapid speciation strip tests
2008	Molecular LPAs for first-line anti-TB drug resistance detection
2010	LED microscopy
2010	Selected non-commercial DST methods (MODS, CRI and NRA)
2010	Xpert® MTB/RIF
2013	Policy update on Xpert® MTB/RIF, with extension to childhood and EPTB

Source: WHO policies are available at http://www.who.int/tb/laboratory/policy_statements/en/.

The WHO policy process has also been used to review other technologies not shown in Table 3. Some received negative policy recommendations (e.g. commercial serological antibody-based TB tests; interferon-gamma release assays [IGRAs] for detection of active TB),[20,21] while others were not endorsed due to lack of adequate evidence (e.g. loop-mediated amplification [LAMP]; LPAs for second-line drugs.[22,23] In 2014, no TB technologies underwent review by the WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB).

WHO policies form the basis for the International Standards for TB Care (ISTC), developed by TB CARE 1, with funding from the United States Agency for International Development (USAID), and endorsed by several international agencies. The 3rd edition of ISTC was published in March 2014;[24] standards related to diagnosis are summarized in Box 1.

Box 1. ISTC, 3rd edition: standards for diagnosis

Standard 1. To ensure early diagnosis, providers must be aware of individual and group risk factors for TB and perform prompt clinical evaluations and appropriate diagnostic testing for persons with symptoms and findings consistent with TB.

Standard 2. All patients, including children, with unexplained cough lasting two or more weeks or with unexplained findings suggestive of TB on chest radiographs should be evaluated for TB.

Standard 3. All patients, including children, who are suspected of having PTB and are capable of producing sputum should have at least two sputum specimens submitted for smear microscopy or a single sputum specimen for Xpert® MTB/RIF (Cepheid Inc.; USA) testing in a quality-assured laboratory. Patients at risk for drug resistance, who have HIV risks, or who are seriously ill, should have Xpert® MTB/RIF performed as the initial diagnostic test. Blood-based serologic tests and IGRAs should not be used for diagnosis of active TB.

Standard 4. For all patients, including children, suspected of having EPTB, appropriate specimens from the suspected sites of involvement should be obtained for microbiological and histological examination. An Xpert® MTB/RIF test is recommended as the preferred initial microbiological test for suspected TB meningitis because of the need for a rapid diagnosis.

Standard 5. In patients suspected of having PTB whose sputum smears are negative, Xpert® MTB/RIF and/or sputum cultures should be performed. Among smear and Xpert® MTB/RIF negative persons with clinical evidence strongly suggestive of TB, antituberculosis treatment should be initiated after collection of specimens for culture examination.

Standard 6. For all children suspected of having intrathoracic (i.e. pulmonary, pleural and mediastinal or hilar lymph node) TB, bacteriological confirmation should be sought through examination of respiratory secretions (expectorated sputum, induced sputum, gastric lavage) for smear microscopy, an Xpert® MTB/RIF test and/or culture.

Source: WHO.[24]

The above-described WHO policy process is the route for policy endorsements at WHO, and donors such as UNITAID, the US President's Emergency Plan for AIDS Relief (PEPFAR) and the Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund) use WHO policy endorsement to guide decisions on TB commodity purchases. However, WHO is not a regulatory body and does not recommend or approve technologies for individual country use. While WHO and ISTC policies outline best practices for diagnostics at the global level, countries implement their own national guidelines and standards. For example, India has developed its national policy, Standards for TB Care in India.[25] All 22 HBCs have national guidelines or standards in place, with heterogeneity evident in diagnostic algorithms used.

5.4. Currently available and pipeline technologies

The 2014 TB diagnostics technology landscape is promising, with several product developers and a robust pipeline of new technologies. The range of technologies that can improve upon the performance of smear microscopy and compete with the GeneXpert continues to expand. In the next two to three years, new products will challenge the Xpert technology, with many designed to operate in more decentralized environments by virtue of smaller size, reduced complexity and cost, increased robustness, use of battery power and improved performance over smear microscopy.

Many of these technologies use innovative and proprietary components to target the same needs of rapid diagnosis of TB at decentralized clinics and microscopy centres. Several fully integrated tests aim to deliver result in less than one hour; this should improve time to treatment and enable POC testing programmes. The automated NAATs solutions also appear to be more suitable for use by minimally skilled users in their intended settings, but larger scale trials of novel tests are still limited.

Several developers have assays for certain drug resistance patterns incorporated into the detection test or as a reflex test upon confirmation of TB disease. This will enable programmes to aim for selective DST—albeit sometimes limited to only one drug (e.g. RIF). Commercial microarray platforms are increasingly becoming cheaper, easier and faster to use. The application of NGS may well become a critical component of molecular detection of drug resistance in the future, and help manage use of new drug regimens expected to emerge in the coming three to five years.

A main bottleneck to the application of NAATs is appropriate evaluation of these tests under field conditions. There have been limited assessments of some new NAATs, with only two evaluations done so far for the LoopAMP™ MTBC Detection Kit (Eiken Chemical Company [hereafter Eiken]; Japan) and the EasyNAT™ TB kit (Ustar),[17,18,26,27] and one each for the Genedrive® MTB assay (Epistem; United Kingdom),[28,29] Truelab™ MTB (using the Truelab™ RealTime micro PCR system, Molbio; India)[29] and FluoroType® MTB assay (using the FluoroType ® platform, Hain Lifescience; Germany).[30] However, the limited evidence generated is inadequate for informing policy. Some of these products have been on the market for at least a few years now and more performance data are needed to inform NTP policies.

Capacity and ability to meet demand can present another concern with widespread scale-up of new technology. Following WHO endorsement and donor-sponsored price-reductions, demand for Xpert® MTB/RIF was so great that manufacturer Cepheid had difficulty fulfilling orders in a timely manner. Many TB diagnostics manufacturers today are relatively small companies with limited experience responding to extremely rapid increases in demand compromising the product quality or service. With these challenges in mind, FIND recently launched its Support for Success programme, through which companies can gain access to a global team of experts to address their special technological and knowledge needs during product development, manufacturing, scale-up and market entry. Taking a different approach, Alere recently accessed a low-interest loan of US$ 20 million from the BMGF to expand production facilities in Jena, Germany, thus expanding manufacturing capacity for the pending release of their HIV viral load and TB assays.

With the expected introduction of new TB drug regimens, there is a need for new technologies for rapid detection of resistance to both existing and new drugs. That is, new drug regimens will require companion diagnostics to ensure a coherent, seamless approach to test and treat. In the medium term, the need for a biomarker-based, low-cost, non-sputum-based test also remains a key priority. Although biomarker discovery is an active area and several potential product concepts (e.g. antigen detection tests; VOC; enzymatic detection) are presently under development, none is likely to be on the market with policy endorsements within the next three to five years. In the longer term, new diagnostic solutions will be necessary to identify those patients with latent infection who are at the highest risk of progressing to TB disease, so that the vast pool of latently infected individuals can be successfully reduced. The pipeline for such tests is currently weak, with very few groups working on such solutions.

5.4.1. CXRs and CAD

Perspective: The use of radiography (i.e. CXRs) to screen for lung abnormities indicative of current or previous TB infection has been in use for decades. A CXR can be used to raise suspicion of active PTB disease or to identify previous infection (e.g. old, healed scars). There are no TB-specific abnormalities, and ≤10% of patients with active PTB may have normal findings on CXRs.[31,32] However, CXRs have shown good sensitivity in identifying individuals with the highest risk of TB, particularly when extended criteria of "any abnormality" and "intentional overreading" are applied.[33] With the prevalence of smear-negative diagnosis in patients with HIV, an abnormal CXR may raise persistent concern for TB using a relatively cheap yet rapid method (although non-specific).[34] However, in HIV-infected people, CXRs have limited sensitivity.[35]

CXR is a diagnostic tool, but can also be used as a triage test, and is used in prevalence surveys as a screening tool. A prevalence survey for TB in Shandong Province, China, in 2010 revealed that around 50% of positive TB cases found in this survey did not present with cough.[36] Persistent cough is one of the primary indicators used in the passive case finding of TB, after which sputum microscopy is performed to diagnose TB. Abnormal CXRs could, therefore, be key to active case finding by identifying cases that otherwise would have not have been diagnosed by conventional, passive case finding. An alternative study investigated the cost–benefit of using CXR as an adjunct diagnostic test in combination with the Xpert® MTB/RIF assay. Preliminary data demonstrated that radiology can be a useful tool for ruling out TB in Xpert-negative individuals, but that this approach was still more costly than performing Xpert® MTB/RIF alone.[37] CXRs are also frequently used to rule out active TB, before initiating LTBI therapy (e.g. IPT) in addition to a symptom screen and possibly a sputum examination.

Historically, several technological hurdles have hindered the widespread application of CXRs in resource-limited settings. X-ray technology itself requires adequate power supply, air conditioning and logistical considerations in moving equipment and film processors in rural areas. In addition, CXR requires radiographers or clinicians to interpret the images for abnormalities, and reproducibility of interpretation has been a challenge. Many studies show wide variation in performance by different readers (radiographer versus clinician), or even the same reader, and training approaches can vary.[34]

Today, CXR is becoming more accessible in remote settings due to technological advances such as powerful yet more compact electronics, high resolution digital imaging instead of film-processed images and appropriate computer hardware/novel image analysis software algorithms. These advances have created a series of systems, including less expensive portable systems, which may improve the ability to perform CXR to investigate PTB in resource-limited settings. However, larger-scale evaluation of these novel technologies for use in active case finding or as adjunct diagnostics, particularly with image analysis software-enhanced CXR, is still limited.[33]

Comprehensive guidance for TB prevalence surveys, the Lime Book, is available from WHO and provides key information on the use of CXR.[33] Several types of X-ray equipment can be used. One option is conventional X-ray (with manual or automated film developing), conventional X-ray with a digital plate or digital X-ray (computed radiography, direct digital systems or slot-scan systems). Conventional X-ray equipment (analog) is much lower in initial cost compared to digital X-ray, but the continued costs of film and processing materials over the lifespan of the equipment means that it is more expensive in the longer term. A cost-benefit analysis of analog versus digital X-rays for general hospital use highlighted lower costs using digital systems.[38] Analog systems have been mounted onto trucks and used as mobile units in rural areas, but as film development is temperature-sensitive, air conditioning is required if temperatures exceed 32°C.

Figure 4. Example of the small size and portability of digital X-ray equipment and its potential for use outside of clinical settings

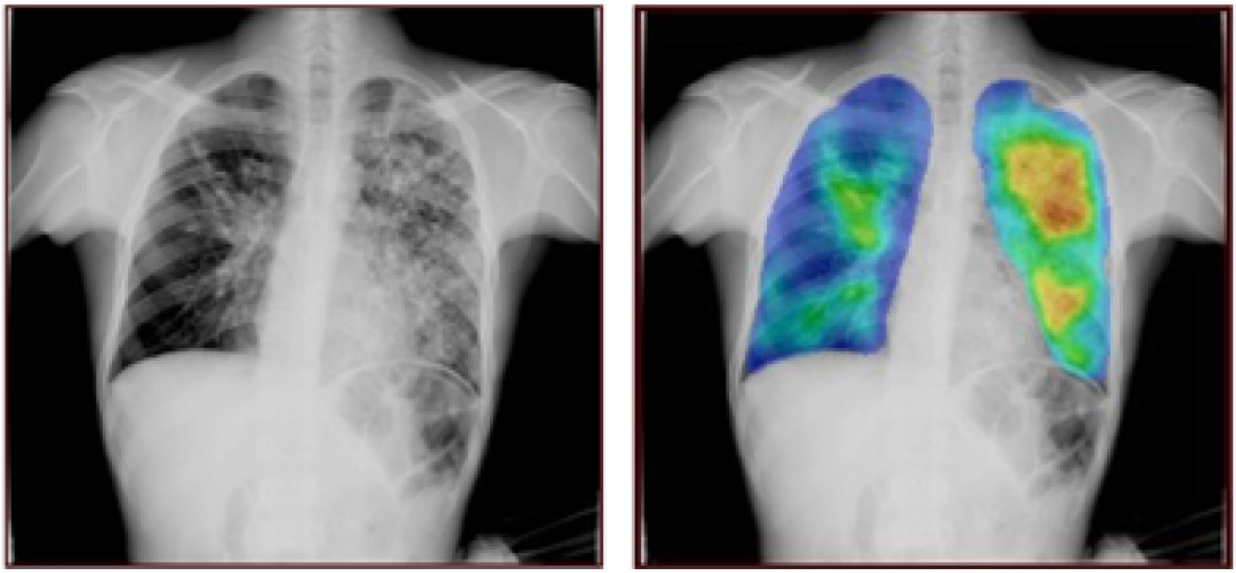
Source: Images produced with permission from MVIP Software and Consulting, Germany (www.mvip.de).

As an alternative to film-based CXR, digital plates can be used with conventional X-ray equipment, removing the need for X-ray film, a film developer and associated reagents (Figure 4), offering reduced complexity and cost, improved image quality, decreased radiation dose, better archiving and the potential for electronic transmission of acquired images (for expert opinion and quality assurance, etc.).[33]

Digital CXR also offers the possibility for use of objective computer-aided interpretation of PTB. While, in principle, effective on analog films as well, in practice, the quality of many analog films is poor (overexposed or underexposed prior to scanning). CAD systems permit uniformity in identifying lung abnormalities and reduce variability in reading. The challenge has been to design appropriate algorithms that have sensitivity similar to trained readers.[39]

A recent comparative analysis of the performance between the CAD tools and human readers showed no significant differences.[39,40] The Diagnostic Image Analysis Group (Radboud University, the Netherlands)—leaders in CAD of PTB using digital CXR—have partnered with Delft Imaging Systems to commercialize their CXR analysis algorithms, currently marketed as CAD4TB (Figure 5). This software can also be applied to analog CXR films when electronically scanned and digitized. The Zambia AIDS Related Tuberculosis (ZAMBART) group have investigated the combined use of a CAD tool with digital CXR to help diagnose TB in areas where there is no radiologist.[41] The results suggested that CAD has the potential to increase the use and availability of chest radiography in screening for TB where trained human resources are scarce.

Figure 5. Example of a digital CXR (left) and CAD4TB interpretation of lung abnormality (right)



Source: Maduskar P et al.[40] (reproduced with permission from the International Journal of TB and Lung Diseases).

MVIP (Germany) is developing DigiPortXCAD, DigiportX TB, an ultra-portable digital CXR from Medex Loncin S.A. (Belgium) and a TB CAD system in collaboration with Damien Foundation (Bangladesh) and the University of Applied Science (Göttingen, Germany). They are currently evaluating this system in Bangladesh.

Advantages: To perform digital CXR, no other materials are required, and recurring costs are low once the initial equipment investment is made. Existing conventional X-ray machines can use digital plates instead of analog film and, therefore, existing systems can be converted at lower cost than new capital investment. Digital X-rays expose each patient to a lower dose of radiation than conventional CXR. Screening can be rapid, with two staff required to screen roughly 40 patients per hour. The use of CAD permits broader application, as trained staff are not required to interpret images: tele-radiology gives each patient and provider rapid feedback after screening and all images can be archived and/or exported to a central repository and remotely interpreted. Mobile screening units permit active case finding in the community, leading to earlier detection and detection of subclinical cases, therefore, potentially limiting TB transmission. Digital CXR can be used as a triaging tool to optimize more expensive confirmatory tests such as Xpert® MTB/RIF. The application of CXR may have a positive impact on health care in resource-limited countries for a broader variety of pulmonary diseases, including pneumonia, chronic obstructive pulmonary disease and lung cancer.

Disadvantages: Current digital X-ray systems are expensive, but this initial capital cost is offset over time, as no other consumables are required for operation. Although units can be loaded onto trucks or containers to be mobile, most equipment is not ruggedized and is, therefore, sensitive to jarring and vibration. A valid warranty or service agreement is recommended and maintenance or repair can be challenging in remote field settings. A generator is necessary for power supply. Tele-radiology requires high-speed or broadband data transmission systems. If human resources review images for abnormalities, intra- and inter-observer variability are common and there is a lack of a universally accepted reporting system. CXR presents only a two-dimensional image of a three-dimensional structure, with parts of the lung obscured by overlapping structures. X-rays create exposure to a small dose of radiation and can increase the lifetime risk of cancer; however, while there is no threshold that is considered safe, the exposure from one CXR equals the exposure from 10 days in natural surroundings, so the lifetime risk is considered to be very small.

5.4.2. Sputum collection and sample processing tools

Perspective: Sputum is the most important clinical sample for TB diagnosis using most existing technologies. To detect PTB, a patient must expectorate a sufficient volume of sputum to enable diagnostic testing by microscopy, culture or molecular tests. For the improved collection of sputum to diagnose PTB, two tools were identified, one currently marketed and the other in development. The Lung Flute, from Medical Acoustics® (USA), is shown in Figure 6.

Figure 6. Lung Flute from Medical Acoustics® to aid sputum collection from patients who are unable to expectorate a sufficient sample

Note: The user blows into the blue mouth piece (left) and the low resonating frequency generated aids the release of sputum located deep in the lung tissue.

Source: Image reproduced with permission from Medical Acoustics®.

A small, plastic device, the Lung Flute is non-invasive and easy for the patient to use. The tool uses sound vibrations to stimulate mucus clearance, effectively thinning and dislodging mucus deep in the lungs to ease production of a sputum specimen. One study assessed the performance of the Lung Flute on TB suspect patients, finding that the Lung Flute permitted 88% of this cohort to subsequently provide a specimen.[42] The device is approved by the United States Food and Drug Administration (US FDA), CE-IVD marked and approved for use in Australia, Canada, India, Japan, Philippines, Republic of Korea and Singapore. The company cites a list price of US$ 10 per unit, but notes that price is related to volume of sales.

Deton Corp. (USA) is developing a cough collection tool for testing by nucleic acid detection. A patient coughs into a bag until at least 1 litre of air has been collected. The hypothesis is that the droplet nuclei produced by this and the TB cells therein are so small that they cannot precipitate, instead remaining as particulates in suspension. The contents of the bag are forced over a device designed to focus the airflow and permit the deposition of particles onto a capture film. This film can then be removed and processed to extract MTB DNA for subsequent testing via polymerase chain reaction (PCR) or a similar diagnostic method. The developers speculate that this tool may enhance sensitivity of MTB diagnosis as other potentially inhibiting materials in sputum are not collected by this method.

For rapid processing of sputum for culture, Salubris Inc. (USA) offers Decomics®, a product that is both CE-IVD marked and US FDA (510k) cleared, and marketed since 2012. This is used to decontaminate and concentrate specimens for culture-based testing from a variety of common specimen types without the need for a centrifuge. The test uses absorbent beads and reagents to liquefy, decontaminate and neutralize samples in less than 25 minutes. A variety of commercial vendors, including Becton, Dickinson and Company (hereafter BD; USA) and Hardy Diagnostics (USA), also offer kits to decontaminate sputum samples for culture, employing derivatives of the N-acetyl cysteine and NaOH methods commonly used to prepare sputum specimens prior to culture.

DNA Genotek Inc. (Ottawa, Canada; a subsidiary of Orasure Technologies, Inc., USA) has developed two products to process sputum. The OMNIgene® SPUTUM is intended to liquefy and decontaminate the sample allowing it to be transported without cold chain to a laboratory for diagnostic testing. Samples can be used for microscopy, culture or nucleic acid analyses. An evaluation of the OMNIgene® SPUTUM by the All India Institute of Medical Sciences, New Delhi, highlighted that no samples were contaminated when tested by culture and the time to positive result was not altered. The company's prepIT® MAX technology is intended to be a high-performance method to release nucleic acids from Mycobacterium tuberculosis complex (MTBC) cells without the need for a mechanical approach, such as bead beating. Samples first

treated by the OMNIgene® SPUTUM method can be subsequently processed with this kit to obtain nucleic acids for further analysis. The current method does rely on ethanol precipitation of DNA, so a centrifuge, heat block and ice are required to process samples. The cost of these products is currently not available.

Akonni Biosystems (Maryland, USA) offers the TruTip™ DNA extraction system to extract TB DNA without requiring a centrifuge. Here, MTB cells are lysed in buffer and the DNA captured, washed and released from a specially designed pipette tip. This requires only a single battery-powered pipettor. Akonni Biosystems has demonstrated the use of the tips with liquid handling systems for higher-throughput extraction of DNA.[43]

Longhorn Diagnostics (Texas, USA) produces PrimeStore MTM®, a proprietary material that kills and lyses MTB cells and then stabilizes the nucleic acids, while being shipped to the laboratory without cold chain.[44,45] The PrimeStore MTM® can either accommodate a swab taken from the sputum sample (100–200 µL sputum) or up to 500 µL of raw sputum. It is intended for use with NAAT-based testing such as LPA or PCR-based methods.[45] Reagents cost US$ 500 for 50 tubes (list price). Recently, Molzym & Co. (Germany) released MTB-DNA Blood, a product used to extract DNA from MTBC cells in 1 and 10 mL of whole blood. The MTB-DNA Blood is based on the Molzym & Co. MolYsis™ technology, which removes non-target human DNA and enriches MTBC. Molzym & Co. also offers SelectNA™ and SelectNA™plus, automated extraction tools for use with this product. After DNA is extracted, it can be applied to a variety of NAATs. The tests cost US$ 15.80 and automation equipment costs US$ 16 725 or US$ 32 780 (list prices). The products are currently not yet CE-IVD marked.

Other sample preparation methods have been developed to capture and enrich MTB cells from sputum, with paramagnetic beads coated with ligands specific to the mycolic acid components of the MTB cell wall. Besides enriching for MTB, washing of beads removes confounding components to limit non-specific background in microscopy. Microsens Diagnostics Ltd (United Kingdom) markets TB-Beads, which can capture MTBC cells directly from sputum for use in microscopy, culture or other diagnostic tests. The capture is rapid, requiring just five minutes, and does not require other equipment such as a centrifuge. The company estimates that 30–40 samples can be processed in one hour. The beads are priced at US$ 1.20 per test for low-resource countries. This product is CE-IVD marked, and two recent evaluations have shown that the beads can improve the sensitivity of microscopy by 25–35%.[46,47] A further study compared TB-Beads' performance to traditional centrifugation to prepare samples for culture, showing similar preparation times per sample and culture data.[48] Therefore, although data are limited, it appears that TB-Beads might have some benefit in laboratories where consistent power is a concern, and could reduce biohazard as there is no opportunity to generate aerosols.

Genetein Co. Ltd, a subsidiary of Precision System Science Co. Ltd (Japan), has developed a similar product, the TRICORE bacterial concentration kit. This has been evaluated in one study where the authors noted that the tool improved the detection of MTBC in paucibacillary specimens.[49] Currently, no other information is available on this product.

5.4.3. Microscopic diagnosis of MTB and associated tools

SSM

Perspective: SSM specifically stains the mycobacterial cell wall with colorimetric or fluorescent dyes for subsequent visualization via microscopy. The original method is now over 130 years old and it remains the primary diagnostic test for pulmonary MTB in HBCs. A positive smear test is not wholly specific for MTB; a positive result indicates the presence of acid fast bacteria (AFB), but does not discriminate between MTBC and other non-tuberculous mycobacteria (NTM). SSM is also used for treatment monitoring with confirmed TB cases on drug therapy. The low cost, relatively rapid time to result, ability to be used in peripheral laboratories and limited need for equipment and reagents supports the continued use of this test, particularly in low-income HBCs.

A recent survey of 22 HBCs indicated that the average cost of SSM is US$ 1.77 (IQR US$ 1.28–2.69) per smear, including various components (e.g. material; labour; equipment and overheads).[2] In this survey, a global estimate of the total number and cost of SSM tests was calculated via input from 22 HBCs: 77.6 million sputum smears performed annually in 42 827 microscopy centres, for a total value of US$ 137 million—with the majority of tests (61%) performed in the BRICS countries.[2]

SSM algorithms use either three or two specimens for diagnosis. The three-sputum algorithm includes two specimens produced by the patient at the microscopy centre (the spot specimens) and a morning specimen, expectorated by the patient at home in the early morning. The latter is considered to contain a higher bacterial load, which improves diagnosis. The incremental improvement with three specimens as opposed to two (a spot and morning) is limited (i.e. 2–5%) and the obligation of patients to present three independent specimens to a clinic on multiple days can lead to patients defaulting from the testing pathway.[50]

A meta-analysis of the incremental diagnostic value of two versus three specimens led to a WHO recommendation in 2007 that two smears are adequate, and only one positive smear (out of two) is required to identify a new case of TB, provided that the microscopy laboratory had effective external quality assurance (EQA) systems and documented good-quality microscopy.[50] Furthermore, in 2010, WHO recommended that same-day microscopy on two samples results in test performance comparable to spot testing on different days. The aim of this policy was to reduce loss-to-follow-up of suspected cases, supported by a randomized trial and meta-analysis demonstrating an insignificant change in performance using the two-spot method as compared to the three-specimen screening process.[51,52] in practice, however, many HBCs still rely on analysis of three specimens: the recent analysis of SSM in HBCs by Kik SV et al. showed that 13 of 22 NTPs in HBCs reported using three smears, while 11 used only two.[2]

Samples for smear microscopy are commonly processed directly, but concentration of MTB via centrifugation or bleach sedimentation may improve sensitivity. Centrifugation does add an extra biosafety requirement for sealed rotor buckets to minimize risk from aerosols. An advantage of bleach sedimentation is that all bacteria are killed, creating a non-biohazardous specimen. An evidence base is developing for the use of bleach sedimentation for improved diagnosis of MTB, but no policy recommendation has been made by WHO.[53-55]

The most common method for SSM uses a conventional light binocular microscope in addition to the Ziehl-Neelsen (ZN) staining method where AFB are stained red with carbol fuschin, while other types of bacteria are stained blue with methylene blue. This requires that the microscope be used at 40x and 100x magnification with oil immersion. The number of visible AFB units are scored to determine the bacterial load based on the number of AFB viewed per field (e.g. 3+: >100 AFB 20 fields; 2+: 1–10 AFB 50 fields; 1+: 10–99 100 fields; +/-: 1–9 AFB 100 fields; 0; negative in 100 fields).[24] Fully trained microscopists can read a maximum of 25–30 slides a day.

The application of FM has led to an improvement over conventional SSM as MTB cells are easier to observe. Staining of AFB with fluorescent dyes is more rapid than the ZN method and, most importantly, each field can be read at a lower magnification thus it is easier for microscopists to identify the AFB. As a result, the number of slides that can be read by a microscopist in one day increases to 50–60. The result follows the same scoring algorithm to note the bacterial load. Studies conducted in reference laboratory settings have shown that light emitting diode (LED)-FMs used for SSM have, on average, 10% greater sensitivity than light microscopy and similar specificity.[56-58]

FM to detect MTB was first reported in 1947,[60] but was not appropriate for use in resource-limited settings due to a variety of factors, including a need for a dark room, bulb longevity and overall cost.[59,60] The advent of LED technology allowed durable, battery-powered, low-cost yet effective fluorescent microscopes that do not require a dark room. FIND partnered with Carl Zeiss Microimaging (hereafter Zeiss; Germany) to develop a reduced cost monocular fluorescent LED microscope, the Primo Star iLED, for use in HBC microscopy centres (Figure 7). The CyScope® TB Fluorescence Microscope and a CE-IVD approved stain are also offered by Partec (Germany) (Figure 7). Alternative strategies include conversion to a LED light source with existing conventional light microscopes for FM use: FluoLED™ (Fraen Corp.; Italy);

Lumin™ Illuminator (LW Scientific; USA); Fluo-RAL (RAL Diagnostics, a partner of bioMérieux, France) and ParaLens™ (QBC™ Diagnostics; USA). FIND currently offers negotiated pricing for the Primo Star iLED at approximately US$ 1640.

Figure 7. Examples of LED-FM products: Zeiss Primo Star iLED (left); Partec CyScope® (right)

Sources: Images reproduced with permission from Zeiss and Partec.

In 2010, WHO endorsed LED-FM for SSM[60] and, in 2011, issued a policy statement recommending fluorescent microscopy as a replacement to conventional light microscopy.[61] Recent studies have investigated the suitability of FM for scale-up, with a focus on operational issues.[56,62] In one study, three FM systems were compared to a traditional mercury bulb FM and, while all were comparable, users showed a preference for the Primo Star iLED and the FluoLED™ due to easier operation.[56] Trainee technicians were more likely to report false-positive as opposed to false-negative results on LED-FM microscopes than reference technicians.[62] These findings point to further requirements for appropriate training, quality management and performance monitoring in the field.[56,58,62,63]

Advantages: SSM is a low-cost, highly specific (~98%) tool for diagnosis of PTB that can be effective with appropriate training. The test can be applied in peripheral facilities, with EQA programmes to improve performance in many settings. FM increases the sensitivity of this test by ~10% over colorimetric light microscopy and increases the number of slides read per day. (However, uptake of the more rapid "front-loaded" algorithms and more widespread use of LED-FM is still necessary as many HBCs still read only three slides per patient using direct ZN microscopy).[2] SSM is also effective for monitoring treatment of TB.

Disadvantages: The primary drawback to SSM is the lack of sensitivity, which is highly variable at 20–80%, especially with paucibacillary specimens (common with poor-quality sputum samples or HIV co-morbidity).[64] In PLHIV, early diagnosis of an MTBC co-morbidity is necessary for effective treatment—TB infections are the greatest cause of death in this subgroup—but SSM performs poorly in this group. Furthermore, although SSM is intended to identify mycobacteria in sputum, it is estimated that 17% of transmission is from patients who are SSM-negative.[65]

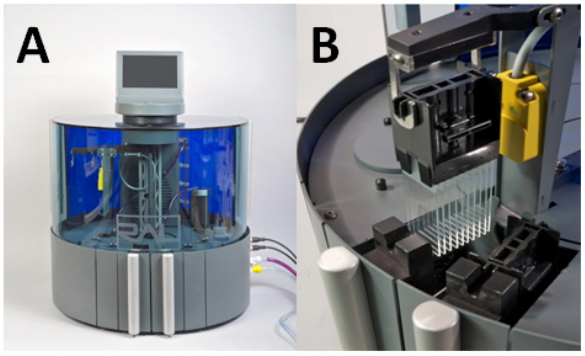
By identifying AFB, SSM is non-specific. SSM also cannot detect drug resistance. The reading of slides is a time-consuming, tedious and repetitive task that requires diligence to be effective. There is a strong impact of user skills on analytical performance and difficulty in training and maintaining skilled personnel to work in microscopy centres. While an effective EQA plan can sustain improved performance, the resources to maintain and coordinate such systems can be extensive.

Automated staining tools and slide processors

For the high throughput and consistent staining of SSM slides, RAL Diagnostics (a partner of bioMérieux, France) offers an automated staining station, the RAL STAINER, and reagents for either conventional ZN or fluorescent microscopy (RAL STAINER COLD ZN and FLUO RAL reagents). The product offers consistency of staining via automation (Figure 8). The machine can be used on all specimen types in laboratories

with electrical power, with applications in larger high-throughput microscopy centres and in smaller facilities to reduce time spent preparing slides. Automated bulk staining of slides has been shown to be a factor in saving significant resources and improving staining results in laboratories with a high workload.[66] The RAL STAINER processes 10 slides at the same time, using two, 10-slide cassettes, requiring 20 minutes. All staining reagents are housed within reagent reservoirs in the machine. Internal air is filtered to prevent airborne release of chemicals. The tool has been marketed since 2013 and costs ~US$ 15 000. The ZN and Auramine reagents are priced at US$ 0.30 and US$ 0.50 per slide, respectively.

Figure 8. A: RAL STAINER standalone slide processor with a touchscreen interface for operation; B: Inoculated smear slides are processed (cartridge with maximum 10 slides capacity)

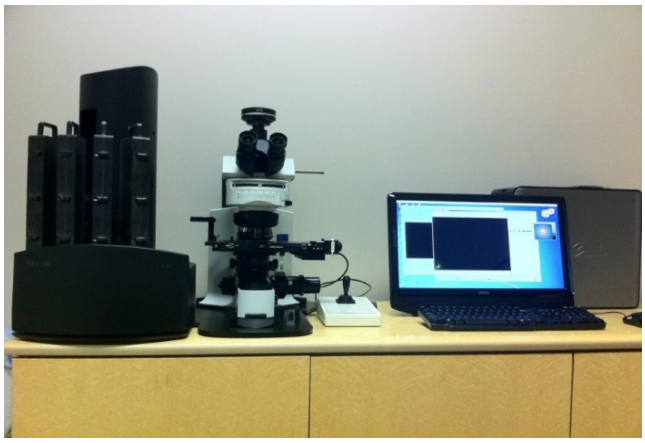


Source: Images reproduced with permission from bioMérieux.

The Aerospray® TB Series 2 from ELITechGroup (Switzerland) is another automated staining platform for SSM and can also be used for conventional or fluorescent staining methods. Slides are prepared via reagents applied from atomizing spray nozzles, limiting the volumes used. This platform can batch process either 12 or 30 slides and requires five minutes processing time. The ELITechGroup notes that the 30-slide system can process up to 400 slides per hour. However, it should be noted that protocols for fixing the smear prior to staining can vary from 15 minutes to overnight with incubation at 65–75°C. Current pricing is unknown.

To address difficulties in reading microscopy slides to a consistently high standard, several groups promote automated approaches to autofocus, identify fields to view[67-73] or scan the entire slide and automatically score AFB counts from selected images.[74] One commercially available automated system is on the market, the TBDx system from Signature Mapping Medical Sciences Inc. (a wholly owned subsidiary of Applied Visual Sciences Inc.; USA) (Figure 9). This system consists of an FM with a digital camera and automatic slide loader (maximum capacity 200). Stained slides are automatically loaded and viewed by the microscope; the digital images of each field per slide are then automatically processed by a proprietary algorithm. The system takes approximately 5 minutes to analyse one slide, 1 hour to analyse 10–12 slides, or 16 hours to analyse 200 slides. Data and results are in a format that can directly populate laboratory information systems. When 3+/2+ AFB are consistently detected, the scoring algorithm can be adapted to read fewer fields and, therefore, decrease the time taken to analyse slides with a high AFB. The system is available without the slide loader, but capacity is limited to only four slides.

Figure 9. TBDx system showing the automated slide loader (left) FM with digital camera and automated stage (centre) and PC operation to operate the reader and employ the scoring algorithm



Source: Image used with permission from Applied Visual Sciences Inc.

The TBDx system is commercially available, and is aimed at high-volume laboratories in HBCs, particularly for triaging specimens prior to diagnosis by GeneXpert to reduce costs or serve as a diagnostic tool in place of SSM. An initial independent evaluation in South Africa showed that the system has an excellent correlation with identifying SSM 2+/3+ versus manual FM performed by a reference microscopist.[74] Compared with culture only, the TBDx had high sensitivity (75.8%), but low specificity (43.5%). TBDx interpretation of scanty slides was affected; investigators suggested a hybrid approach where positive and negative slides scored by TBDx were acceptable and that scanty slides were later reviewed by a microscopist, still reducing workload by over 50%. They noted that if the sensitivity of the TBDx could be increased to 70%, then specimens requiring Xpert® MTB/RIF confirmation would drop to 43% or 35%, while still detecting 76% of all culture-positive cases.[74] The developers are revising their scoring algorithm to analyse scanty specimens and are now partnering with FIND to further assess the feasibility of this tool in resource-limited countries. The microscope and computer are offered at US$ 23 000 with the optional 200-slide robotic loader at US$ 21 000. The software license is based on two components: the length of the licensing period and the type of hardware purchased. Applied Visual Sciences Inc. notes that this could be < US$ 1.00–2.00 depending upon the length of license.

BD is currently developing a fully automated instrument for semiquantitative analysis of AFB in sputum that also incorporates the staining procedure (Figure 10). The system standardizes specimen preparation using a single-use disposable cartridge that houses unitized reagents.

Figure 10. Automated slide reader in development by BD

Source: Image courtesy and © Becton, Dickinson and Company. Reprinted with permission.

A slide-reading algorithm scores the reading of slides; developers claim that sensitivity of the prototype is similar to that of LED-FM performed by a microscopist. The system is being designed for ease of use so as to suit relatively unskilled workers. Data input and operation are via a touchscreen interface. Preparing the slides takes approximately 70 minutes. The overall test takes two hours to complete, and up to 40 slides a day can be batch processed by a battery-powered machine that has a small footprint of approximately 1 square foot, or 930 square centimetres. The developers anticipate that this product will be commercially available in 20–24 months.

There has been significant interest in the application of mobile phone technology with onboard high-resolution cameras to LED-FM in resource-limited settings.[75,76] The CellScope TB Microscope uses this technology (Figure 11), with developers at the University of California (Berkeley, USA) intending for this technology to replace LED-FM. Their device uses the camera of a tablet connected to an optical chamber containing LED filters and lenses to read auramine-O stained slides. It takes approximately four minutes to read each slide,[76] and data are shown on the screen of a tablet postioned on the top of the instrument. With the reader viewing a digital screen rather than looking into the microscope, the device has the potential to make slide reading less tedious. Sensitivity and specificity of SSM diagnosis by new users were within 15% of those achieved by experienced technicians using conventional LED-FM.

The use of a tablet device allows all images to be archived and also sent via wireless networks for confirmatory review. Currently, the device relies upon manual reading of each field, but algorithms to automate the process are in development. The device is battery-powered, weighs approximately 2 kilograms and has a footprint of 400 centimetres. Commercial release of this product is expected in 2017 at a price comparable to other quality FMs currently on the market.

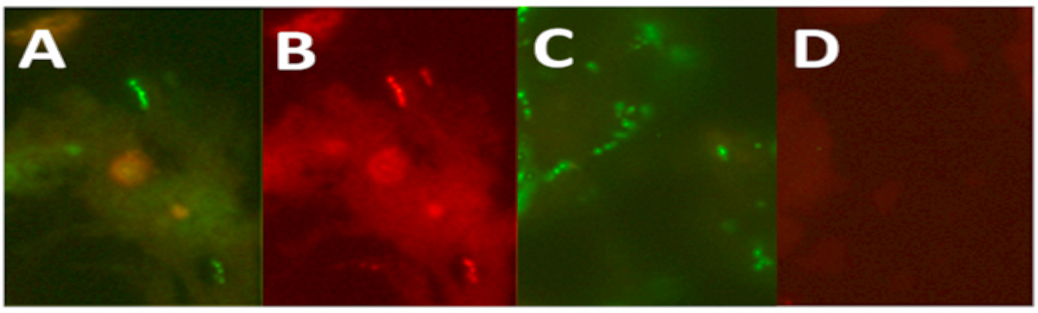
Figure 11. CellScope TB Microscope with a tablet on top for image capture, analysis and viewing

Source: Image used with permission from the University of California, Berkeley.

Fluorescence in situ hybridization (FISH)

FISH of ribosomal RNA (rRNA), messenger RNA (mRNA) and whole cells with targeted oligonucleotide probes is a method that has been widely used to rapidly and directly detect pathogens in medical specimens via FM (Figure 12). rRNA is an excellent candidate target molecule as it is single stranded and, therefore, amenable to hybridization via a sequence-specific complementary probe. Regions of rRNA can be species specific (including for MTBC), and as the copy number per cell is significant (e.g. 10^4), amplification prior to detection is not required.[77] Improvements to the detector probe chemistry have increased the sensitivity of the method, predominantly via the use of peptide nucleic acids (PNAs), which act as DNA mimics. The polypeptide backbone of PNAs permits better hybridization thermodynamics and kinetics that increase probe binding efficiencies and, therefore, sensitivity.[78] PNAs are also hydrophobic in nature, and it has been proposed that this enables PNA probes to more easily transverse the hydrophobic mycolic acid-based cell wall and hydrophobic cell membrane and therefore encounter their rRNA target.[78]

Figure 12. Two-colour FISH using fluorescence labelled probes of a cultured sample derived from a mixed infection of MTB and *M. abscessus*



Notes: Panels A and C reflect the detection of mycobacteria in the green fluorescence channel using Mycobacterium genus specific probe. The MTBC specific probe used in the orange fluorescent channel can then discriminate MTB (panel B) from an NTM (panel D).

Source: Image used with permission from ID-FISH Technology Inc.

While commercial assays to other bacterial pathogens are commercially available, existing research on the application of FISH for MTBC diagnosis and identification has been limited. ID-FISH Technology Inc. (USA) is developing their FISH FM assay kit with assistance from FIND. Their FISH assays are designed for the speciation of culture-positive samples and for testing sputum. Each assay uses two probes; one specific for mycobacteria and a second that can identify MTBC (Figure 12). Batched processing of up to 10 samples takes two hours and ID-FISH Technology Inc. recommends a maximum of 30 slides be prepared per day per technician.[79] An incubator, centrifuge and clean water are required. Slides are read via FM, and ID-FISH Technology Inc. currently claims a sensitivity of 1000–5000 cells/mL. Assays for mycobacteria, MTBC, *M. avium* complex, and *M. kansasii* are in their development pipeline. The cost per test for the MTBC assay is US$ 5 and is currently commercially available for culture confirmation purposes. The sputum diagnostic has not yet been commercialized.

Advantages: Automated slide preparation may provide more smears of more consistent quality for integration with the automated reader systems or allow staff to read slides. Where staffing is a constraint, lower-throughput slide readers such as the fully integrated and automated BD device may meet the need to produce smear test results without experienced personnel. Automated microscopy platforms with screening algorithms may alleviate labour and training requirements by identifying smear-positive or smear-negative samples and leaving only the scanty slides for analysis. There is the potential to run such readers overnight as, in principle, supervision is not required, and this may reduce laboratory technician time, while increasing throughput. FISH assays can be employed in microscopy centres where FM is already utilized and, unlike FM, a FISH assay can discriminate between AFB and MTBC improving specificity for PTB.

Disadvantages: Systems are expensive to purchase and maintenance contracts will be necessary. More widespread evaluation of these technologies is under way, but their performance is not yet fully understood within intended-use settings.

5.4.4. Culture-based tools for the diagnosis of TB and DST

Perspective: Mycobacterial culture remains the current gold standard diagnostic test for the detection of MTB from sputum and other clinical specimens. Unlike SSM, which has a lower limit of detection of 10^4 cells/mL,[80,81] culture methods can detect lower levels of MTB as low as 10–100 cells/mL.[81] Therefore, when possible, smear-negative sputum specimens should be further assessed by culture to confirm the absence of MTB. In addition to its higher sensitivity, MTB culture can be used for further diagnostic purposes, notably phenotypic DST to inform treatment regimens, to provide sufficient DNA for speciation, genotyping using rapid molecular DST or for molecular epidemiologic studies.

All culture methods are laboratory based as infrastructure and trained personnel are required for the specimen processing, inoculation, incubation and monitoring of cultures. Given the highly contagious nature of MTB, culture laboratories are required to have biosafety level 2 capacity requiring laminar flow hoods to allow handling of materials. When cultures of MTB are routinely manipulated for additional phenotypic, molecular or biochemical testing, then the use of a biosafety level 3 laboratory is mandatory.[82] These requirements limit most culture-based diagnosis of MTB and DST to reference laboratories. The use of biosafety level 2-only facilities can widen the use of culture methods to other laboratories, but not third-tier facilities such as microscopy centres.

Sputum specimens are processed prior to culturing to decontaminate the specimen from other commensal microorganisms and to concentrate the MTB cells. This typically involves N-acetyl L cysteine and NaOH to liquefy and decontaminate the sputum specimen. This is then neutralized and the MTB cells concentrated via centrifugation in sealed containers to prevent risk of aerosolization. The cell pellet is then re-suspended in a small volume of saline to provide the inoculum. The decontamination step is important to limit the growth of other microbiological contaminants in the culture that would require reprocessing and significant delay in reporting of results.[83] NaOH is the most commonly applied decontamination method but there is a trade-off to its use. Higher concentrations of NaOH (e.g. 4% total volume) produce reduced contamination, but more MTB cells are killed.[84,85] Prolonged exposure to lower NaOH concentrations also has a similar bactericidal effect.[86] Culture media are also supplemented with antibiotics to further limit contamination.

Culture can be performed with solid or liquid media using automated or manual methods to generate results. Solid culture is the primary manual method of culture with Löwenstein-Jensen (L-J) medium, egg-based media being the most popular. In low- and middle-income countries, L-J is routinely used for MTB culture and DST due to its low cost and stability for several weeks if refrigerated. Other media for solid culture and DST are available, including natural (e.g. blood agar[87]) or synthetic media (e.g. Middlebrook 7H10 and 7H11).[88] A wide variety of microbial media manufacturers sell powdered synthetic media in addition to L-J slants. Although solid culture is a sensitive test method for MTB, its suitability for diagnostic purposes is limited by the time it may take to generate a positive result or rule out infection. A recent study noted an average time to result of 21 days when using L-J culture with sputum specimens.[89] When L-J is used for DST purposes, the average time to result is even longer, with one group reporting 63 days from the original receipt of the specimen.[90]

Semi-automated and liquid culture

Liquid culture can be used with any specimen type. The primary advantage of liquid culture is that the growth of MTB cells is more rapid (10–14 days) than culture on solid media, permitting faster diagnosis. In addition, a variety of liquid culture systems are commercially available that can automatically detect positive cultures and permit higher test volumes, greatly simplifying yet expanding the throughput of culture-based testing (Figure 13).

In 2007, a WHO Expert Group endorsed the use of liquid culture for the identification of MTB and for DST based on the performance of the BD mycobacterial growth indicator tube (MGIT™) system, noting that other liquid culture systems give similar performance.[91] The endorsement, however, does come with the caveats that testing should be performed in laboratories with uninterrupted power supply for critical equipment and appropriate infrastructure and biosafety procedures to prevent laboratory-acquired infections.

Incubators with automatic reading of the media bottles are made by BD, bioMérieux and Thermo Fisher Scientific (USA). Each of these technologies is US FDA-approved for the detection of MTB. The basic principle of each technology is similar whereby the media bottle is prepared and then inoculated with processed sputum, scanned via a barcode and then inserted into the machine. The measurement of growth of MTB in the bioMérieux BacT/Alert® 3D system is based on the increase of CO_2 that is detected via the change of reflectance from a sensor disc at the base of the tube, which gradually changes from green to yellow. The BD Bactec™ MGIT™ technology is based on the depletion of O_2, where initially present O_2 is quenching fluorescence from an indicator at the bottom of the vial and consumption of O_2 is gradually reducing such quenching. The Thermo Fisher Scientific VersaTREK® system measures growth by automatically monitoring the rate gas production (in this case CO_2) within the headspace of the culture bottles. All systems notify the laboratory personnel when a positive culture is detected or when the tube has been incubated for the appropriate time without indicating growth.

Figure 13. Three commercial liquid culture platforms: BD (MGIT™ 960, left); bioMérieux (BacT/Alert® 3D 120, centre); Thermo Fischer Scientific (VersaTREK® 528, right)

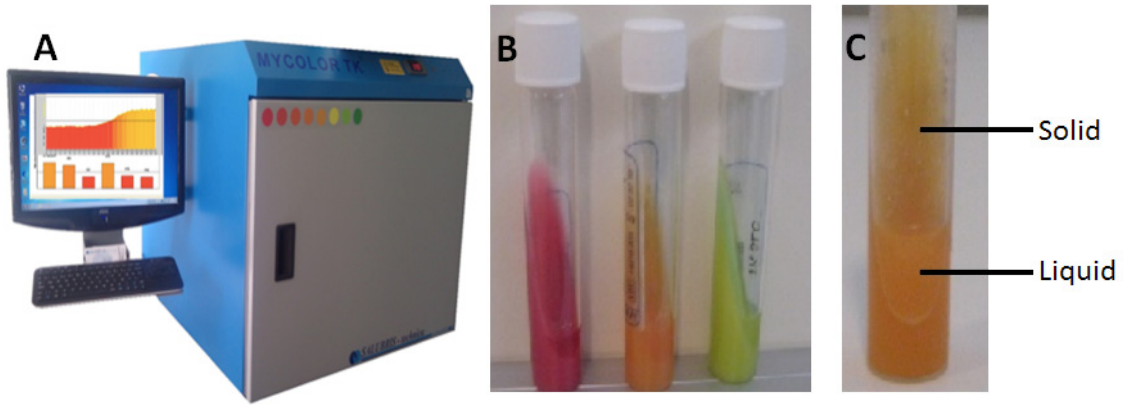
Sources: BD MGIT™ 960 image courtesy and © Becton, Dickinson and Company. Reprinted with permission. bioMérieux BacT/Alert® 3D image reproduced with permission from bioMérieux. Thermo Fischer Scientific VersaTREK® 528 photo courtesy of Thermo Fisher Scientific. Copying is prohibited.

BD markets the Bactec® systems, which use MGIT™. The Bactec MGIT™ 360 and 960 instruments are available; the Bactec MGIT™ 960 can host up to 960 bottles at any point in time, which translates to approximately 8000 MGIT™ tubes per year. FIND has negotiated a pricing agreement with BD for purchase by HBCs; the list of materials and their cost are available at http://www.finddiagnostics.org/about/what_we_do/successes/find-negotiated-prices/.

The BacT/Alert® 3D Mycobacteria Detection Systems is manufactured by bioMérieux and comes in different modules and sizes, covering the entire range of 60 to 1440 bottles in incubation at any point in time. The Thermo Fisher Scientific VersaTREK® Automated Microbial Detection System also comes in two models that are both modular. The 528 version (hosting up to 528 bottles at any point in time) has greater throughput and can process 4700 culture bottles per year. All three culture systems are US FDA-approved for mycobacterial identification.

Salubris Inc. offers a series of products that also perform with automated TB culture (Figure 14). They manufacture: TK MEDIUM® SLC; a biphasic, culture medium that can be incubated and automatically monitored in the MYCOLOR TK® instrument (Figure 14); and TK MEDIUM SLC-L, a liquid formulation.

Figure 14. A: MYCOLOR TK automated incubator; B: TK colorimetric media with changes from red to orange (mycobacterial positive culture) red to green (contaminated culture); C: biphasic format of TK media with both solid and liquid media



Source: Images reproduced with permission from Salubris Inc.

TK MEDIUM® is an egg-based solid medium similar to L-J.[92] Unlike the liquid media used in other automated platforms, the TK media do not require any additives, creating a simplified workflow and reducing risk of contamination.[92] The media contain dyes that react upon the growth of microorganisms (Figure 14).[93] Upon mycobacterial growth, the original red colour turns orange and then yellow. The colour change is indicated before colonies are visible on the agar, improving the time to detection. Contamination by fungi or Gram-negative bacteria produces a green pigment; some Gram-negative bacteria can produce orange/yellow. The TK SLC medium is biphasic having both solid and liquid media (Figure 14). The TK Mycolor instrument detects and monitors the colour changes (from red to yellow or to green) and this system provides growth curves over time that are also analysed to predict the type of growing microorganism.[92] The TK Mycolor instrument is priced at ~US$ 15 000 and TK SLC Liquid® costs US$ 2–3 depending on volume. The TK Anti TM PNB kit (susceptibility testing to first-line drugs plus TB complex NTM differentiation) is listed at US$ 15–18.

The performance of TK MEDIUM® was evaluated against L-J culture in one large study where 16 303 specimens were assessed.[92] The authors reported that the median time to detection of the TK MEDIUM® was 15 days as opposed to 25 days using L-J. However, the early colorimetric detection of growth with TK MEDIUM® was on average 12 days, while for L-J it remained at 25 days. The TK media also produced more positive cultures than L-J, 12.63% versus 11.69%, respectively. The authors noted that contamination with L-J was observed in 5.33% of all slants, while only 0.55% using TK MEDIUM®.[92] A comparative

assessment between this system and the other liquid systems has not been performed, but recently a new liquid medium, TK SLC Liquid®, was compared to MGIT.[94] Overall, the performance of both media was similar, but MGIT™ had a faster median time to result, 7.7 days as compared to 15.1. Contamination was much more prevalent in the MGIT™ tubes (13.7%) compared to TK SLC Liquid (1.3%), which may be due to the preparation steps prior to inoculation of MGIT.

Manual non-commercial culture-based assays

Given that the application of automated culture-based testing requires significant infrastructure and purchase of capital equipment, a variety of simplified, manual culture methods using commercial media are available. BD and Salubris Inc. both note that their culture tube methods can be used manually. The BBL™ MGIT™ tubes can be incubated in a traditional incubator and growth indicated by shining a long-wave ultraviolet lamp onto the reactive disc on the tube base. Positive tests emit a vivid orange fluorescence and at the meniscus; negative tests remain non-fluorescent. With the Salubris Inc. TK MEDIA, the laboratory technician visually notes the colour change of the media as an indicator of mycobacterial growth (Figure 14B).

Other non-commercial culture-based methods include the microscopically observed drug susceptibility (MODS) assay, the nitrate reductase assay (NRA), colorimetric redox indicator (CRI) assay, phage-based assays and the thin layer agar (TLA) assay. Of these, MODS, NRA and CRI were endorsed by WHO as non-commercial methods for mycobacterial culture and DST in 2010.[95] These tests are proposed as direct or indirect tests for rapid screening of patients suspected of having MDR TB. Their use must be in clearly defined programmatic and operational conditions, in reference laboratories and follow strict laboratory protocols. In this section, we describe current perspectives on the performance of these assays and the commercial products on the market or in late-stage development.

The MODS method was developed by the Peruvian National Tuberculosis Control Program to improve upon existing diagnostic tests for MTBC.[96-98] The application of MODS is in diagnostic laboratories where routine manipulation of culture is not performed. The principle is a rapid and sensitive assay that can identify MTBC in addition to phenotyping resistance to RIF and INH. The test uses 24-well plates in which four wells address a single patient specimen: two wells are drug free, while the other two wells contain RIF and INH, respectively. After inoculation, the plates are inserted and sealed in zip lock bags and then incubated. Mycobacterial growth is identified by using an inverted light microscope to observe microcolonies in the liquid media. MTBC is morphologically different from many of the mycobacteria in that they have a chording morphology.[98] Alternatively, para-nitrobenzoic acid (PNB), a compound that inhibits the growth of MTBC, but not of NTM, can be added to one (drug-free) well to discriminate between NTM and MTBC.[99]

The performance of MODS is faster than with solid culture.[89] One study noted MODS detection of MTBC took a median of 9 days as opposed to MGIT™ (16 days) and solid culture with 7H10 (29 days).[100] A different study using MODS on smear-negative and smear-positive specimens noted median culture times of 11.7 and 9 days, respectively.[101] In a recent meta-analysis, the MODS assay had a sensitivity of 98% for RIF resistance and a specificity of 99.4%. The mean turnaround time was about 10 days.[102] While MODS is an effective and relatively low-cost assay, it requires that materials are procured from multiple vendors, which can present a challenge for many low- and middle-income countries. To circumvent procurement and supply issues of variable components, Hardy Diagnostics offers a CE-IVD marked MODS Test Kit™, which includes all necessary reagents for a single MODS plate and a colored guide to simply plate preparation prior to inoculation. To limit the risk of spills and cross-contamination, they have replaced the loose plastic lid with a tightly sealing silicone lid.

A comparative evaluation of the Hardy Diagnostics MODS Test Kit™ and conventional MODS has recently been completed in Peru.[103] The authors reported that the sensitivity and specificity achieved with the MODS Test Kit™ were 99.3% and 98.3%, respectively, when compared to conventional MODS. The concordance for direct DST for RIF and INH was 97.9% with the MODS Test Kit™ and conventional MODS. The median time for detection including RIF and/or INH resistance was 8.5 days for the MODS Test Kit™

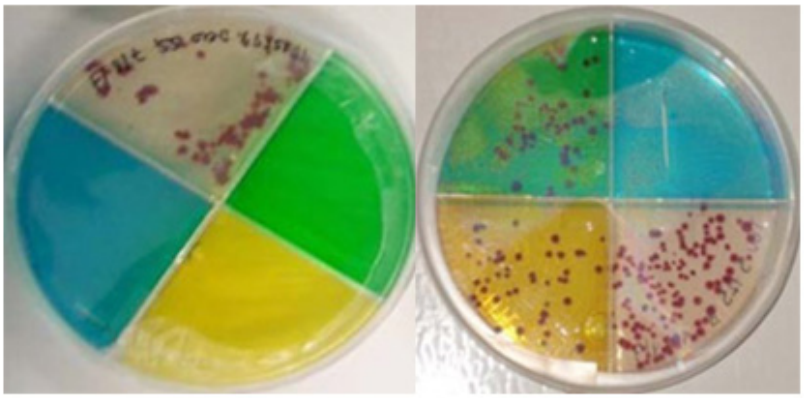
compared with 10 days for the conventional MODS method. A standard operating procedure for MODS has been developed by the culture and DST subgroup of the STOP TB Partnership NDWG, for use by countries wishing to apply MODS to their TB diagnostics programme.[104]

NRA, also known as the Griess method, relies on the growth of MTBC to reduce nitrate to nitrite.[105] This method uses L-J slants that contain potassium nitrate and the reduction of nitrate is detected using the Griess reagent, a chemical substrate that in the presence of nitrite produces a coloured reaction by turning from pink to purple. An advantage of this test is that it can be applied before colonies are visible on the slant, therefore, reducing time for detection.

The principle of CRI is very similar to NRA and is based on the reduction of a coloured broth indicating the growth of mycobacteria. A dye is added directly to the culture media and observed on a daily basis for growth. While CRI can be used for the direct culture of MTBC,[106] the literature shows a preponderance of studies using CRI as a rapid DST method after the initial culture isolation of MTBC. A meta-analysis review of studies investigating CRI for rapid screening of RIF and INH resistance showed a median time to result was within 7–14 days and that most studies had very high sensitivity and specificity.[107] NDWG has also published standard operating procedures for the application of NRA and CRI for diagnosis and DST.[108,109]

TLA uses a solid medium such as Middlebrook 7H11 and utilizes the microscopic detection of early mycobacterial growth.[110] Colony detection is typically much faster than L-J medium at 7–14 days; a time to result similar to liquid culture, but at reduced cost.[111,112] Duplicate plates can be prepared with PNB and, therefore, discriminate MTBC from other mycobacterial species. While not currently endorsed by WHO, there are two commercial developments under way to develop TLA products for MTBC culture. Nanologix Inc. (USA) is producing the BioNanoPore (BNP™) Ultra-Fast Identification Technology. This is a TLA plate for initial colony incubation that is overlaid with a nitrocellulose filter also pre-coated with agar. After a preliminary incubation period, the membrane is removed and then applied to a chromogenic dye-based TLA plate. After 20–30 minutes, the dye is reduced with a dark purple to black colouration of the bacterial colonies on the membrane.[113] Nanologix Inc. has also developed a packaging method in which TLA plates are flat packed to limit catastrophic plate damage during shipping as compared to conventional vertically stacked plates. Nanologix Inc. also adds an inert gas to prolong media stability.

Figure 15. Rapid Colorimetric DST assay



Notes: Food colouring dye is used to discriminate each quadrant for MTBC (clear) and DST (yellow, RIF; green, INH; blue, CFX).[114] The addition of STC indicates the presence of viable microcolonies as dark red spots. The left-hand plate indicates a drug-susceptible MTBC isolate, while the right-hand image indicates an MDR TB isolate that is not XDR.

Source: Images reproduced with permission from FIND and Imperial College, London.

Researchers at the Imperial College (London; supported by FIND) have developed the Rapid Colorimetric DST, a TLA plate that can identify MTBC and phenotype MDR and XDR MTB (Figure 15). The TLA plate is divided into four colour-coded quadrants that contain the growth media only (Middlebrook 7H11), or 7H11 media augmented with RIF, INH and ciprofloxacin (CFX), respectively.[114] The CFX component is a proxy for the identification of fluoroquinolone (FLQ) resistance as the resistance mechanisms to different FLQs are typically the same.[115] The test was developed to be used in minimally resourced settings where traditional laboratory culture is not routinely performed. The test consists of a collection pot to liquefy and stabilize sputum for up to five days at ambient temperature for transport to a laboratory. The contents of the pot are directly transferred onto the plate without further processing. The lid of the plate is locked to minimize biohazard risk and then stored in a zip lock bag to further protect personnel.[114] Growth is monitored using a conventional light microscope. As microscopic review of microcolony formation is tedious, a CRI (2, 3-diphenyl-5-2-thienyl tetrazolium chloride [STC]) is added to indicate microcolony growth to highlight plates to be assessed for growth.[116]

The performance of the Rapid Colorimetric DST assay was assessed on a panel of MTB isolates that had been previously tested using MGIT™.[114] Overall, the assay was found to perform well although the time to result at 13 days was slower than the MGIT™. The test showed excellent sensitivity for the three drugs tested, with the INH assay showing highest sensitivity. The authors noted that the low cost and simplicity of use make this an affordable alternative to automated DST culture. However, more studies are required where parallel performance is assessed using direct analysis of sputum. FIND is currently assessing possible partners to commercialize this assay.

Drug susceptibility testing products

Information on drug susceptibility is important in determining effective treatment for patients who do not respond to first-line therapy. Contacts may also be treated appropriately and it offers epidemiologic data on the prevalence of drug-resistant MTB within a given population or region. BD, Thermo Fischer Scientific and Salubris Inc. have a variety of media products for detecting resistance to RIF, INH, pyrazinamide (PZA), ethambutol (EMB) and streptomycin (STR) using their automated culture platforms (Figure 13 and Figure 14). For extensively drug-resistant (XDR) TB, the critical concentrations to detect drug resistance to second-line drugs have been established using the Bactec™ MGIT™platform.[117]

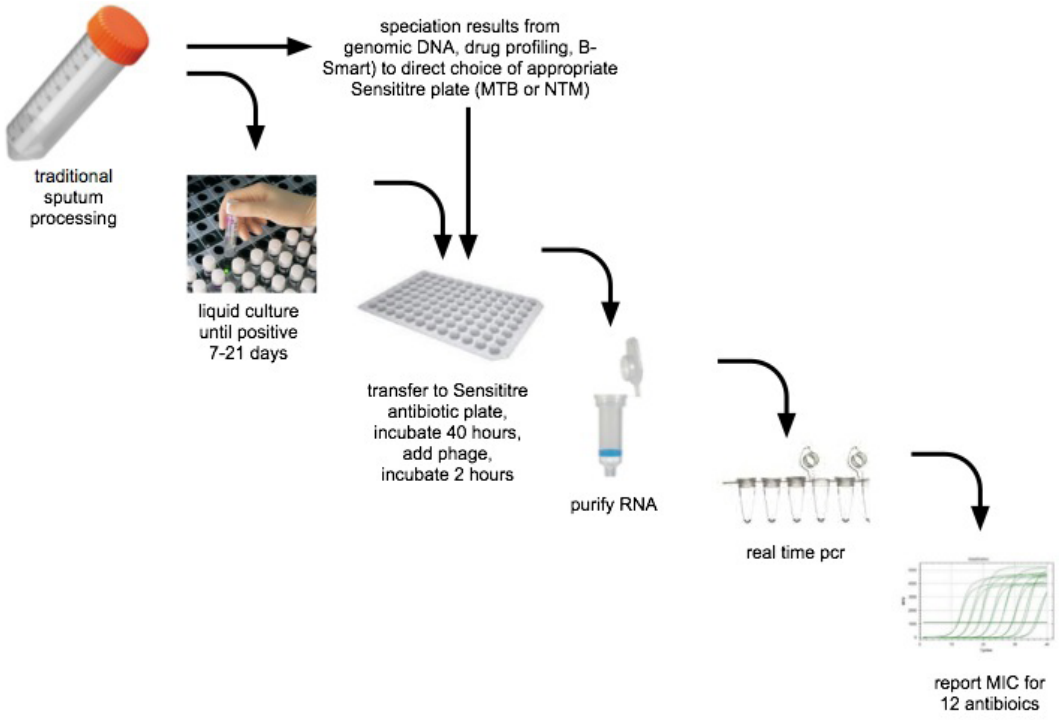
There is evidence that non-commercial assays can be used for DST. A recent multicentre study from six countries used direct NRA to screen for MDR and XDR TB from smear-positive sputum samples.[118] Performance was comparable with reference methods and the median time to result varied by setting with 21 days being the longest. The MODS assay format can also be used to screen for XDR TB. One prospective study investigated using MODS to screen for resistance to ofloxacin (OFX) and kanamycin (KM) in archived clinical isolates.[119] The sensitivity of the assays was 96.2% and 91.5% for OFX and KM, respectively. The specificities were 100% and 98.7%, respectively, and the median time to result was only seven days. Similarly, another study reported on the assessment of CRI for rapid identification of drug resistances to RIF, INH, OFX, KM and capreomycin in comparison to MGIT™.[120] The authors noted comparable performance between sites and noted that with results in one week the CRI assay is more rapid than the solid agar DST methods (from three to six weeks). The turnaround time for NRA was the same as for MGIT™, but the test was less expensive to employ.

Figure 16. Thermo Fischer Scientific Sensititre® AIM™ System (left) and Sensititre® Vizion™ (right) to automate 96-well plate inoculation and for the analysis of drug sensitivity during incubation

Source: Photo reproduced with permission from Thermo Fisher Scientific. Copying is prohibited

The DST methods described above use predetermined critical concentrations of each drug that indicate sensitivity or resistance. Many reference laboratories use the agar proportion method where the isolates are exposed to a range of increasing drug concentrations to more precisely establish the level of resistance to each drug. Typically, laboratories will use solid media to perform this. This method is time consuming to prepare plates and read the plates for results, and growth on solid media is typically slow. Thermo Fischer Scientific offers three CE-IVD marked liquid DST plates for use with their Sensititre system: the MYCOTB, RAPMYCO and SLOMYCO to assess minimum inhibitory concentration (MIC) for proportional drug susceptibility analysis of MTB culture isolates, rapid or slow growing isolates of MTB, respectively. These tools use 96-well plates that contain 12 first- and second-line drugs in ranges of at least seven doubling dilutions for each. Thermo Fischer Scientific offers semi-automated tools to perform rapid plate inoculation and reading of the plates (Figure 16). The Sensititre® AIM™ is a small touchscreen-operated inoculating station; the Sensititre® Vizion™ is a plate imager that displays an image of the susceptibility plate on a computer screen and provides the template of drug dilutions and colour-coded interpretive results to reduce errors. The plate image is visually scored, but plates can also be manually scored via an inverted mirror.[121] The current cost of equipment and reagents are not known.

Figure 17. Principal processes in the B-SMART™ assay (Sequella Inc.)



MIC, minimum inhibitory concentration.

Notes: MTBC cells are first cultured and then infected by a bacteriophage containing an engineered reporter system that produces a transcribed RNA target. This target can then be extracted and measured using reverse transcription PCR or other nucleic acid amplification methods. MTBC cells killed by the drug do not host the replication process and, therefore, the detector product is absent in drug sensitive isolates.

Source: Image reproduced with permission from Sequella Inc.

Another commercial assay in development for the identification of both MDR TB and XDR TB by DST is B-SMART™ from Sequella Inc. (USA). This assay uses a genetically engineered bacteriophage to measure viability of MTBC in the presence of any antibiotic of choice. Phage infection of live MTBC cells generates an RNA molecule in large amounts, the circular Surrogate Marker Locus (cSML) (Figure 17). This is detected via reverse transcription PCR.[122] More recently, Sequella Inc. has described a simplified system using magnetic bead purification of the cSML, and the labelling of amplicons via nucleic acid sequence-based amplification (NASBA) for detection via a nucleic acid lateral flow assay. If antibiotics are effective against the pathogen, the bacterial cell cannot support phage infection and there is no cSML synthesis. If the pathogen is drug resistant, cells are infected and the cSML is efficiently synthesized. The test is designed to provide detection of multidrug/XDR TB directly from sputum within two days, but is also being developed to speed comprehensive minimum inhibitory concentration determination for major anti-TB drugs using Sensititre plates. It is aimed at the reference laboratory as bacterial culture manipulation is necessary. The detection of viability can be achieved as early as 16 hours and definitively at 40 hours post incubation; it is, therefore, considerably faster than phenotypic DST. Although the B-SMART™ assay incorporates a molecular test, there is no specific target allele for genotyping drug resistance and so specificity is improved. The product release is due in 2015.

Rapid speciation tests for culture-positive specimens

While all of these culture systems can identify the growth of mycobacteria, the discrimination between MTBC and NTM must be confirmed. PLHIV are more susceptible to an NTM pulmonary infection, typically when CD4 counts are $< 50/\mu L$.[123] NTM are acquired from the environment and not considered contagious. Pulmonary disease via an NTM infection can be difficult to treat and, therefore, early identification may improve treatment outcomes.[124-126]

Traditional biochemical testing to confirm MTBC is time consuming, and other approaches such as high-pressure liquid chromatography or NAATs require further equipment (NAAT-based speciation tests are discussed in depth later). The use of PNB to discriminate MTBC from NTM requires an extra culture to be performed. Three rapid MTBC speciation assays employ immunochromatographic strips to detect MPT64, a protein secreted only by MTBC. These are available from BD (MGIT™ TBc Id), SD BIOLINE (TB Ag MPT64 rapid) and TAUNS (Capilia™ TB-Neo).[127] The MGIT™ TBc Id test is for liquid culture only, while the other two assays can be used to confirm MTBC from either liquid or solid cultures.[128] The Capilia™ TB-Neo is an improved design on the Capilia™ TB, which was endorsed by the Expert Group in 2007.[127]

Advantages: Culture-based methods still offer the greatest level of sensitivity when detecting MTBC, and the growth of a culture can allow for the speciation of mycobacteria and phenotypic DST. All culture methods described here can be used for DST or provide DNA for genotypic methods (described below). When high-volume testing of cultures is necessary, then automated equipment can allow greater throughput. There is also the development of manual assays for use in settings where automated tools are unaffordable or impractical. Many non-commercial assays have similar performance to the automated liquid culture systems and positioning them closer to the patient may improve early detection of MDR TB. There is continued commercial interest in the manufacture of MODS, CRI, TLA and phage-based assays.

Disadvantages: Culture-based diagnosis for TB is slow, typically taking from one to three weeks with liquid media (and much longer for solid cultures) and requiring the resources to permit safe processing and manipulation of cultures. Careful decontamination of specimens during processing prior to inoculation is critical as prolonged exposure to NaOH can also kill MTBC cells. The slow turnaround time reduces the clinical impact of culture results. Training and strict adherence to protocols to decontaminate specimens is important for gaining consistent test results. Non-commercial cultures can be poorly standardized and EQA systems are required, along with extensive training and standardization. Newer commercially available tools such as TK media, MODS and BioNanoPore (BNP™) plates still have limited evidence of performance in resource-limited settings. High cost of commercial liquid cultures and DST has been a major barrier for their scale-up in developing countries, especially given associated costs to install appropriate biosafety infrastructure and equipment.

5.4.5. Biomarkers to detect active TB or indicate LTBI

Perspective: Many of the diagnostic tools described in this report focus on the use of sputum as the specimen from which to diagnose PTB. While sputum collection is non-invasive and relatively easy to perform, it can still present problems with an inadequate sputum volume, an excess of saliva, paucibacillary samples and the required processing steps before use in a diagnostic test. A direct and rapid method for the diagnosis of all active infections of TB (including EPTB and paediatric TB) using a non-invasive or minimally invasive specimen such as urine or fingerstick whole blood would be of huge benefit for the early diagnosis of MTB in peripheral settings.

While highly effective, low-cost and lateral flow-based rapid diagnostic tests (RDTs) are available for other infectious diseases such as HIV or syphilis, similar RDTs for MTB are ineffective.[129] There is a variety of commercially available serodiagnostics, antibody-based tests for TB in different formats (e.g. enzyme linked immunosorbent assays [ELISAs]) or RDTs. Unfortunately, evidence-based reviews indicate that none has adequate performance[129,130] or that these tests are not cost effective when compared to other conventional tests for MTB.[131] Based upon such evidence, WHO took the unprecedented step of releasing a policy statement that recommended against use of commercial serologic ELISA or RDT assays for TB diagnosis.[20]

The WHO policy statement encouraged further work in this area, however, and did not rule out new biomarkers for TB diagnosis. There is significant investment, research and investigation into the discovery and utility of novel biomarkers in this area. For example, the BMGF has invested over US$ 10 million in TB diagnostic biomarker research. Biomarkers and assays to detect them currently being developed for TB diagnostic use can be roughly categorized into five areas:

1. assays that detect T-cell immune response when stimulated with TB antigens;
2. serologic assays that directly detect antibodies to MTB antigens;
3. detection of VOCs indicating the presence of MTB within the patients (e.g. breath or urine VOC tests);
4. antigen detection assays that specifically detect mycobacterial derived compounds or antigens;
5. assays based on inflammatory markers for EPTB.

Immune response-based tests for LTBI

In most individuals, initial MTB infection is eliminated or contained by host defenses and remains latent. Although latent and active (i.e. symptomatic; infectious) TB disease are part of a dynamic spectrum, people with LTBI are generally considered to be asymptomatic and not infectious. However, latent TB bacilli may remain viable and "reactivate" later to cause active TB disease. Identification and treatment of LTBI can substantially reduce the risk of development of disease, and is an important TB control strategy, especially in low-TB incidence settings where reactivation of LTBI often accounts for the majority of non-imported TB disease.

The goal of testing for LTBI is to identify individuals who are at increased risk for the development of active TB; these individuals would benefit most from treatment of LTBI (also termed preventive therapy or prophylaxis). There is no diagnostic gold standard for LTBI and all existing tests are indirect approaches that provide immunological evidence of host sensitization to TB antigens. There are two accepted, but imperfect, tests for identification of LTBI: tuberculin skin test (TST) and IGRA. Both tests depend on cell-mediated immunity (memory T-cell response), and neither test can accurately distinguish between LTBI and active TB disease. A detailed recent review of these tests is available elsewhere.[132]

TST, performed using the Mantoux technique, consists of the intradermal injection of 5 tuberculin units of purified protein derivative PPD-S or 2 tuberculin units of purified protein derivative RT23 (these two types of purified protein derivative are considered equivalent at these concentrations). In a person who has cell-mediated immunity to these tuberculin antigens, a delayed-type hypersensitivity reaction will occur within 48–72 hours. The reaction will cause localized induration of the skin at the injection site, then the transverse diameter is measured (as millimetres of induration) by a trained individual and interpreted using risk-stratified cutoffs (e.g. lower cutoff is used for HIV-infected people).

Several companies make commercial tuberculin products, including the Statens Serum Institute (SSI; Denmark), TUBERSOL® by Sanofi Pasteur (France) and Aplisol® by JHP Pharmaceuticals (USA).

TST has several known limitations. False-positive and false-negative results can occur. There are two important causes of false-positive results: NTM infection and prior bacillus Calmette-Guerin vaccination. False-negative TST results may occur because of limited sensitivity in particular patient subgroups (e.g. immunosuppressed individuals [due to medical conditions such as HIV infection or malnutrition] or those taking immunosuppressive medications), or because of pre-analytic or analytic sources of test variability.

IGRAs are in vitro blood tests of cell-mediated immune response; they measure T-cell release of interferon-gamma (IFN-γ) following stimulation by antigens specific to MTB—early secreted antigenic target 6 (ESAT-6), culture filtrate protein 10 (CFP-10) and the TB7.7 peptide antigen (only QFT). These antigens are encoded by genes located within the region of difference 1 (RD1) locus of the MTB genome. They are more specific for MTB than purified protein derivative because they are not encoded in the genome of any bacillus Calmette-Guerin vaccine strains or most species of NTM other than *M. marinum*, *M. kansasii*, *M. szulgai* and *M. flavescens.*

Two commercial IGRAs are available in many countries: the QuantiFERON®-TB Gold In-Tube (QFT) assay (Cellestis/Qiagen; Carnegie, Australia); and the T-SPOT® TB assay (Oxford Immunotec; Abingdon, United Kingdom). QFT is approved by the US FDA, Health Canada, Japan, Russia Federation and Taiwan and is also CE marked, while the T-SPOT® TB assay is approved in Europe (CE marked), the USA, China and Japan. More recently, commercial IGRAs have emerged from India (Immucheck TB Platinum, Immunoshop India Pvt Ltd) and China (e.g. TB-IGRA, Beijing Wantai Biological Pharmacy Enterprise Co. Ltd; ASACIR TB (A.TB), Haikou VTI Biological Institute). There is very little published evidence about the accuracy or quality of these newer commercial IGRAs, and there are no policy endorsements of these newer IGRAs.

The extensive evidence base on LTBI tests has been reviewed elsewhere.[132] In brief, published evidence suggests that both TST and IGRA are acceptable, but imperfect tests. They represent indirect markers of MTB exposure and indicate a cellular immune response to MTB. Neither test can accurately differentiate between LTBI and active TB, distinguish reactivation from reinfection or resolve the various stages within the spectrum of MTB infection. Both TST and IGRA have reduced sensitivity in immunocompromised patients, and have low predictive value for progression to active TB. To maximize the positive predictive value of existing tests, LTBI screening should be reserved for those who are at sufficiently high risk of progressing to disease. Such high-risk individuals may be identifiable using multivariable risk prediction models that incorporate test results with risk factors and using serial testing to resolve underlying phenotypes. In the longer term, basic research is necessary to identify highly predictive biomarkers.

Currently, Autoimmun Diagnostika (hereafter AID; Germany) is developing an assay to measure IGRA and Il-2 expression to discriminate latent for active TB. As with the other IGRA assays, the test requires whole blood and the enrichment of the buffy coat cells prior to exposure to the MTB antigens ESAT-6, CFP10 and purified protein derivatives of MTB for stimulation. Essentially, it is the same test principle as with the IGRAs described above. The test takes from two to three days from specimen collection to the test result; the actual time required for sample and test preparation takes from five to seven hours. AID notes the process can be semi-automated after the buffy coat enrichment step. They offer the AID *i*Spot Reader to screen the test and automated software to score test results. This is a laboratory-based test whose key requirements include electrical power, clean water, a laminar flow hood, cell culture reagents, a centrifuge and a CO_2 incubator. The intended market prices are €63 (euros) per test and €33 900 for the AID *i*Spot Reader. AID aims to release this product in 2015.

In 2011, WHO published its policy on use of IGRAs in low- and middle-income countries.[21] The policy states that:

- there are insufficient data and low-quality evidence on the performance of IGRAs in low- and middle-income countries, typically those with a high TB and/or HIV burden;
- IGRAs and TSTs cannot accurately predict the risk of infected individuals developing active TB disease;
- neither IGRAs nor TSTs should be used for the diagnosis of active TB disease;
- IGRAs are more costly and technically complex to do than TSTs; given comparable performance but increased cost, replacing TSTs by IGRAs as a public health intervention in resource-constrained settings is not recommended.

Advantages: IGRAs are more specific for LTBI than TSTs, and the in vitro format of the assays is more convenient.

Disadvantages: IGRAs and TSTs cannot distinguish latent from active TB, and both are imperfect markers of latent infection. Both tests have limited predictive value for progression to active disease. IGRAs require venous whole blood thus phlebotomy is required. The testing is laboratory based and requires instrumentation and trained staff, contributing to the overall high cost of the test. Recent studies show highly variable results, especially with repeated, serial testing of healthcare workers. The test is also less accurate in certain populations, including children, immunocompromised patients and people who have recently been infected.

5.4.6. Serodiagnostic assays for detection of MTB antigens or immune response to MTB

The most comprehensive and advanced development work to design a serodiagnostic assay for the rapid detection of antibodies to MTB has been led by FIND and partners with a diagnostics platform being developed by MBio Diagnostics Inc. (hereafter MBio; USA) using their unique multiplexed immunoassay platform. To identify which MTB proteins most commonly elicit an immune response, a preliminary study screened over 500 serum samples from a global distribution of individuals who were either healthy or diagnosed with active or LTBI disease.[133] An MTB protein array comprised of 4099 proteins (over 99% of the predicted MTB proteome) was used to identify the antigens most commonly reactive to sera from cases of active TB. From this study, a small number of antigens were found.[133] MBio is developing a multiplexed ELISA with 57 MTB antigens and 31 control spots into a fluidic channel integrated onto a single disposable cartridge (Figure 18).

Figure 18. MBio automated portable reader and disposable detection cartridges

Source: Image reproduced with permission from MBio.

The initial design requires only 10 µL of whole blood or 5 µL of serum or plasma, which is mixed with a diluent and 150 µL added to the cassette. No pumping is necessary as capillary flow draws the sample over the array spots followed by wash and labelling steps. MBio has recently demonstrated that 150 µL of whole blood can be added directly to the cartridge without dilution. The antibodies that bind to the TB antigen array spots are detected via a labelled secondary antibody to human antibodies. Detection is achieved via fluorescent measurement of each antigen and control spot in a small portable MBio reader (Figure 18). The assay illumination approach is a variation on planar waveguide technology, which uses low-cost lasers, optics and imaging sensors that are now ubiquitous in cell phones and consumer electronics.134 The scoring of test results is interpreted automatically by the instrument and the time to read a cartridge is less than one minute. The turnaround time to process one sample is under one hour. The instrument can rapidly analyse each cartridge, and multiple cartridges can be batch processed. The system takes less than one hour to install, and training requires one day.

To validate the performance of the equipment and assay, phase 1 validation studies were performed in Peru and Viet Nam where 200 patients were screened. Whole blood, plasma and serum samples were tested from each patient and the data compared with data derived from the same specimens analysed in a reference laboratory using a multiplexed ELISA. The concordance of both assays was greater than 75% for all antigens and the predetermined performance characteristics were met. The assay is now through phase II validation with a larger cohort of 700 participants. The data are currently under review and if results are positive, then a diagnostic set of MTB antigen targets will be determined.

Other groups are also looking to develop RDTs using detection of either MTB-specific antigens or antibodies. TB Biosciences (USA) is currently developing a visually scored immunochromatographic strip test based on antigen discovery work performed at the New York University School of Medicine. They noted

that antibodies from cases with early TB and advanced TB and for TB/HIV co-morbidity are only observed in a small subset of MTB proteins.[135-137] To be effective, since a single antigen-based test does not have acceptable performance, ideally a pooled group of antigens should give greater performance.[136,137] TB Biosciences focused on three characterized antigens and the reactivity of synthetic peptides (derived from each protein) was assessed for reactivity with sera.[138,139] They were able to establish the dominant epitopes of each protein antigen. As synthetic peptides are chemically synthesized, scaled production is more uniform in terms of lot quality and more cost effective when compared to using recombinant proteins. TB Biosciences has created an RDT using these antigens. A preliminary evaluation in India has just been completed with 400 participants and the data are currently being assessed. TB Biosciences anticipates product release in Q2 2015.

Urine is an ideal specimen type as it is non-invasive, easy to collect, and biomarkers and metabolites from systemic infectious diseases are excreted into this matrix. Commercial TB diagnostic products are available to detect the presence of lipoarabinomannan (LAM) in urine. LAM, a 19 kDa glycolipid, is a key component of the mycobacterial cell wall and has been linked to pathogenicity.[140] LAM is produced by actively growing cells and degradation of the cell wall also releases LAM into the blood where it is ultimately expelled in urine. Alere markets the Determine™ TB LAM Ag (an immunochromatographic strip) that targets LAM in urine with polyclonal antibodies. Another Alere LAM assay product, the LAM ELISA Clearview™ is scheduled to be discontinued soon.

The Determine™ TB LAM Ag is an RDT that was commercially launched in 2013 by Alere and takes under 30 minutes to perform and requires 60 µL of urine per test. The user visually scores the reaction. A meta-analysis of LAM immunoassays noted that they are inadequate for diagnosing TB in HIV-negative patients.[141] The performance of the Determine™ TB LAM Ag assay to detect TB in PLHIV has been shown to have high specificity (>98%), but markedly lower sensitivity.[142] However, the same study noted that there was a correlation of increasing sensitivity with decreasing CD4 cell counts. For example with CD4 counts >200 cells/µL the sensitivity was only 4%, but this increased to 66.7% when CD4 counts were <50.[142] The same study demonstrated incremental improvement in sensitivities when the Determine™ assay was combined with other tests such as smear microscopy or Xpert® MTB/RIF for all groups with CD4 counts under 200 cells/µL. Another group has since also reported that the use of the Determine™ TB LAM Ag and Xpert® MTB/RIF provides better sensitivity.[143] Current evidence also suggests that the Determine™ assay has poor performance in diagnosing paediatric TB and, unlike adults, should not be used even within an HIV-infected subpopulation.[8,11,144] FIND is currently working together with academic partners to assess the data available on the Determine™ TB LAM Ag in preparation for a possible WHO review.

Other groups are assessing the utility of urine-based biomarkers to inform on active TB disease.[145,146] While the early data suggest that biomarkers other than LAM can be used to diagnose active TB from urine, a platform and manufacturer are as yet unknown and it is unlikely that a test will be marketed until after 2016. FIND is currently working with Standard Diagnostics (Republic of Korea; a subsidiary of Alere; USA) to investigate the early development of an RDT to detect LAM in sputum.

Advantages: The detection of TB LAM Ag is rapid and can aid in the diagnosis of TB in HIV-infected patients with low CD4 counts who are particularly at risk of mortality if not treated. Rapid diagnostic strip tests are commonly used in developing countries thus user familiarity with the basic test format is high. The assay can also indicate EPTB infections, which are more common in PLHIV, particularly in those with advanced immune suppression.[147,148] EPTB is difficult to diagnose in many cases, especially in PLHIV. An effective rule in/rule out assay can permit more rapid treatment of suspected cases.

Disadvantages: LAM assays have low sensitivity in patients with CD4 counts greater than 200 cells/µL. LAM assays are not specific for MTBC as all mycobacteria synthesize LAM, and other bacteria, dust, dirt and faeces can also possibly confound test results.[149,150]

Other biomarkers for MTB

MTBC expresses beta lactamase, an enzyme that degrades penicillin by cleaving the β-lactam ring. A group at Texas A & M University (USA) developed fluorogenic β-lactam substrates that generate fluorescent products when cleaved by β-lactamase.[151] Structural analysis of the binding region of MTB β-lactamase has allowed scientists to create β-lactam analogues that are specific to the MTB enzyme that cannot be digested by β-lactamase produced by other species of bacteria.[152] The authors noted the successful detection of live MTB in less than 10 minutes, even in unprocessed human sputum. This group is developing a rapid assay for MTBC infection by using the presence of β-lactamase in sputum via their Reporter Enzyme Fluorescence (REF) system.[153] Recently, new data were released by this group describing a cephalosporin-based molecule for which BlaC had 120 000 times greater specificity compared to TEM-1 β-lactamase. An assessment of this molecule with 50 clinical samples showed detection of MTBC with 90% sensitivity and 73% specificity within one hour.[154] A product based on this technology, the TB REaD™ POC assay, is now under development via a partnership of Global BioDiagnostics (GBDx; USA) and FIND with support from the Wellcome Trust (United Kingdom) and the BMGF.

The test is targeted for use as a TB diagnostic or alternatively for triage at the peripheral health-care level where an estimated 60% of suspected cases seek care. The time to result is aimed at 30 minutes with a maximum of five steps to generate a result. While the test will be qualitative, a semiquantitative approach is conceivable. An automated, battery-powered reader is being developed to automate result interpretation and data will be stored and uploaded via wireless networks. The test is anticipated to be released by 2016.

Other groups continue to investigate potential biomarkers that can indicate active TB and discriminate this from LTBI. ProteinLogic (UK) has developed their Immiprint system, a multiplexed antibody array, to detect and quantify the expression of soluble forms of cluster of differentiation (sCD) antigens from serum. [155] CD antigens are expressed on cells of the human immune system in specific patterns relating to the specific challenge of an infection or disease;[156,157] TB infection creates a unique fingerprint of sCDs for active and latent TB. In 2103 they were awarded a £1. 2 million (GBP) grant by the UK Technology Strategy Board with which to translate the sCD biomarkers onto that multiplexed ELISA format in collaboration with Microtest Diagnostics (UK). The current work is still in the development stage but the company aims to have a product released in 2016. More upstream developments efforts have shown that down regulation of CD127 occurs in TB antigen–specific CD4 T-cells and several groups have noted the potential of this for use in a minimally invasive diagnostic assay for MTB infection.[158,159] Very recently, a group reported on the proof of concept testing of the T-cell activation marker–tuberculosis (TAM-TB) assay to diagnose TB in a pediatric population.[160] The assay uses standard flow cytometric procedures and equipment to detect fluorescently labeled tuberculin stimulated CD4 T-cells and concomitantly measure counterstained CD127 on the same cells. The study cohort, though quite small in size demonstrated that the TAM TB assay had a sensitivity of 83% for TB infection with a specificity of 96.6% using TB culture as a reference standard. The authors noted that 5 other cases of TB were identified by the TAM-TB assay but were culture negative. The clinical symptoms were strongly suggesting of TB infection and it should be noted that culture identification of TB in from pediatric patients can suffer from poor sensitivity.

5.4.7. VOCs

Perspective: MTB cells produce a variety of metabolic products in vivo, including methylated derivatives of n-alkanes, naphthalene and benzene that create chemical signatures, which if accurately detected can indicate MTB infection.[161,162] The assessment of the MTB metabolic products may also have potential for assessing treatment outcomes.[163] Detection of VOCs has been performed in urine, sputum and breath. The detection of MTB infection via breath is especially compelling as it is minimally invasive, provides a rapid result, does not require sample preparation and reagents, and unlike many NAATs does not present a risk of product contamination of equipment or the test site. VOCs could aid in the improved diagnosis of MTB in children and PLHIV, two groups that present significant challenges for TB diagnosis using current tools. The potential of using VOCs has been demonstrated using giant African pouched cane rats trained to identify VOCs from TB-infected sputum. This has shown similar or better performance to the performance of smear microscopy.[164,165] APOPO, a Belgian NGO, is leading a social enterprise that researches, develops

and implements detection rat technology for humanitarian purposes such as TB detection and landmine action. The proposed simplicity of VOC breath-based detection modalities could provide screening for TB diagnosis in the community by minimally skilled workers.

Pipeline technologies: There are several developers working this space, including Menssana Research Inc. (USA), the eNose Company (Netherlands), Rapid Biosensor Systems (United Kingdom), Metabolomx (USA) and Next Dimension Technologies (USA). All are developing different core technologies to detect MTB at the POC using VOC tests. The Menssana Research Inc. product, the Breathscanner™, is a tool that measures alveolar breath samples that are subsequently separated by gas chromatography and detected with flame ionization or surface acoustic wave detection. Menssana Research Inc. has also developed Breathlink™, a cloud-based application for the collection, concentration and analysis of VOCs detected by the Breathscanner™. The test takes minutes to perform. A study in 2012 assessed the performance of the mobile tool (with data uploading to a cloud-based analysis system) in three countries with a total of 251 participants who were screened.[166] Overall, performance was scored at 71.2% sensitivity and 72% specificity, which was similar to performance of the laboratory-based system.[161] The authors hypothesized that cutoff values on the mobile system can be created to maximize the negative predictive value to 99% with a corresponding positive predictive value of 13%. Although the device is CE-IVD marked, independent validation studies are required.

Figure 19. Hand-held Aenose VOC monitor for TB diagnosis produced by the eNose Company

Source: Image reproduced with permission from eNose Company.

The Aenose monitor analyses VOCs via an array of two different types of metal-oxide sensors. The device measures chemical adsorption and desorption dynamics at the sensor surfaces by passing exhaled air followed by clean air over the sensors in a process that takes 10 minutes. An initial process takes five minutes in order to permit the patients to flush any environmental influences from the lungs. The use of sensors allows miniaturization of the technology, creating a small hand-held device, as compared to gas chromatographic tools used in the Breathlink™ (Figure 19). In addition, the developers claim that this technology is cheap to make at scale. The original technology had the patient exhale at least 1 litre of air into a collection bag, but the new device directly monitors the breath of the patient. A study compared the performance of a prototype device to smear- and culture-based diagnosis of MTB. The data demonstrated a performance similar to smear microscopy with a sensitivity of 76.5% and a specificity of 87.2%.[167] Currently, there are four other field studies being performed with this technology in HBCs.

Rapid Biosensor Systems is developing the TB Breathalyzer, which uses a disposable collector and reaction tube that is read via a battery-powered reader. The patient coughs into a collection tube and then an internal concentric plunger is suppressed to collect the sample from the sides of the tube. Subsequent twisting of the plunger smears the sample across a (labelled) peptide-coated prism at the base of the tube.[168] With TB infection, Ag85B is produced by MTB cells in the sputum and the antigen can displace

the labelled Ag85B peptide epitope bound to an antibody-coated surface on the prism. The device measures the loss in fluorescence by evanescent wave fluorimetry and then scores the result. A field evaluation of the TB Breathalyzer showed it was accepted by patients and it took 10 minutes to screen each patient. The culture component was not performed and the authors compared it to smear microscopy where it had a sensitivity of 79%.[168]

Advantages: The primary advantage of using VOCs to diagnose MTB is that it is a non-invasive sampling method and can give a rapid time to result. Improved diagnosis of paediatric TB may be enhanced by such tools. By analysing the headspace from specimens such as urine, EPTB can also be diagnosed. There are increased cost savings as extra materials and reagents are not necessary for preparation of the test analyte(s). The equipment can be easy to use and does not need highly trained personnel. Other diseases, such as lung or breast cancer, may be assessed with the same tool providing wider utility, especially when considered as an initial screen for patients presenting with a persistent cough. Sensor-based technologies can be produced at scale and, unlike gas chromatographs, do not need daily calibration prior to use.

Disadvantages: It is unclear if VOCs can detect EPTB, and their performance in cases of paucibacillary load has not yet been assessed. Other risks are to the integrity of these tools under adverse environmental conditions with unskilled users in the intended settings as this too has not yet been established. The nascence of these technologies is reflected by limited evaluation data on any of the prototype products.

Assays based on inflammatory markers for EPTB

Several systematic reviews have shown that non-specific, inflammatory markers such as adenosine deaminase (ADA) and free, unstimulated IFN-γ may have a potential role in the detection of extrapulmonary forms of TB—TB pleuritis, TB ascitis and TB pericarditis (http://tbevidence.org/2011/11/reviews-on-ada-ifn-gamma-for-extra-pulmonary-tb/). However, the application of these tools has been constrained by lack of validated, standardized, commercial assays. For example, there are several in-house biochemical assays for ADA, with a variety of cutoffs, and unclear interpretational criteria.

Antrum Biotech (Pty) Ltd (hereafter Antrum Biotech; South Africa), a spin-off company from the University of Cape Town, is developing an ELISA product, the InterGam Ultrasensitive Rapid Immuno-suspension Assay (IRISA™-TB) for initial diagnosis of EPTB by detecting unstimulated interferon gamma (uIFN-γ) from patient specimens. This group notes that pleural TB, pericardial TB, abdominal TB or TB-meningitis can all be diagnosed by this method. Current conventional diagnostic methods for these manifestations of EPTB disease are typically slow (culture) or insensitive (microscopy). Unlike the IGRAs, which require blood collection followed by in vitro stimulation of macrophages and CD4 cells to MTBC antigens, this assay measures the residual level of IFN-γ in a specimen, e.g. pleural or pericardial fluid.[169,170] In addition, IGRAs are suitable for indicating exposure to MTBC, the IRISA™-TB assay is a diagnostic test.

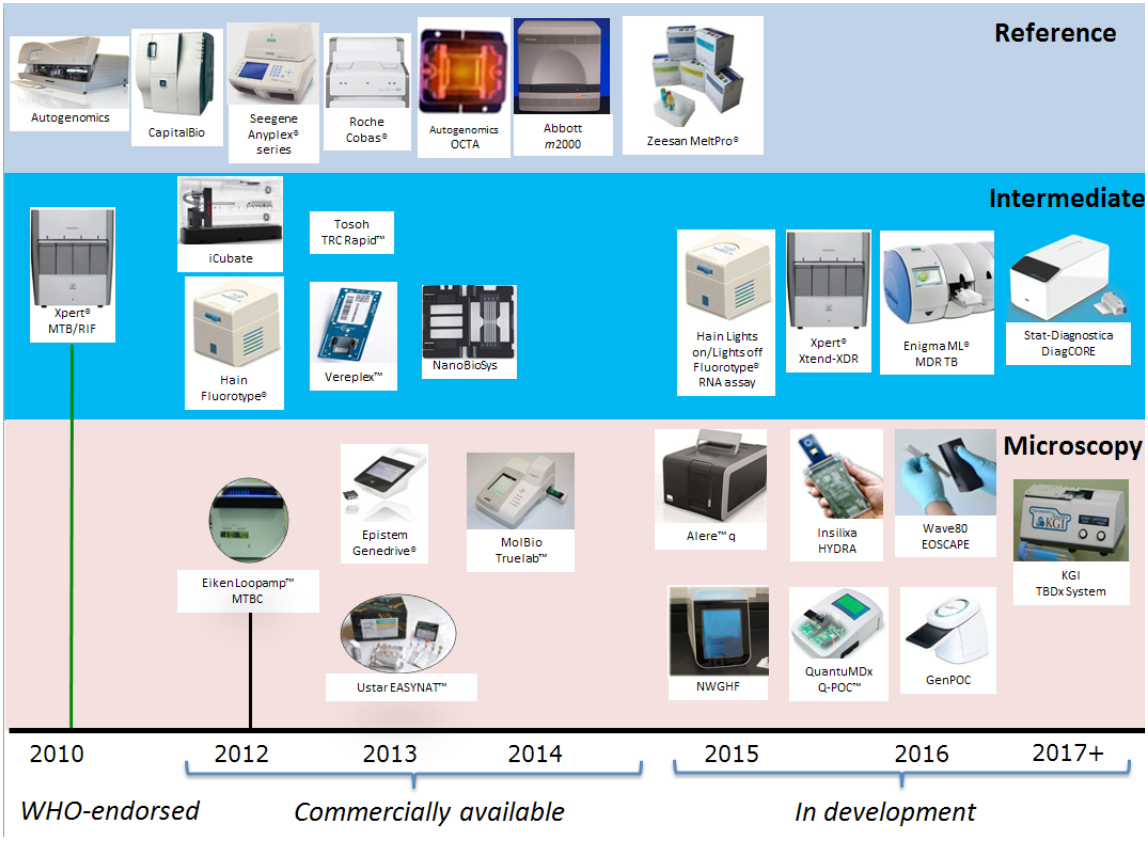
Two recently published studies have assessed the performance of the IRISA™-TB assay in identifying pleural TB and pericardial TB. [169,170]The initial findings of these studies were that the IRISA™-TB assay had superior accuracy for the diagnosis of microbiologically confirmed PTB when compared with the Xpert® MTB/RIF test or the ADA. Two further evaluation studies are currently being performed to better assess the performance of this assay. Antrum Biotech notes that the test complexity makes it suitable for intermediate level facilities (e.g. hospital laboratories) and requires instrumentation associated with ELISA assays (e.g. plate shaker; washer; reader). The test is currently not CE-IVD marked, but this is in process. No pricing is available for the test, which Antrum Biotech aims to release onto the market in Q4 2014/Q1 2015.

5.4.8. NAATs and sequencing methods

The amplification and detection of nucleic acids (either DNA or RNA) have been shown to be a rapid, highly sensitive and specific method for diagnosing infectious diseases. As noted earlier, NAATs are currently the largest group of diagnostic technologies and products being offered by manufactures or in development (see Table A1 in the appendix). In addition, the presence of many alleles associated with resistance to first- and second-line drugs can be detected providing genotypic DST. A pipeline of the automated and semi-modular NAATs for MTB diagnosis and/or genotyping DST is shown in Figure 20.

NAATs have greater performance than the other tests for MTB, excluding culture.[171] While culture-based methods remain the gold standard for TB diagnosis and phenotypic DST, the time to result and the need for significant laboratory infrastructure and logistics limit their application to centralized facilities. Traditionally, there have been a large number of NAAT "home brew" assays that utilize PCR; however, they produce highly inconsistent results.[172] Since the release of UNITAID landscape reports in 2012 and 2103 there has been an ever increasing number of NAATs being commercially released or in late-stage development.[173,174] These are designed to target a variety of applications in the MTB test continuum and can be discriminated by their intended location of use, application, throughput and cost or the scale and complexity of the technology; all of which indicate NAATs' utility for MTB detection and/or genotypic DST (Table 4).

Figure 20. Current and emerging automated, semi-modular or non-integrated TB NAATs; their intended laboratory location and their release or anticipated time to market



Note: Only the Xpert® MTB/RIF within this group has received WHO endorsement, while the LoopAMP™ MTBC Detection Kit is currently the only test undergoing evaluation for WHO Expert Group review.

Table 4. Summary of the NAATs relating their role in TB diagnosis in terms of intended location of use, throughput and other key factors

Test	Location	Throughput	Function	Complexity	Hardware cost	Cost/test
LPA	Ref./Int.	Moderate	MTB/NTM/ Dx/DST#*	Moderate	Moderate	Moderate
Automated batched PCR	Ref.	High/moderate	MTB Dx	High	High	Low
High-income country NAATS	Ref./Int.	High/moderate	MTB/NTM Dx	High	High	Moderate
Microarrays	Ref./Int.	Moderate	MTB/NTM Dx DST#	High	High	High/ moderate
Modular NAATs	Ref./Int.	Moderate	Dx/DST	Low	High	Moderate
POC assays	Int./Per.	Moderate/low	Dx/DST*	Low	Moderate/ low	Low

Dx: diagnosis; Ref., reference laboratory; Int., intermediate laboratory; Per., peripheral facility.

Some tests are available to rule in other common types of NTM.

* DST may be part of the same test or as a reflexive test after MTB infection is confirmed.

The performance of most NAATs is identical to culture when smear-positive sputum is tested. However, with smear-negative/culture-positive specimens, performance is highly variable, and currently even the most developed NAATs have reduced sensitivity compared to culture (while still more sensitive than smear microscopy). NAATs can be used on many specimen types, but development efforts have been focused on sputum and/or liquid cultures to enable additional genotyping for drug resistance and rapid high-throughput testing or to supplant or complement smear microscopy, the largest market for NAATs.[2] Paucibacillary sputum specimens with lower numbers of target bacteria present a challenge, which is compounded by the presence of other materials that limit cell lysis and/or by carry over into the test reaction where they can inhibit amplification. Mycobacteria are also difficult to lyse due to their cell wall physiology and these factors can limit the availability of target nucleic acids.

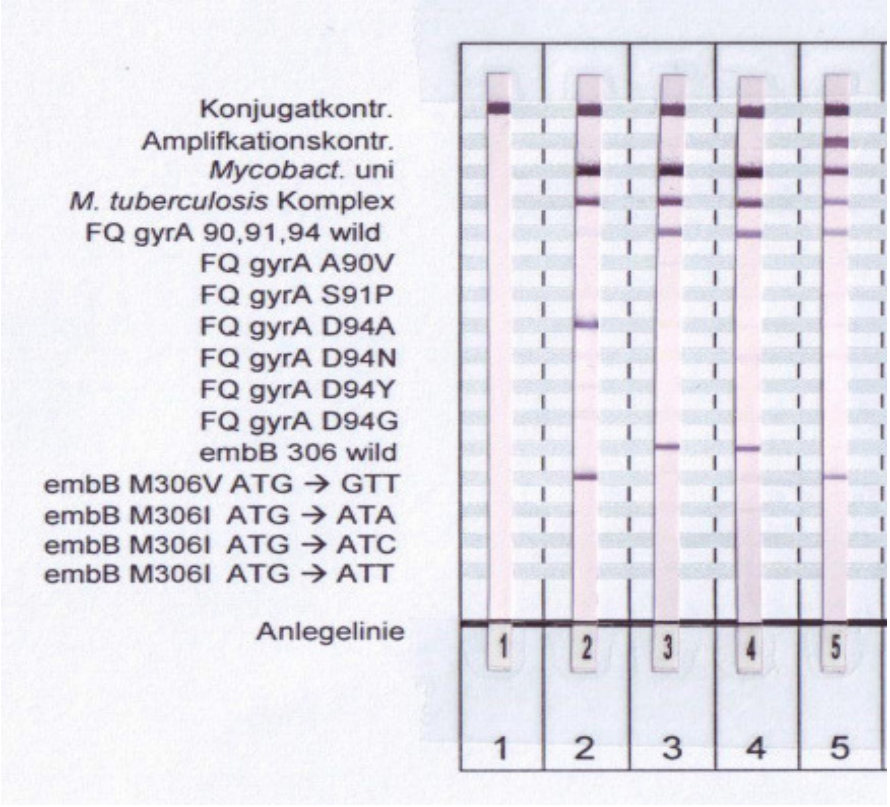
With NAATs, MTBC genomic-specific targets are exponentially amplified by a variety of methods: PCR; transcription-mediated amplification (TMA); LAMP; NASBA; strand displacement amplification (SDA); facilitating detection.[171] Test results can rely on automated readers that measure fluorescence or luminescence, simple visual assessment of fluorescence or banding on specific test strips. These processes can be either separate (open), or fully or partially integrated and automated. The high sensitivity of NAATs adds complexity to testing, especially when using open systems. Maintaining amplicon-free environments is especially critical. Ideally, this requires separate rooms or areas and diligent quality control to prevent nucleic acid contamination of the laboratory. Some NAATs have been endorsed by WHO and/or by national regulatory agencies. Many others are undergoing evaluation or are in late-stage development. The following section presents current and pending NAATs, highlighting the technology, intended use and unbiased evaluations of performance of each one.

LPAs

Perspective: LPAs were the first NAATs endorsed by WHO in 2008 for use in the molecular detection of MTBC and drug resistance from smear-positive patients at risk of MDR TB.[175] The underlying principle of the LPA is a multiplexed DNA hybridization assay that can interrogate a variety of amplified genetic targets in a single, relatively simple test format. LPAs have been developed for the diagnosis of MTBC, speciation of NTM, genotypic drug resistance screening or a combination these (Table 5). Genomic DNA is first extracted from a culture isolate or specimen (typically smear-positive sputum). DNA targets are selectively amplified and labelled with biotin via asymmetric PCR, and the single-stranded amplicons subsequently applied to test strips. Specific oligonucleotide probes are printed on nitrocellulose sheets, which are then cut into test strips. Each amplicon specifically binds only to its complementary probe on the strip, while unbound amplicons are removed in subsequent wash steps. Bound amplicons are detected by the attachment of a label to the biotinylated amplicons, typically a streptavidin-linked enzyme that creates a colorimetric reaction. A series of dark bands mark the regions of the strip where amplified DNA has bound (Figure 21). The result can be interpreted visually against a scoring chart or using a reader (Table 5). The strips also include amplification and hybridization controls to for quality control.

LPAs are designed for use in reference and intermediate-tier laboratories and can be manual or semi-automated for the DNA extraction, hybridization and washing and finally reading and scoring of test results. A thermocycler is required to generate labelled amplicons. Originally, the tests were noted for difficulty in consistent performance due to frequent contamination of the test areas and subjective reading of the strips. Improved quality control procedures, restricted access to dedicated molecular areas, stricter adherence to standard operating procedures and more automated processes for hybridization can reduce contamination events.

Figure 21. Example of visually scoring the banding patterns on LPAs exhibited by drug-susceptible and drug-resistant strains of MTBC



Source: Image reproduced with permission from AID.

Table 5. Current LPA products and associated equipment marketed for MTBC diagnosis, mycobacterial speciation and genotypic DST

Developer	Test name	Myco/ MTBC/ Spec	DST	CE-IVD	Automated hybridization	Test reader	Released
AID	TB Resistance Module INH/RIF	Y/Y/N	RIF/INH	Y	N/A	Genoblot	Q2 2013
AID	TB Resistance Module FLQ	Y/Y/N	FLQ/EMB	Y	N/A	Genoblot	Q2 2014
AID	TB Resistance Module AMG	Y/Y/N	AMG	Y	N/A	Genoblot	Q2 2015
Fuji-Rebio Europe	INNO-LiPA MYCOBACTERIA v2	Y/Y/Y	No	Y	Auto-LiPA 48	N/A	N/A
Fuji-Rebio Europe	INNO-LiPA Rif.TB	N/Y/N	No	Y	Auto-LiPA 48	N/A	N/A
Hain Lifescience	GenoQuick® MTB	N/Y/N	No	Y	GT-Blot 48	GenoScan®	2010
Hain Lifescience	GenoType® MTBDRplus 2.0	Y/Y/N	RIF/INH	Y	GT-Blot 48	GenoScan®	2012
Hain Lifescience	GenoType® MTBDRsl	Y/Y/N	FLQ/AMG/ETB	Y	GT-Blot 48	GenoScan®	2009
Hain Lifescience	GenoType® Mycobacterium CM	Y/Y/Y	No (Q4 2104)	Y	GT-Blot 48	GenoScan®	2004
Hain Lifescience	GenoType® Mycobacterium AS	Y/Y/Y	No	Y	GT-Blot 48	GenoScan®	2004
LG Life Sciences	AdvanSure Mycobacteria GenoBlot Assay	Y/Y/Y	No	N/A	N/A	Genoblot	N/A
LG Life Sciences	AdvanSure MDR TB GenoBlot Assay	Y/Y/N	RIF/INH	N/A	N/A	Genoblot	N/A
NIPRO Co.	NTM/MDR TB	Y/Y/N	RIF/INH	N/A	N/A	N/A	2012
NIPRO Co.	INH	Y/Y/N	INH	N/A	N/A	N/A	2012
NIPRO Co.	PZA	Y/Y/N	PZA	N/A	N/A	N/A	2012
NIPRO Co.	FLQ	Y/Y/N	FLQ	N/A	N/A	N/A	2012
Vircell	SPEED-OLIGO® DIRECT MTB	Y/Y/N	No	Y	Not required	N/A	N/A
Vircell	SPEED-OLIGO® DIRECT Mycobacteria	Y/Y/Y	No	Y	Not required	N/A	N/A
YD Diagnostics	MolecuTech REBA Myco-ID	Y/Y/Y	No	N/A	REBA processor	REBA Scan	N/A
YD Diagnostics	MolecuTech REBA MTB-MDR	Y/Y/N	RIF/INH	N/A	REBA processor	REBA Scan	N/A
YD Diagnostics	MolecuTech REBA MTB FQ	Y/Y/N	FLQ	N/A	REBA processor	REBA Scan	N/A
YD Diagnostics	MolecuTech REBA MTB KM	Y/Y/N	AMG	N/A	REBA processor	REBA Scan	N/A
YD Diagnostics	MolecuTech REBA MTB XDR	Y/Y/N	FLQ/AMG	N/A	REBA processor	REBA Scan	N/A

Myco, all mycobacteria; MTBC, MTBC only; Spec, speciation of mycobacteria other than MTBC; N/A, not available; Y, yes; N, no. For other abbreviations, please see the list at the start of this report.

The Hain Lifescience Genoquick® MTB and Vircell (Spain) SPEEDOLIGO® assays are included in Table 5, although not LPAs. Both tests take only from one and a half to three hours to generate a result as dual-labelled PCR amplicons are captured and detected via a nucleic acid RDT. LPAs require a hybridization step that increases the time to result by approximately two hours. Commercially produced assays are included in Table 5 that are used to speciate NTM, to diagnose MTBC, MDR TB and XDR TB or to genotype resistance to specific drugs typically related to XDR (e.g. FLQ or aminoglycosides [AMGs]). The primary benefit of LPAs is that, in addition to MTBC detection, they can measure a multitude of targets in parallel and, therefore, are well suited to interrogate a high number of genetic modifications for DST genotyping or speciation and results are typically available within 24 hours at a laboratory. LPAs can use a variety of specimens, but culture isolates or smear-positive sputum samples give excellent performance due to adequate amounts of template DNA. The HAIN Lifescience MTBDRplus 2.0 is a modification of the original assay endorsed by WHO in 2008.[175] By improving the efficiency of the PCR reaction, the manufacturers claim improved sensitivity with smear-negative/culture-positive sputum. One study noted the MTBDRplus 2.0 had similar performance to Xpert® MTB/RIF when comparing the assays.[176]

In March, 2012, a WHO Expert Group reviewed the performance of the Hain Lifescience GenoType® MTBDR*sl*, the first LPA to genotype alleles associated with resistance to second-line drugs (FLQ, AMG/cyclic peptides and EMB). The test is intended as a low-cost and rapid tool to identify XDR TB from MDR TB-positive specimens at the reference laboratory level to supplant the phenotypic DST methods. After review of pooled data, the Expert Group decided that the performance of the GenoType® MTBDR*sl* was insufficient to replace phenotypic DST methods, as it could rule in many XDR TB cases, but had insufficient performance to rule out XDR TB.[23] However, the Expert Group noted that given the high assay specificity for detecting resistance to FLQs and second-line injectables, the results of the Genotype MTBDR*sl* assay could be used as a rapid test to guide the implementation of additional infection control precautions pending the results of phenotypic DST results. A recent study from Democratic Republic of Congo noted that a common mutation in FLQ-sensitive strains could be misinterpreted as a marker for FLQ resistance and that there is a need to improve the design of this assay to better inform when such alleles are encountered.[177] There is limited peer-reviewed evidence describing the performance of most of the tests in Table 4 in HBC settings. FIND currently is in the process of conducting a non-inferiority study comparing the Hain Lifescience MTBDRplusv1.0 to the MTBDRplus v2.0 and the MDR products from Nipro Co. and YD Diagnostics. Furthermore, FIND supports the late phase development activities for the PZA assay by Nipro Co. (Figure 2). Currently, FIND offers negotiated prices for the Hain Lifescience MTBDRplus v2.0 and MTBDR*sl*. In addition, other ancillary equipment for performing these tests is also offered (http://www.finddiagnostics.org/about/what_we_do/successes/find-negotiated-prices/mtbdrplus.html). Further negotiations with companies are ongoing.

Advantages: LPAs are sensitive tests that can be used for a variety of purposes, including MTBC diagnosis, speciation of other NTM and genotyping common drug resistance alleles (especially for RIF) within a 24-hour period. The assays can interrogate a large number of targets within a single assay and they are comparatively low cost. The tests have relatively low instrumentation costs and can use generic equipment (e.g. thermocyclers) and can be applied to semi-automated systems for higher throughput in addition to automatic scoring and archiving of the test results.

Disadvantages: LPAs can be performed only in reference and intermediate facilities due to the primary requirements of trained personnel and reagent/equipment storage. To reduce the potential for contamination, a series of dedicated sites within the laboratory are necessary. The performance of LPAs with smear-negative/culture-positive specimens is not recommended for tests other than the Hain Lifescience MTBDRplus v2.0. Manual reading of the LPA strip tests needs to be carefully interpreted to avoid error. As with other genotypic methods for DST, not all resistance genotypes can be identified for many drugs (e.g. PZA); conversely, silent mutations in drug-sensitive strains can be misclassified as drug resistant leading to use of expensive yet unnecessary treatment options.[177] There is a limited evidence base demonstrating many of the newer products' performance in HBCs.

Commercial NAATs for use in reference and intermediate-tier laboratories

As noted in Table 4, the complexity or required cost and infrastructure for instrumentation of many of the current commercial NAATs excludes them from routine use in microscopy centre settings where the burden of testing is greatest. Historically, the first NAATs for clinical and diagnostic use were aimed at the high-income country markets for TB where the capacity to perform complex test methods was available due to greater resources, fully equipped reference laboratories and a lower burden of TB disease. Technologies aimed at first- and second-tier laboratories are marketed for high-volume testing for MTB. In addition, a growing range of technologies and products for genotypic DST are available using LPAs, microarrays or molecular beacons to assess either culture derived isolates or smear-positive specimens. This section is divided into the first commercial MTBC assays, automated MTBC detection platforms and microarray platforms. A pipeline showing the release and intended release data for NAATs other than LPAs is shown in Figure 20.

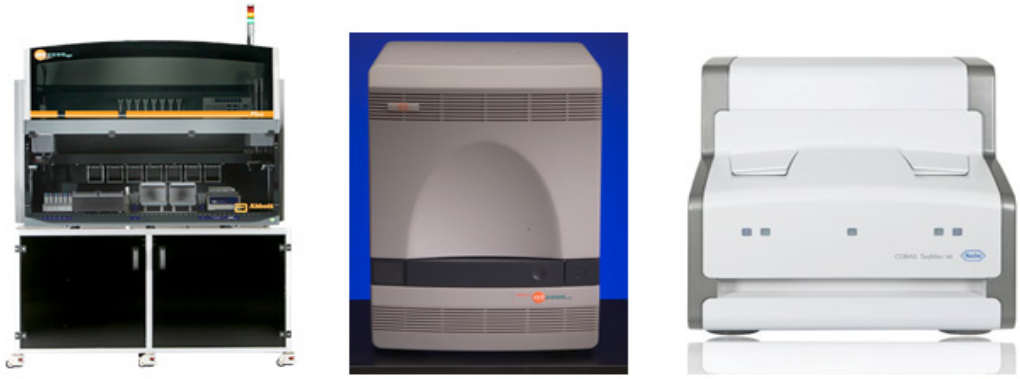
Early commercial MTBC assays: Historically, the Gen-Probe rapid identification test for MTBC (approved in 1986), the Syngene SNAP MTBC test (approved in 1990), the Roche Amplicor MTB test (approved 1996) and the BD ProbeTec ET Direct TB assay (approved in 2001) were the first TB NAATs to be approved for

use by the USA. None of those assays nor their successors have been endorsed by WHO. Newer versions such as the Hologic Gen-Probe® (USA) Amplified MTD (AMTD) and the Roche COBAS® TaqMan® MTB assay can be used on platforms capable of sample batch processing for confirmation of culture-positive samples or sputum specimens as well as for confirmation of smear-negative samples. The AMTD assay involves a single-tube system in which MTBC cells are first lysed, then the 16S rRNA target is isothermally amplified by TMA.[171] A specific probe indicates the presence of TMA amplicons. The single-stranded TMA amplicons bind to and stabilize the complementary fluorescent probe to create a double-stranded molecule that is otherwise (intentionally) degraded when single stranded, the hybridization protection assay.[178] Test reactions are read after amplification using the Hologic Gen-Probe® Leader® Luminometer to detect the presence or absence of luminescence in each reaction tube. In addition to MTBC diagnosis, Hologic Gen-Probe® also offers other US FDA-approved tests to speciate mycobacteria derived from culture. The Accuprobe tests use the same probe detection technology and instrumentation for MTBC, *M. avium, M. avium complex, M. gordonae, M. intracellulare* and *M. kansasii*. However, these assays are used to confirm culture isolates and do not amplify the nucleic acids, but instead can utilize sufficient target RNA from the lysed cells.

The BD ProbeTec ET™ Direct TB assay is a method that targets IS6110 using another isothermal amplification method, SDA.[171] The amplicon detection system incorporates a DNA oligonucleotide probe that binds to one strand of the target amplicon and is subsequently cleaved.[179,180] This event separates a fluorophore from its quencher so a fluorescent signal is generated in positive reaction. The assays incorporate an internal control that informs on the presence on inhibitory materials in the test reactions. The reactions are monitored in real time and the entire process takes under two hours and up to 94 samples can be batch processed.[181] This is excluding the sample preparation and lysis, which is manual and involves both centrifugation and heating prior to amplification. There is an extensive body of peer-reviewed studies assessing the performance of both technologies.[182]

Automated centralized MTBC Dx platforms: While the Hologic Gen-Probe® and BD technologies offer high-throughput testing, they require manual processing. Both Roche Diagnostics (hereafter Roche; Switzerland) and Abbott (USA) have developed NAATs that are amendable with their existing automated extraction platforms permitting automated extraction, reaction preparation and manual transfer to their amplification platforms (Figure 22). These assays are intended for positioning in reference laboratories based on their overall cost and need for infrastructure and operation by skilled personnel. The larger test volumes offer savings in terms of bulk purchases of reagents or in equipment lease systems, and both platforms have assays developed for other diseases such as HIV viral load. Both technologies can also use manual extraction methods for reduced throughput and potentially could be positioned in intermediate laboratories.

Figure 22. Systems for high-throughput testing for MTB: Abbott m2000sp automated sample extraction and reagents preparation station (left) and m2000rt RealTime PCR machine (centre); Roche COBAS® TaqMan® 48 Analyser (right)



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The Roche COBAS® TaqMan® MTB Test is a real-time PCR assay for the qualitative detection of MTBC DNA.[183,184] The assay is CE-IVD marked and has further regulatory approval in Canada and Japan. The platform can be integrated with the COBAS® AmpliPrep Instrument to automate sample preparation. The assay amplifies an MTBC specific target in the 16s RNA gene and can use the Roche Cobas® TaqMan® 48 Analyzer that can run up to 96 reactions via two independent PCR units (e.g. 2 x 48; see Figure 22). The results of amplification are automatically analysed. Roche estimates that over 100 samples can be processed per day and note that results from PCR for 1–44 tests takes approximately four hours. Other studies have also noted high specificity when compared to other NAATs in this group.[185,186]

Abbott recently released the *m*2000 RealTi*m*e MTB assay. The technology is currently available as a research use only tool and a CE-IVD mark is anticipated by Q3 2014. Automated extraction can be performed with the Abbott *m*2000sp instrument (US$ 162 000) (Figure 22) or with other automated or manual methods. The automated extraction takes under five hours with 94 samples and two controls and can also prepare a plate for PCR amplification. MTB target is detected by real-time PCR using the *m*2000rt instrument (US$ 45 000) (Figure 22) and the data are analysed with Abbott MaxRatio technology. Combined, the time to result is seven hours. Abbott is currently also developing a reflex assay for MTB RIF/INH Resistance assay that will be CE-IVD marked in Q4 2014. Further specifications are in Appendix 1. There are no peer-reviewed data on this assay.

NanoBioSys Inc. (Republic of Korea) is developing technologies for rapid batched preparation and analysis of specimens. They offer a semi-automated sample extraction platform, the UltraFast LabChip Sample Prep G2 test that can process up to 12 samples simultaneously in only 15 minutes. NanoBioSys Inc. also uses a microfluidic chip, the LabChip G2-3, to analyse up to 10 samples via real-time PCR in under 30 minutes. Reaction mixture preparation and sample addition is manual. Therefore, with this test format relatively high throughput for a semi-automated procedure is possible. NanoBioSys Inc. is developing larger capacity PCR chips to test 16, 48 and 96 samples. There are no regulatory data on these products. In a recent evaluation study of the LabChip G2-3 on 247 clinical specimens it was noted that the test had sensitivity and specificity of 94% and 83.3%, respectively, when compared to smear-positive/culture-positive samples. With smear-negative/culture-positive, the sensitivity and specificity were 63.3% and 95%, respectively.[187] The test performance data were similar to conventional real-time PCR, but the authors noted a run time of only 27 minutes using the LabChip G2-3 in which 45 PCR cycles were performed. Current pricing is also not known.

The TRCRapid® M.TB assay is offered by Tosoh Bioscience (Japan). There is limited information on this product, but the assay is approved for use in Japan and several independent studies have described the product[27] or evaluated its performance using clinical specimens.[188-190] The assay is based on an isothermal

amplification method, transcription-reverse transcription concerted (TRC), and the assay targets MTBC 16S rRNA and also hosts an internal amplification control to indicate interfering substances. Reactions and data analysis are performed in the TRCRapid®-160 analyzer with the time to result from starting sample preparation being from one to two hours, depending on the RNA extraction method used. It has been proposed that 16S RNA may be an indicator of cell viability and so this assay may have potential use for treatment monitoring. One study reported a specificity of 90.7% with smear-positive/culture-positive specimens, while with smear-negative/culture-positive samples the sensitivity was 44.8%.[189] A larger multicentre evaluation using a more detailed nucleic acid extraction protocol reported that the sensitivity of TRC-2 was higher than that of culture with 86.7% versus 80% when clinical indicators of TB infection were included.[188] Current pricing or intended markets are not known.

There are also assays for the diagnosis or genotyping of MTB from culture or clinical specimens that can be performed using existing laboratory equipment. A real-time thermocycler must be able to perform melt curve analysis, which is a useful approach to discriminate allelic differences in the amplified DNA. These tests are intended for use in reference-tier laboratories as the reagents need cold chain storage and skilled personnel. These assays can be used for detection of MTBC from sputum and concomitant genotypic DST or for confirmation of culture results; essentially, they are competitive products compared to LPAs in terms of their utility, cost and range of targets. In researching this landscape, we identified many companies that offer real-time PCR assays for TB diagnosis, but there are generally no peer-reviewed data describing performance. In addition, most of these offer only detection of MTBC. There are several products available that offer MTBC detection and/or drug resistance genotyping with CE-IVD, Republic of Korea Ministry of Food and Drug Safety and/or China Food and Drug Administration (CFDA) approval for manufacture.

Seegene (Republic of Korea) has developed a suite of NAAT assays around their key technologies to permit highly multiplexed PCR and novel probe designs to improve the detection of each amplified product via melt curve analysis. The assays have been developed to run on the CFX96™ real-time thermocycler (Bio-Rad Laboratories; USA). In addition, Seegene provides novel software (MuDT) to analyse data and generate results from their assays as the analysis software on the CFX96 platform cannot effectively interpret the data generated. The Anyplex™ series are real-time PCR assays that include the Anyplex™ MTB/NTM for MTBC and NTM, the Anyplex™ plus MTB/NTM/MDR TB for MTBC, NTM and genotyping RIF and INH resistance, and finally the Anyplex™ II MTB/MDR/XDR that detects MTBC, genotypes MDR via interrogation of 7 INH resistance mutations and 18 RIF resistance mutations and the interrogation of 13 alleles associated with XDR (7 alleles for FLQ and 6 for injectable drugs). Even though highly multiplexed, the assays' designs are such that the primary sample can be run to detect MTBC and MDR and then be reflexed to determine XDR status if the sample is MTBC and MDR positive. Seegene estimates a maximum throughput of 96 patient specimens and controls in six hours. Only the Anyplex™ MTB/NTM has been assessed via a prospective evaluation on existing DNA extracted from clinical specimens.[191] The authors noted that the assay had a very similar performance (96.4%) to the Hain Lifescience MTBDRplus v1.0 assay with sensitivity/specificity for MTBC and NTM of 100%/96% and 100%/97%, respectively. The tests are currently CE-IVD marked and their pricing is available through the distributors.

Xiamen Zeesan Biotech Co. Ltd (hereafter Zeesan Biotech; China) has developed the MeltPro® Drug-Resistant TB testing kits for genotyping MTBC culture for first- or second-line drug resistance. These are real-time PCR assays to genotype for resistance to RIF, INH, FLQ and second-line injectables via independent kits. The assays have recently been approved by CFDA in 2013 and Zeesan Biotech plans to release them in Q4 2014. As with the other mentioned kits, DNA extraction is excluded from the kit and samples must first be processed with a suitable method. Overall, the time to result is four and a half hours and the developers note 48 samples per test run can be processed. Each assay uses two separate reactions to test the sample (e.g. the INH assays uses *inhA* promoter/*katG315* and *ahpC*/*inhA96*). A study compared the performance of the INH kit to phenotypic resistance testing with DNA sequencing to confirm test results.[192] Wild type and INH mutant DNA were mixed to establish test performance in heteroresistant MTBC populations and assay performance in five different brands of real-time PCR machines were also assessed. Of the 437 INH-resistant isolates screened, 397 mutation-containing isolates were correctly identified. Of the discordant samples, DNA sequencing confirmed that 31 isolates had no mutations in the entire region sequenced and

5 had mutations outside of the MeltPro target regions and 4 were heteroresistant. Data and time to result were different for the five brands of PCR machine, but all were compatible with the use of this assay. Currently, Zeesan Biotech aims to market the tests at US$ 16 per reaction.

Advantages: The commercial platforms and assays described above permit high-throughput testing of MTBC specimens or screening of culture isolates as well as confirmation of smear-positive and smear-negative (in part) results. Identification and a range of DST results can be obtained within one or two days compared to several weeks with culture-based methods, allowing early and appropriate treatment initiation to be prescribed or adjustment made and hence improving outcome. Due to the scale of testing that can be achieved there may be cost savings from bulk purchase of reagents. The test data are scored automatically thus user interpretation of results is not necessary and data are recorded and archived for quality control analysis and epidemiologic purposes.[192] Some of these tests house internal amplification controls that identify inhibiting substances in the sample extracts. The equipment may also be used for HIV diagnosis and viral load testing. The kits can be used on existing real-time PCR machines, defraying startup costs for new testing regimes.

Disadvantages: These platforms are expensive, and designed for use in reference laboratory settings and can only adequately serve urban/tertiary settings. High-volume sample processing and testing is very complex to perform; therefore, highly trained staff are necessary to run the laboratory. Reagents also require cold chain and so space is also required to house refrigerators and freezers; this equipment may need to be purchased in addition to generators and universal power supply units that can prevent shut down during a loss of electrical power. Apart from equipment costs, maintenance contracts are necessary. More evaluations on these technologies and assays are required to ensure that performance is acceptable or optimal to justify the investment required for their implementation.

Microarray platforms: Microarrays-based systems use sequence-specific single-stranded DNAs, typically spotted onto a glass slide or chip in a precisely defined orientation or array. These arrays can be used to interrogate asymmetrically PCR-amplified or melted PCR amplicons derived from MTBC genomic DNA for a wide variety of genotypic targets to aid speciation and drug resistance screening. The role of microarrays is primarily not for TB detection and the principal advantage is that they can interrogate a much greater number of targets than LPAs. Therefore, they potentially offer greater discriminatory power for a wider variety of drug resistance markers in a single test. Culture or smear-positive sputum can be used to provide DNA for extraction and subsequent amplification via PCR.

Asymmetric PCR is used to first amplify the target region(s) and to then create an excess of the labelled DNA strands, which is complementary to the DNA oligonucleotide(s) printed on the array. The amplicon mixture is applied onto the array under precisely defined salt concentration, temperature and washing conditions to optimally hybridize the labelled DNA strands with their corresponding target spot on the array. If a labelled DNA sequence is complimentary to a spot, then hybridization occurs. Microarrays are analysed in a reader and the signal intensity from each spot (typically fluorescence, chemiluminescence or electrical impedance) is measured to indicate if labelled DNA has bound or not. These are scored *in silico*. To give greater confidence of results, multiple spots of the same target sequence are often printed on arrays with the expectation that each will give similar levels of detection when probed with the same sample. The tests are complicated to perform and require sensitive and complex equipment, but some groups are developing more robust products that may be used outside of reference laboratories.

The INFINITI®, a US FDA-approved platform from AutoGenomics (USA) has an MDR TB assay and a second assay, the MTBC-OCTA, to detect MTBC from eight samples per array. These tests still require manual extraction and PCR amplification of the DNA sample, but the remaining steps to process the microarray and interpret data are automatically performed by the INFINITI®platform.[193] The MDR TB assay is intended for the detection of MTBC in addition to genotyping for resistance alleles to RIF, INH and PZA and 48 samples take six and a half hours to prepare and analyse. The cost is US$ 95 per array. The MTBC-OCTA assay is a higher-throughput array that simultaneously screens eight specimens for MTBC on one chip. With this assay, 240 samples can be screened in eight hours. The MTBC-OCTA arrays are US$ 40 per test and the INFINITI reader is US$ 227–447.

Another partially integrated system has been developed by Veredus Laboratories Pte Ltd (Singapore), offering the VerePLEX™ Biosystem and a TB detection kit, VereMTB™ Detection Kit (Figure 23). The extraction of DNA is not automated and must be performed beforehand. However, PCR, hybridization and detection are all performed on the array chip within the instrument thereby reducing time and limiting opportunities for error. The VerePLEX™ Biosystem is modular and can process five arrays simultaneously. The overall time to result is three and a half hours and 10–15 samples can be processed in eight hours. The chip is designed to detect MTB and can speciate nine other NTM. In addition, the test is designed to detect RIF and INH resistance. Due to many of the components being integrated, this technology is easier to use than other arrays on the market and could have utility in mid-tier laboratories. The platform and assay were released in 2012 as research use only.

Figure 23. VerePLEX™ Biosystem and VereMTB™ Detection Kit: A: VereMTB™ Detection chip; B: VerePLEX™ Biosystem and reader

Notes: Both PCR amplification and microarray hybridization are performed in the VereMTB™ Detection chip. The VerePLEX™ Biosystem includes a five-unit processing station for the on-chip PCR and the subsequent hybridization and washing of the chip. On the right is the reader that interrogates amplicon binding to the array.

Source: Images reproduced with permission from Veredus Laboratories Pte Ltd.

CapitalBio Corp. (China) offers two microarray products: one for the speciation of common mycobacteria (Mycobacterium Identification Array Kit); and a second product that identifies MTBC and also genotypes drug resistance to RIF and INH (*M. Tuberculosis* Drug Resistance Detection Array Kit). Both assays are CE-IVD marked and also have been approved for clinical use by CFDA. The tests require extra core equipment, including a thermocycler, hybridization oven and microarray scanner. CapitalBio Corp. offers software to automatically interpret the array data. The tests can be used with culture isolates or from smear-positive isolates. The assays take six hours. The sensitivity and specificity of identification array demonstrated 99.6% and 100%, respectively, on culture isolates and 10% with sputum samples, but poorly with other specimen types.[194] A comparison of the MDR array to culture DST using culture isolates and sputum samples demonstrated high performance for RIF resistance detection (91.8% for isolates and 94.6% for sputum) and INH detection (70.2% for isolates and 78.1% for sputum).[195] The NTM assay costs US$ 25 and the MDR TB assay is US$ 21. Other equipment costs are estimated to be US$ 75 000.

Akonni Biosystems offers the TruArray® MDR-TB assays that can identify MTB and *M. avium* and detect alleles that confer resistance to RIF and INH. The data from these assays are read by the company-produced instrumentation, the TruDiagnosis® Systems. Akonni Biosystems is developing tools for use outside of tier-one facilities and has recently published a study describing the performance of a portable microarray analyzer.[196,197] The assay was designed to identify MTB and *M. avium* and includes assays for detection of resistance to EMB and STR in addition to RIF and INH.

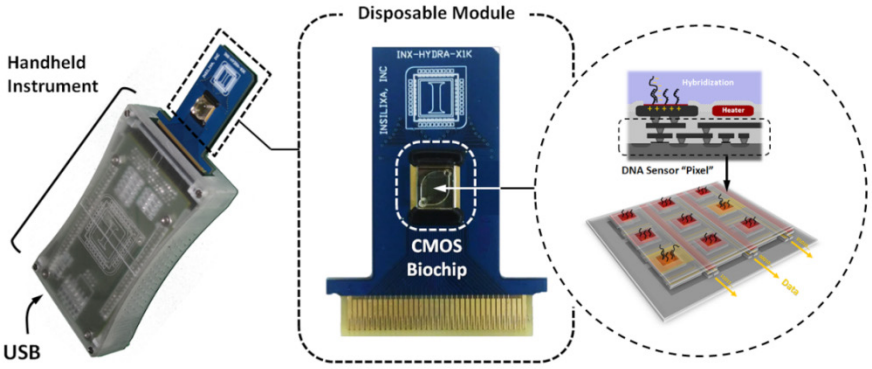
The TruArray® uses a single-tube multiplexed asymmetric PCR reaction that incorporates a fluorescent label on short, single-stranded amplicons for hybridization. The reaction is added directly to the hybrid-

ization reaction mixture and has relatively short hybridization and wash times. They propose that the assay would work well with smear-positive sputum samples as it has a low analytical limit of detection (110 copies of MTB). The reader is portable, but the methodology is still relatively complex and Akonni Biosystems is working to create more simplified workflows to allow use in lower-tier facilities. A single worker can process 24 samples in eight hours and more significant time savings may be made by reducing the hybridization time from three hours to 15 minutes.[197] The market release date for the simplified array technology is currently unknown. Akonni Biosystems is a collaborator on a recently awarded US$ 29 million grant from the NIH to refine this technology into an integrated device that will target MTB and drug resistance and investigate urine as a specimen type in order to better diagnose EPTB.

iCubate (USA) currently offers their technology for mycobacterial testing via an automated system. The cassette-based MYCO assay can identify mycobacteria and speciate MTBC as well as nine other NTM. In addition, the same cassette offers genotyping of 45 alleles associated with resistance to RIF, INH, EMB, and STR. The amplification of a large number of alleles is via a novel method to grossly multiplex and amplify target DNA, amplicon rescue multiplex PCR.[198] This method separates the PCR reaction into a first, target-specific primer-driven amplification and a second, target-independent common primer-driven amplification where the targets are amplified and labelled. The amplicons are interrogated via a low density microarray. The assay has a platform for automated bead-based sample preparation, PCR, hybridization and washing of the microarray, the IC processor. The platform is random access and can process from one to four cartridges. The manufacturers note that these can be linked for up to 12 units permitting processing of 48 samples simultaneously. The microarrays are read and analysed via the IC Reader that, the manufacturers notes, takes seconds to analyse a sample. The test is currently listed as research use only and no pricing information is currently available.

Stanford University (USA) and Insilixa Inc. (USA) are currently in the late-stage development of the HYDRA 1K, a fully integrated, hand-held and highly multiplexed genotyping tool for DST (Figure 24). The technology differs from most other microarrays in that it utilizes complementary metal-oxide semiconductor (CMOS) technology to heat each individual spot on the array. The group is developing real-time polymerase chain reaction assays for a 1024 spot microarray in which DNA amplification and binding to the target spots is measured during the thermal cycling. After amplification, a melt curve analysis is performed on each array spot to further differentiate between mutant and wild type sequences that have bound to each probe. Analysis is performed on the device. All reagents are stored in the CMOS chip, but DNA extraction is manual. The current assay is being developed to detect first- (RIF, INH, PZA and EMB) and second-line drugs (including injectables), in total over 250 single nucleotide polymorphisms. It is also the first genotypic array to include the known resistance alleles to bedaquiline (BED). By using existing high volume, precision manufacturing technology from the microelectronics industry, the group predicts low costs for readers and arrays cassettes in the range of US$ 200 and US$ 15, respectively. The developers note that the assay will enter into field testing in Q2 2015.

Figure 24. Hydra 1K hand-held platform and chip (left)



Notes: Disposable arrays (centre) are inserted into the reader and PCR amplification is measured in real time on each detector pad of the CMOS biochip (centre) that contains integrated heating elements throughout to enable both PCR and also melt curve analysis post-amplification (right). Test data are downloaded from the instrument via a USB port (left).

Sources: Image reproduced with permission from Stanford University and InSilixa Inc.

Advantages: The use of microarrays for TB diagnosis and genotypic DST offer a single test that can simultaneously interrogate a wide variety of targets or genotypic mutations, typically greater than those screened via LPAs. In addition, greater confidence in test results can be achieved by using multiple spots for each target assessed. Testing can provided information on drug susceptibility in 24 hours rather than phenotypic culture-based DST that can take weeks or months until results are known. Analysis of the test data is automated unlike some LPAs and reduces user error in determining results. Some arrays can detect MTB, in addition to first- and second-line drugs in a single test. The development of integrated and/or modular platforms shows that there is potential to perform rapid genotypic DST outside of reference laboratories with some having the potential for use in microscopy centres.

Disadvantages: The complexity of some arrays on the market means that at least some of the present solutions are complex to perform and require trained staff in higher-tier laboratories. This complexity is also typically reflected in relatively high cost for both hardware and tests. All the arrays use dedicated equipment and throughput is limited by multiple steps in processing for some tests. Sample preparation is still manual for all the array technologies, but more automated systems could be used. There are very limited performance data available for these tests and no evidence base for any array technology has been reviewed by WHO.

Modular, cartridge-based, automated NAATs

In high-income countries, there has been a significant expansion in the market to simplify the diagnosis of infectious diseases using fully integrated and typically modular NAAT-based technologies. These technologies permit random access testing where a relatively unskilled user adds the specimen to a cartridge that is then inserted into a machine and the processes of extraction, amplification, quality control and scoring of results are fully automated. Over 30 companies are currently offering or developing automated platforms for modular testing for the diagnosis of infectious diseases. However, many are currently not developing MTB assays to integrate onto their platforms. Here we report only on platforms that are reported to offer or are developing MTBC assays (Figure 20).

FIND and the University of Medicine and Dentistry of New Jersey (USA) in collaboration with Cepheid successfully developed a combined TB and RIF resistance assay in a cartridge for use on the GeneXpert platform, the Xpert® MTB/RIF test.[199,200] Briefly, all components of the test are performed in a single disposable cartridge. The only steps for the user are liquefaction and decontamination of the sputum sample, which is then pipetted into the cassette. The cassette test is then scanned for its barcode and inserted into the machine. The test takes a further two hours to generate a result as to TB positive or negative and RIF resistance detection. The assays use real-time PCR and five "sloppy molecular beacons" to identify poten-

tial mutations in the target region that indicate the potential for RIF resistance.[201] Internal controls provide quality control for the extraction, amplification and detection processes.[171]

The design of the GeneXpert system is modular and Cepheid offers the system in a variety of configurations, with modules per machine ranging from 1, 2, 4 and 16. In addition, an even larger system, the INFINITI series is available with configurations of either 48 or 80 modules. The INFINITI platforms have very high throughput with the manufacturer noting >1300 or >2000 tests per day with the 48 and 80 modules systems, respectively. WHO endorsed the Xpert® MTB/RIF assay in 2010, and issued an updated policy recommendation in 2013. While the initial recommendation suggested the use of the Xpert® MTB/RIF as an initial diagnostic test in individuals suspected of MDR or HIV-associated TB only, the new recommendation now suggests the use of the Xpert® MTB/RIF rather than conventional microscopy and culture as the initial diagnostic test in all adults suspected of having TB (conditional recommendation acknowledging resource implications, high-quality evidence).[202,203] Furthermore, the new guidelines recommend that:

- Xpert® MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in all children suspected of having TB (conditional recommendation acknowledging resource implications, very low-quality evidence.[202]
- Xpert® MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test for CSF (cerebrospinal fluid) specimens from patients suspected of having TB meningitis (strong recommendation given the urgency for rapid diagnosis, very low-quality evidence).
- Xpert® MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture or histopathology) for testing specific non-respiratory specimens (lymph nodes and other tissues) from patients suspected of having EPTB (conditional recommendation, very low-quality evidence).

Other updates on the scale-up of this assay are described in section 6.

Development work with this platform is ongoing. The collaborative groups that developed the Xpert® MTB/RIF assay are currently looking to expand the detection modality of the GeneXpert to include the capacity to discriminate 10 fluorescent dyes rather than the current 6 dyes. This is intended for use in a reflexive assay, the Xtend-XDR, to screen TB/RIF-positive specimens for MDR and XDR resistance alleles. The Alland group at Rutgers University (previously the University of Medicine and Dentistry of New Jersey) received US$ 1.4 million from the NIH for the development of the Xtend-XDR to include genotyping of resistance to FLQ, INH and aminoglycosides (AMGs [amikacin and KM]).[201] The test will add value to treatment decisions by confirming MDR via genotyping for INH and in addition provide information on two of the second-line drugs used to treat MDR. The Xtend-XDR prototype is anticipated to undergo initial verification soon in both China and the Republic of Korea.

Modular, cartridge-based NAATs in development

Enigma Diagnostics (hereafter Enigma; United Kingdom) is developing a modular instrument, the Enigma® mini laboratory (ML) system which is CE-IVD marked. In principle, the technology is similar to the GeneXpert in that all reagents are stored in a single cartridge and the ML system performs automated sample extraction, real-time PCR and analysis of the cartridge once inserted into the instrument. The Enigma® ML system uses Resonsence® probes that enable more rapid amplification using real-time PCR.[204] Enigma was recently awarded funding of £1.4 million (pounds sterling) from the Technology Strategy Board (United Kingdom) to develop a drug resistance assay in partnership with the Imperial College and HSR Ltd using the ML system.

Stat-Diagnostica (Spain) is planning to develop an MTB assay on their DiagCORE platform. The system will be integrated and use real-time PCR to amplify extracted DNA. No further information is currently available.

Advantages: The inherent simplicity of automated modular systems has allowed high-performance testing to be performed by minimally trained staff with high confidence in the correct test result. The sealed cassettes prevent contamination and the need for dedicated areas in which to perform testing. The time to result is under two hours and can potentially provide results to patients the same day (in contrast to culture or LPAs). Patient specimens do not have to be batched but can be individually interrogated according to demand. The combination of all core test functions in an enclosed single-test device reduces the potential to contaminate future tests or the test area and equipment. All test data can be stored and potentially uploaded to central facilities. This enables full data review by national programmes and in addition permits the assessment of all test components in order to measure the performance of equipment, reagents and the proficiency of laboratory staff. Other assays are being developed for these modular systems. For example, Cepheid is introducing an Xpert HIV viral load assay and is developing a reflexive assay for XDR TB as previously described. In addition, the Xpert system and other NAATs in development may be positioned outside of reference laboratories and in some cases may be placed in microscopy centres if infrastructure and facilities are sufficient.

Disadvantages: The technical complexity of the modular NAATs creates several limitations to their use outside of a traditional laboratory. The first is that equipment has a dependency for use with mains electricity and typically cannot be run from batteries for continued and extensive use. In addition, the equipment is relatively sensitive and needs protection from environmental extremes, including heat, humidity and dust. The GeneXpert assay currently requires a minimum of 1 mL of sputum to be processed. The high cost of goods in terms of the instrumentation, maintenance, calibration and test cartridges is seen as an ongoing concern by low- and middle-income countries. The current cost of Xpert® MTB/RIF cartridges was managed only by a concerted effort from donors in a buy down, and most country programmes cannot purchase significant numbers of cartridges and machines without assistance from the donor community. It is unclear how the emerging competing technologies can address similar cost concerns.

NAATs developed for use in lower-tier laboratories and microscopy centres

While the Cepheid Xpert® MTB/RIF assay has been ground breaking in the introduction of high performance molecular testing for MDR TB to facilities outside of the upper-tier laboratories, a variety of competitive products are emerging (Figure 20). There is scope for more robust, lower-cost technologies and assays to provide MTB diagnosis in more austere environments and to compliment or replace smear microscopy as the primary test in microscopy centres. Traditionally, these technologies have been described as the "fast followers" to GeneXpert although, to date, none have yet received endorsement from WHO. These technologies fall into two groups: those tests that are in production; and those that are in late-stage development and expected to enter the market in one to three years (Figure 20). Description of the underlying technologies to these assays has been described in previous UNITAID landscape reports.[174,205] Here, we note recent developments related to these technologies, including independent evaluations and, in some cases, certification by local regulatory agencies.

The LoopAMP™ MTBC Detection Kit (Eiken) was started in development, in 2007, as a low-cost NAAT for rapid diagnosis of PTB. It involves manual processing of extracted DNA from sputum samples followed by instrumented amplification; up to 14 specimens can be batch processed in two hours. The results are manually scored using visual determination of fluorescence of test reactions when compared to positive and negative control reactions. The test was launched in 2011, is CE-IVD marked and now approved for use in Japan. The performance of this assay was presented to a WHO Expert Group for review in 2013 based on its performance in a three-country evaluation. The Expert Group declined to endorse this assay as a replacement test for AFB microscopy, citing insufficient evidence.[22] In light of this finding, Eiken and FIND have created a larger study with 14 countries representing global diversity in terms of ethnicity, geography, MTB and high- or low-HIV prevalence. Each study site is testing at least 500 participants using the Loopamp™ MTBC Detection Kit in addition to Xpert and LED smear microscopy with liquid and solid culture as reference standards. FIND anticipates presenting a report to the Expert Group for their review in Q1 2015. The earliest evaluation of the Loopamp™ MTBC Detection Kit on clinical specimens had noted that the assay performed better with raw rather than processed sputum.[27] Since then, Eiken has modified

the assay to use a greater specimen volume (60 µL) of raw sputum and a more recent study was done to assess the performance of the Loopamp™ MTBC Detection Kit versus the Roche Cobas® TaqMan® assay using specimens from TB-confirmed cases (48 total).[206] While both assays gave 100% correlation with smear-positive samples, the Loopamp™ MTBC assay correctly identified 80% of the smear-negative as compared to 64% with the Cobas® TaqMan® even though the input volume for the Cobas® TaqMan® assay was greater (100 µL).[206]

A recent two-site clinical evaluation in China led by PATH in collaboration with the Chinese Centers for Disease Controls and Prevention has recently been published. In the study, 1329 suspected TB carriers provided specimens screened by the Loopamp™ MTBC Detection Kit, smear microscopy and LJ culture.[17] The results showed the Loopamp™ MTBC assay performed better than smear microscopy. On smear-negative/culture-positive samples, the sensitivity of the Loopamp™ MTBC assay was 53.8% and the overall sensitivity and specificity were 70.7%, and 98.3%, respectively. The authors also noted that catastrophic error due to contamination was very low at 0.21%. This is an important consideration given that the assay is open in format and potentially more prone to contamination of the test areas as compared to modular (i.e. closed) systems. The Chinese Government requires testing of three specimens and the performance of the assay was best when all three specimens were tested by the assay. However, in light of this requirement, further studies are needed to assess the cost-effectiveness of the test with different specimen combinations. Currently, the targeted cost per test is estimated to be US$ 6 and, with instrumentation at US$ 1000, makes it a potentially attractive solution for more decentralized settings.

The Genedrive® Mycobacterium iD® Test-kit is manufactured by Epistem. The test uses real-time asymmetric PCR with highlighter probes to analyse sputum and other liquid specimens for MTBC and is currently the only fast follower that may offer genotyping for RIF resistance (note: this test result is currently shielded from the user). Two of the three assays are MTBC specific and the third is a control. The sample preparation uses a unique paper-based lysis method that takes 5–20 minutes. Paper discs (1 millimetre) are punched from the lysis tool and placed in each of the three reaction wells in the cartridge, rehydrated and then inserted into the device (Figure 25).

Figure 25. Epistem Genedrive® instrument with test cartridge

Source: Image reproduced with permission from Epistem.

The entire test time is under one hour and the Genedrive® instrument is battery powered with all test functions being performed automatically. Result scoring is automatic and the device can store data from 1000 tests. The first evaluation on this technology with clinical MTB specimens has been published.[28] This study was very limited in terms of accessing sufficient fresh clinical specimens, but a parallel assessment with the Xpert® MTB/RIF assay suggested potentially similar performance. Epistem is targeting India and South-East Asia as their market entry point. The test is CE-IVD marked and currently in application for

approval by the Drug Controller General of India (DCGI). The device is currently undergoing multicountry evaluation with FIND and the National Institute of Allergy and Infectious Diseases (NIAID) Tuberculosis Clinical Diagnostics Research Consortium as well as a number of single-centre studies for potential endorsement by WHO. The costs of reagents and device are not known, but quoted as being competitive for these markets.

Epistem recently announced a collaborative funding agreement with the Global Health Investment Fund, LLC (GHIF, London) to support the rollout of its TB assay (http://ghif.com/press-release/). Under the terms of the agreement, Epistem has issued to GHIF a five-year convertible bond totaling US$ 8 million (£4.7 million). As part of the collaborative funding agreement, GHIF and Epistem have made global access commitments to mutually support and facilitate the introduction, distribution and sale of the Genedrive® platform and the expanding menu of infectious disease assays under development for low- and middle-income countries.

The Molbio Truelab™ RealTi*me* micro PCR System consists of a DNA extraction tool and a second amplification and detection instrument (Figure 26). It was released in Q3 2013. Both are battery powered and can operate without charge for one day (processing 12 samples). Test data can be printed from a battery-powered printer that is connected via Bluetooth to the amplification device. Molbio supplies all kits and materials necessary for the assay.

Figure 26. Key instruments and components of the Molbio Truelab™ RealTi*me* micro PCR System: A: Trueprep™, partially automated nucleic acid platform; B: Truelab™ UNO real time PCR analyser with integrated android phone controller; C: TrueNAT™ chip-based MTB assay

Source: Images reproduced with permission from Molbio.

The test assays are performed in the Truelab™ UNO real-time microPCR analyser, using the TrueNAT™, a small, chip-based, disposable reactor that houses the lyophilized reagents and heating element for thermocycling. There is two-colour channel detection with an MTBC assay and internal human DNA control. Molbio is currently developing a reflex drug resistance test for the platform and is working on integrating the sample processing into a single device with the analyser. The use of an android-based cell phone in the Truelab™ Uno to operate the test device also permits data storage for 5000 tests and enables use for global positioning satellite, general packet radio service, global system for mobile communications, Wi-Fi and Bluetooth. There has been one peer-reviewed evaluation of the Truelab™ RealTi*me* micro PCR System with clinical sputum specimens from 226 patients with suspected PTB.[29] In this study, the sensitivity of the TrueNAT™ MTB assay was 99.12% with smear-positive/culture-positive specimens and 75.86% among the smear-negative/culture-positive specimens, specificity was 100%. The assay has been CE-IVD marked and as of Q2 2012 license to manufacture the TrueNAT™ MTB was granted by the Directorate of Food and Drugs Administration, Goa State, India. Molbio is currently targeting India, Asia and Africa as their primary markets. The current price for the instrumentation is US$ 7000 and the tests at US$ 14 each; however, lower prices will be offered for public sector procurements.

The Ustar EasyNAT™ TB is a manual assay based upon their proprietary isothermal cross-priming amplification (CPA) technology and is intended for batch testing in microscopy centres.[26,207] The EasyNAT™ TB assay was released in Q2 2014, but has been a research use only product since 2009. It is CE-IVD marked and was granted CFDA approval in February 2014. The test result is displayed via an RDT-based detec-

tion of hapten labelled MTBC DNA amplicons in a sealed cassette to prevent test site contamination. The primary specimen type is sputum and Ustar offers a syringe-driven extraction tool for low-volume testing and recommends using a commercial DNA extraction kit with a centrifuge for high-volume testing. Up to 30 tests per day is the recommended throughput. Ustar notes that a heat block or thermocycler is required to incubate the CPA assays. A multicentre study of the EasyNAT™ TB assay has been recently completed in China where TB clinics enrolled 2200 TB-suspected cases and sputum specimens were tested by smear microscopy, L-J culture and the EasyNAT™ TB assay.[18] Compared to culture, the sensitivity and specificity of the CPA test for MTB detection within this group was 84.1% and 97.8%, respectively; with smear-negative/culture-positive cases the sensitivity was reduced to 59.8%. A failure rate of 0.8% indicated that trained users were able to prevent contamination of the test sites. Currently, Ustar is looking to market this test in China, India, Indonesia and Thailand and note that reagents cost US$ 8 per test. This excludes the DNA extraction components and the need for a thermocycler or heat block and centrifuge as other equipment.

Figure 27. Hain Lifescience FluoroCycler® 12: a 12-sample real-time thermocycler

Source: Image reproduced with permission from Hain Lifescience.

Hain Lifescience released the FluoroCycler® 12, in 2012, a real-time PCR diagnostic platform for MTBC diagnosis in addition to other infectious diseases (Figure 27). This device can amplify 12 reactions in a batch and up to eight of these platforms can be linked in parallel to a controlling computer. Sample preparation can be manual or automated using the GenoXtract® also offered by Hain Lifescience or via other generic tools. In 2012, Hain Lifescience also released the FluoroType® MTB real-time PCR assays for use with the FluoroCycler® 12. The detection of products is via HyBeacon probes that bind to their target sequence in an amplicon during PCR to generate a fluorescent signal. HyBeacon probe binding to the appropriate amplicon is further confirmed via melt curve analyses after PCR is complete.

Proprietary software analyses the test data to generate a result and the entire process takes four hours if automated extraction is used.[30] A recent prospective study on the performance of the assay in a low-TB prevalence setting showed that the assay had a mean sensitivity of 84.1% as compared to culture. With smear-positive samples the sensitivity was 100%, while smear-negative was lower at 56.3%. The authors noted that a positive predictive value of 100% in smear-positive isolates would also have utility in discriminating NTM from MTBC and the failure rate of the test was 0.7%.[30] The technologies used in this assay suggest that this product will compete for use in the same types of test facilities where Xpert may be used as some degree of power and infrastructure is necessary to house the equipment and reagents. A further FluoroType® MTB assay is in development using MTBC RNA as the target molecule. This is intended to be used for treatment monitoring of MTB infected patients under therapy. The initial clinical data on the

evaluation of this product will be released in Q4 2014. Hain Lifescience is also developing drug resistance genotyping assays using Lights On/Lights Off probes for use with the FluoroCycler®. The price of available tests and equipment is unknown.

Other NAATs in development

Development efforts from several groups aim to create technologies to compete with the tools described above. Many of these are fully integrated, with DNA extraction being incorporated into the test device. Alere is currently redesigning their Alere™ q platform, a fully integrated tool that they have developed for HIV viral load testing (Figure 28). This is a battery-powered, standalone device that can receive a test cartridge to provide an estimation of viral load from a sample in less than one hour.[208] The Alere™ q TB diagnostic system includes a rapid and sensitive test for case detection followed by an immediate reflex test for full drug resistance analysis to enable a test-and-treat paradigm of TB suspects. The technology is being developed for use at all levels of the health-care system, but with a specific emphasis on microscopy centres. Redesign of the Alere™ q platform is required to accommodate a larger cartridge. While HIV viral load testing only requires 5–10 µL of blood, detection of TB from sputum necessitates that multiple millilitre volumes are processed and so the next generation of q is being designed to process cartridges for either test. This development effort is being supported by a US$ 21.6 million grant from the BMGF.

Figure 28. Alere™ q instrument for TB testing that is currently in development

Notes: The sputum sample is collected in a cup that is then screwed onto the test cartridge (bottom of left image). The cartridge contains all reagents onboard and is then inserted into the machine for sample processing, DNA amplification and analysis (right image).

Source: Images reproduced with permission from Alere.

Once a sputum sample is collected, the entire sample handling and processing workflow is fully automated by the Alere™ q instrument, including liquefaction, mycobacterial enrichment, cell lysis, purification, DNA amplification, detection, and results interpretation and reporting. The Alere q can utilize either PCR or isothermal amplification technologies to drive nucleic acid amplification. This level of robust automation that has been achieved in the development is intended for safer, quicker, more sensitive and more economical identification of TB patients. The time to result for the TB case detection at this stage of development is currently less than 20 minutes from specimen collection to result. Collectively, this will enable immediate diagnosis of TB and identification of appropriate therapy regimens resulting in significant reductions in time to therapy and loss-to-follow-up. For TB DNA amplification, the isothermal nicking enzyme amplification reaction (NEAR) assay developed by Ionian Technologies Inc. (a subsidiary of Alere; USA) is used.[171] The NEAR technology can accurately amplify DNA targets in under five minutes. At this time, both the Alere™ q instrument and the NEAR TB assay chemistry are fully integrated into a single test cartridge that will soon be deployed for field testing in intended use settings along with the companion reflex drug resistance test that will use PCR with competitive reporter monitored amplification that can genotype drug resistance alleles in real time.[209]

Figure 29. GenePOC test cartridge and instrument: A: GenePOC test cartridge; B: GenePOC instrument (carousel); C: touchscreen interface

Source: Images reproduced with permission from GenePOC Inc.

GenePOC Inc. (Quebec, Canada) is developing a real-time PCR assays for MTBC and resistance genotyping (RIF/INH and FLQ) using their real-time PCR platform and integrated cassettes (Figure 29). Their device can extract and amplify DNA from samples in one hour or if sample preparation is performed manually, then time to result is 30 minutes. Up to 64 samples per machine can be processed in an eight-hour day. There is currently no information on the automated extraction technology. The amplification platform can house eight cartridges at one time on a carousel. The operating system is onboard and a touchscreen interface is used for data input and viewing results. The device requires mains electricity. The cost of instruments and cartridges is intended to be under US$ 10 000 for the device and the test cartridge to be competitive with the subsidized Xpert® MTB/RIF cartridge cost. The time to market is unknown and currently no evaluation data are available.

The Northwestern Global Health Foundation (NWGHF, USA) is developing a low-cost MTB assay using a real-time platform, the Savanna Molecular Platform under development by Quidel Inc. (USA). A reflex assay to genotype resistance to RIF is also in development. The system incorporates a heat block to first lyse MTBC cells in the sputum specimen that is then transferred to a cartridge for amplification and analysis. Up to 13 samples per machine can be processed in eight hours. The system does require mains electricity for the heat block and the instrument will have mains or battery option. The cost per device is estimated at under US$ 12 000 with cartridges at under US$ 10. The targeted date for release is in 2016.

QuantuMDx, a United Kingdom-based company, has been awarded over US$ 1.69 million to develop MTBC and MDR assays for their Q-POC™ platform. This is a small hand-held unit with a graphical user interface operated by a touchscreen. The Q-POC™ processes cartridges that contain all of the necessary reagents and sensors required to process a sample. DNA amplification via asymmetric PCR is rapid (28 cycles in 4 minutes) with a test result in 10–15 minutes. The Q-POC™ technology is unique in that detection of the amplified DNA is via their binding to complementary oligonucleotides attached to nanowires. As DNAs bind, the surface charge on the wire is altered that causes a change in resistance of the nanowire. These can be easily calibrated prior to amplification and then individually monitored. QuantuMDx notes that tens or even thousands of detector nanowires can be placed into the single fluidic channel of their cartridge. This feature offers the potential to simultaneously multiplex and test a large number of targets without using fluorophores or complex optics and instead using a single small cartridge that is cheap and simple to manufacture at scale. The specimen preparation will be predominantly automated, with lysis and amplification occurring in the cartridge. The Q-POC™ interprets the test results and is battery powered. The estimated release date for the Q-POC™ assay is availability in Q2 2016. The cost of instrumentation and tests are currently not available.

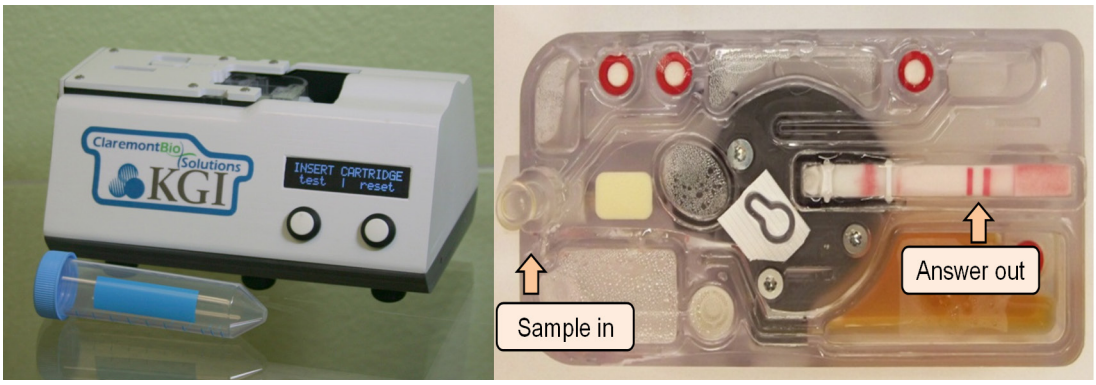
Wave 80 Biosciences (hereafter Wave 80; USA) is continuing to develop their EOSCAPE-TB System to detect MTBC and a reflexive test to genotype RIF and FLQ resistance. This requires only an external liquefaction buffer and all of the reagents for DNA extraction and DNA amplification are stored in a disposable cartridge. The MTBC DNA is amplified via NASBA, an isothermal amplification method. The test is fully integrated and automated. The extraction and amplification is performed in a small battery-powered processing unit that can be used in parallel to prepare and test multiple specimens simultaneously. To score the result, the processing unit is docked into the EOSCAPE analyser. Tests take one hour to complete. Wave 80 is developing the EOSCAPE-1 Analyzer for diagnosis and a second analyzer, the EOSCAPE-Mplex

Analyzer, for multiplexed reaction detection; both are battery powered. Wave 80 estimates that up to 50 specimens a day can be processed. The product is anticipated for release in 2016. The cost of instrumentation and tests are currently not available.

Hain Lifescience is developing single-tube real-time drug resistance assays for MDR and XDR for use with their FluoroCycler® real-time PCR machine. They have purchased the sole rights from Brandeis University (USA) to use linear-after-the-exponential (LATE) PCR, with Lights-On/Lights-Off probes and prime safe technology. LATE PCR is an asymmetric PCR design whereby primer concentrations and changes in annealing temperature bias the increased amplification of the DNA strand to which sequence-specific probes can bind.[210] An advantage of the Lights-On and Lights-Off probe technology is that it uses a single fluorescent channel to detect multiple mutations. Each Lights-On probe consists of a quencher and a fluorescent moiety, while the Lights-Off probe has only a quencher moiety.[211,212] With a matching target sequence the signals from all contiguous Lights-On probes create a composite fluorescent contour that represents a sequence-specific fluorescence signature. Probe binding is affected in the presence of mutations and creates a unique signature. By mapping the fluorescent signatures produced by different drug resistance alleles, it is possible to screen for all of these using only a single fluorescence channel, greatly reducing the complexity of instrumentation and therefore the cost of the reader in addition to saving on assay components. The devices and software are currently under development by Hain Lifescience. The technology will be presented to validation sites throughout Q3 2014 via collaboration with Stellenbosch University (South Africa). The assays will be available as CE-IVD marked products by Q2 2015.

The Keck Graduate Institute (KGI, USA) in collaboration with Claremont BioSolutions (CBIO), the University of Washington, PATH, the Seattle King County TB clinic, Leardon Solutions (all USA), and Ustar Biotechnologies (China) recently received a second NIH award to complete the development of a fully integrated tool for the rapid diagnosis of PTB, the TBDx system. This tool comprises of a compact, inexpensive, battery operated instrument and a disposable cartridge about the size of a mobile phone that can execute pathogen lysis, nucleic acid extraction, isothermal DNA amplification, and lateral flow detection in a single integrated system with minimal user input. Disinfected sputum is first passed through a microbead-beater which lyses the bacteria and extracts nucleic acids, based on CBIO's PureLyse® technology. This novel solid-phase extraction method does not require chaotropic salts or organic solvents and therefore significantly simplifies nucleic acid preparation.[213] All pumping steps within the cartridge are facilitated through inexpensive electrolytic pumps (epumps), based on water electrolysis that generates hydrogen and oxygen gases, which exert pressure on a fluid downstream. A unique, 6-channeled omnivalve controls fluid flow during nucleic acid extraction. The nucleic acids are eluted and channeled to the amplification chambers which contain lyophilized reagents. After amplification the reaction mix is pumped to an integrated lateral flow strip which detects labeled DNA amplicons if MTBC DNA is present.[214] All of these procedures are controlled via the instrument's embedded electronics. KGI is currently evaluating LAMP and CPA amplification methods incorporated into this cartridge. The completion of this technology is anticipated in 2018 and current cost estimates (ex-works) are US$ 150 for the instrument and US$ 7.67 per test cartridge.

Figure 30. KGI TBDx system: instrument (left) and test cartridge (right)



Notes: The instrument controls all processes in the cartridge, which is inserted onto the top of the instrument. This provides power for bead beating, pumping via epumps and heating for DNA amplification. The test cartridge contains all reagents and sample waste is stored on board in a reservoir. The test result is visually scored via lateral flow strip detection of labelled MTBC amplicons.

Source: Images reproduced with permission from KGI.

Great Basin Corp. (USA) has developed a standalone fully automated analyser for use with their isothermal technology based on blocked-primer-mediated helicase dependent amplification (bpHDA) whose design prevents extension from misprimed sequences to increase sensitivity and specificity of the amplification reaction. They have developed a thin film microarray printed with specific target oligonucleotides. As complementary DNA is amplified, it can bind to these and then be detected by a thin film biosensor. The reaction takes only 30 minutes to complete.[215] The group has developed a benchtop assay, the TB ID/R that detects both MTBC and RIF resistance,[216,217] but they have currently postponed development to focus on other products in their technology pipeline. A collaboration between TwistDx Ltd, the London School of Tropical Hygiene & Medicine and PATH recently described the development of two real-time assays for MTBC via IS6110 or IS1081, both of which can detect MTBC in under 20 minutes.[218] The assays use real-time recombinase polymerase amplification utilizing the battery-powered Twista™ real-time reader that can read eight reactions at one time.[218,219] TwistDx Ltd is currently not marketing these assays, but is looking to partner with technology developers to integrate them onto a test platform.

Sequencing methods

The development and application of NGS has fundamentally altered genomic research. The rapid development of this technology is intended to enhance performance and bring down DNA sequencing costs, thus widening the spectrum of possible applications. NGS is already being used within the medical diagnostics scene, including in some clinical laboratories in connection with MTBC. In the case of MTBC, NGS offers great utility for epidemiologic purposes when compared to the current typing methods used, including IS6110 restriction fragment length polymorphism, spoligotyping and mycobacterial interspersed repetitive unit–variable number of tandem repeats.[220] The current methods identify polymorphic genetic regions, but these comprise less than 1 % of the total MTBC genome. Recently, NGS methods were applied to a longitudinal molecular epidemiological study where NGS data from 2031 patients were compared with traditional genotyping data. The primary finding was that the genome-based analysis correlated better with contact tracing information and spatio-temporal patterns of the pathogen's spread.[221]

A further advantage of deep sequencing is the ability to detect heteroresistant or mixed strains in an infection. When using phenotypic methods for drug resistance screening, subpopulations of drug-resistant cells may not grow sufficiently to be detected and so patients are misclassified and incorrectly treated. Specific regions associated with drug resistance can be amplified by PCR and multiple amplicons sequenced to inform on homo- or hetero-resistant strains. Daum LT et al. recently described a standardized protocol using NGS on full-length genes associated with drug resistance to better characterize MDR and XDR MTB isolates.[222] By sequencing the entire gene of each target, less common and novel mutations associated with drug resistance can be identified as coverage is more complete and not limited to a small locus, e.g. the

81 base pair RIF determining region for RIF resistance.[223] However, mutations in the target region of drug resistance may be silent and, therefore, novel mutations have to be carefully screened by phenotypic drug resistance testing to ensure that they are significant. Another group has analysed whole genome sequences to inform on the microevolution of subpopulations in MDR TB isolates collected from patients over time and study the acquisition of new phenotypic resistances that occurred in a stepwise manner.[224] This data demonstrated that second-line drugs used for treatment do act on MDR TB strains by exerting an evolutionary pressure that selects for further drug resistance conferring mutations. By providing entire genome sequences, the application of NGS is key to better understanding resistance mechanisms to current drugs and will be an invaluable tool in understanding the evolution of drug resistance as new antibiotics and treatment regimens are approved for use.

With the impending introduction of new drugs and treatment regimens, it is clear that screening for the emergence of resistance alleles is necessary to aid treatment and to inform diagnostic developers on new targets for rapid genotypic screening of drug resistance. In addition, even with the current treatments available, a complete understanding of the drug resistance alleles and biochemical mechanisms relating to drug resistance is by no means fully understood for most antibiotics.[225,226] In principle, this information may in turn create the potential for drug developers to circumvent existing mechanisms to specific drugs. During the monitoring of clinical trials, it is often assumed that each infection is derived from a single strain as genotyping methods are limited in their sensitivity to highly discriminate between reinfection and relapse. As a recent example, NGS was used on participants of the REMox drug trial to better understand the rates of mixed infection that could be incorrectly identified as reinfection and to confirm relapse cases versus reinfection.[227] In a cohort of 47 participants, 33 were classified as relapsed cases, while 6 were classified as being reinfected by NGS. However, if mycobacterial interspersed repetitive unit–variable number of tandem repeats test data were used, four of these would have been misclassified as relapsed cases.

The relatively low cost yet high performance platforms with small laboratory footprints such as the MiSeq (Illumina, USA), Ion Personal Genome Machine® (PGM™) System (Life Technologies Incorporated; USA) or 454 FLX Junior (Roche, Switzerland) are suitable for use in many research laboratories and many of the performance and applications of commercial platforms have been compared and assessed.[228] The Illumina MiSeqDx, a derivative of the MiSeq, was approved for clinical use by the US FDA in November 2013. Life Technologies Incorporated, the developer of the Ion Torrent system, is also submitting their Ion PGM™ platform for US FDA approval in Q4 2014. Qiagen (Germany) is preparing to release their platform, the GeneReader in 2015 and intends to create a fully automated workflow from sample preparation to sequencing that may be of benefit to super national reference laboratories. The GnuBIO is another NGS system from Bio-Rad Laboratories, soon to enter the market. The GnuBIO system will use microfluidic droplets in which to perform the sequencing reactions, reducing reagent costs and allowing the user to generate tailored data rather than large arbitrary datasets.

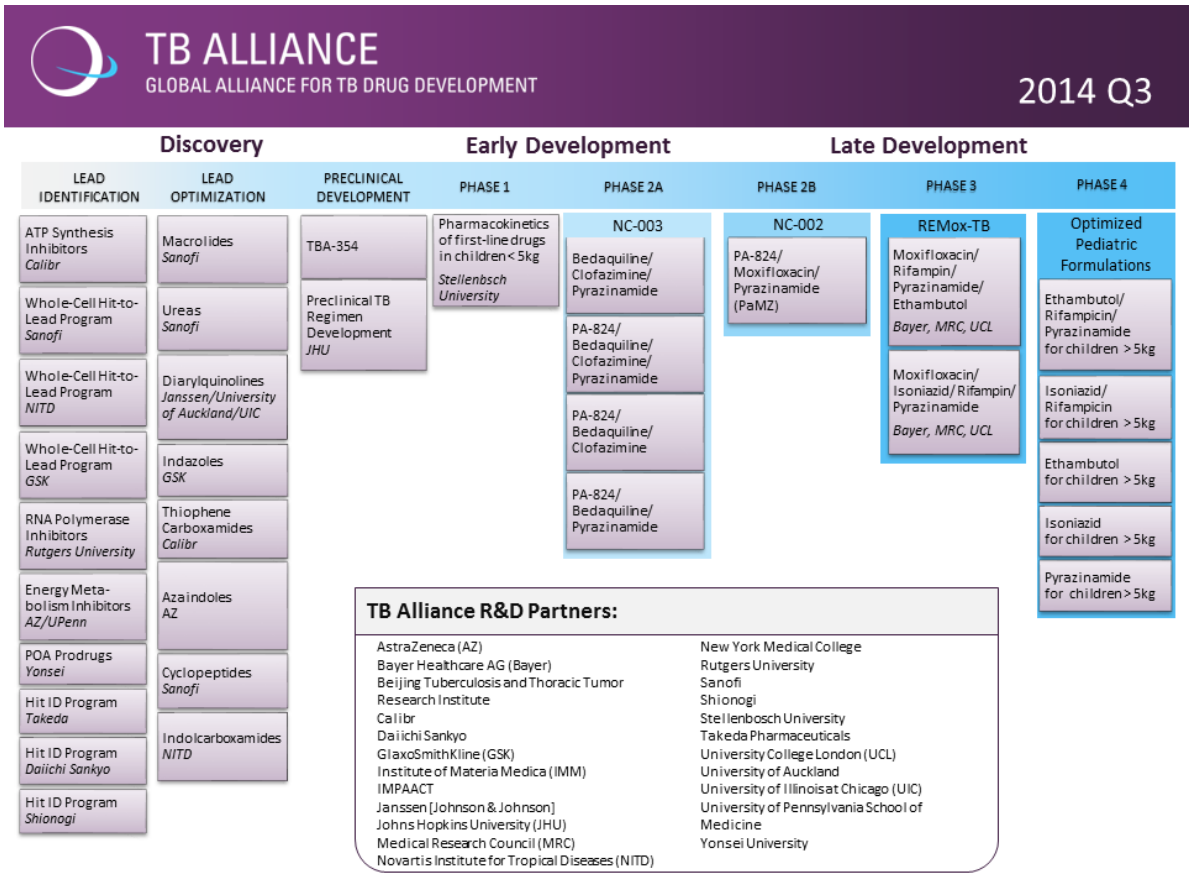
Future and emerging NGS platforms even smaller in size are in development by groups such as QuantuMDx and Oxford Nanopore Technologies (both United Kingdom). Oxford Nanopore Technologies offers two platforms, the GridION™ and the MinION™. As the company's title suggests, a single strand DNA (or RNA) is pulled through a protein-based nanopore and each base subsequently identified in sequence by virtue of a characteristic change in an electric current as the strand is drawn through the pore. The GridION™ is developed as a modular system where sequence read capacity can be incrementally increased by adding further modules or "nodes" to create a parallel system. The disposable cartridges used in this tool contain all reagents necessary to sequence the DNA strands. The MinION™ is being developed as a single-use, disposable sequencer. Both platforms use proprietary software to operate the devices and interpret the raw data.

A significant challenge with all systems is not just with the underlying complexity of technology itself, but also with having the capacity to store, manipulate, annotate and analyse the sequence data. In terms of data uploading and storage, cloud-based systems appear to be the most appropriate.[229] Many developers and researchers now offer the computational algorithms to compile raw sequence data into contigs prior to assembly of fully annotated genomic sequences.[230,231]

A workshop jointly organized by of the Stop TB Partnership NDWG and the Critical Path to TB Drug Regimens (CPTR) was held on 3-4 February 2014 in London.[232] The primary goal of this meeting was to bring together experts to landscape and develop a framework for the coordination of TB diagnostics research, in particular to develop standards for the capture of data and the sharing of data on the molecular basis of drug resistance, i.e. data derived from DNA sequencing of MDR and XDR TB isolates. Currently, there are three accessible national data repositories in which TB genomic sequence data are stored: the National Center for Biotechnology Information (USA); the European Nucleotide Archive; and the DNA Data Bank of Japan. These groups form the International Nucleotide Sequence Database Collaboration, where all genomic data can be accessed and used by national groups, researchers and industry. Currently, the Critical Path Institute (USA) is leading a working group to develop protocols and the architecture for a new sequence database specifically designed to receive and store sequence data relating to drug resistance in MTBC. This database will be available to researchers and developers.

Studies suggest that sequencing may facilitate rapid identification and monitoring of diverse MDR and XDR TB strains and could potentially be integrated into selected regional and reference settings as new TB drug regimens emerge in the three to five-year horizon. Figure 30 shows the pipeline of new TB drugs and regimens, and the need to align new drugs with new diagnostics has been described elsewhere.[233] To improve sequencing and molecular tests, research is needed to establish the genetic basis for resistance to existing as well as new drugs and to associate critical mutations with clinical consequences. Additionally, surveillance is needed to establish the background level of resistance as well as a better understanding of what is wild type for regions, countries and locales.

Figure 31. Pipeline of new TB drugs and regimens



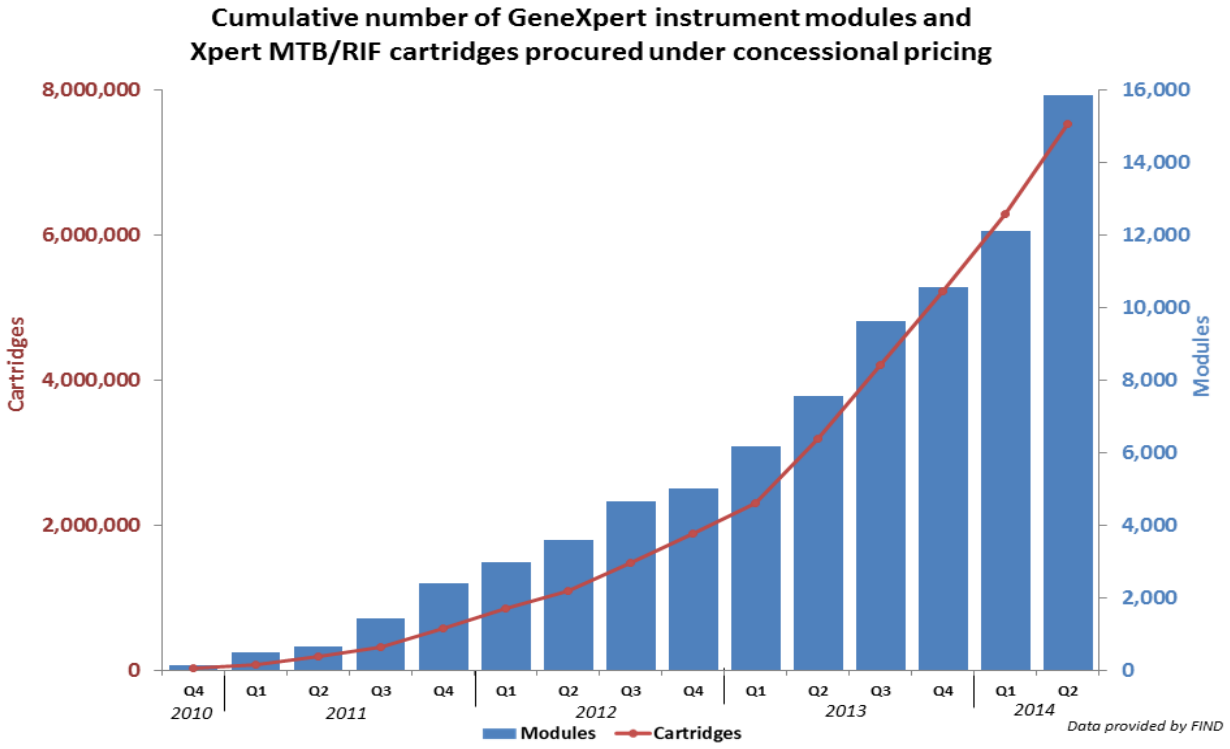
Source: Reproduced with permission from the Global Alliance for TB Drug Development (http://www.tballiance.org/pipeline/pipeline.php).

6. Market landscape

6.1. Market overview including Xpert rollout, field experiences and challenges

Globally, the rollout of Xpert® MTB/RIF continues to be the most important, measurable shift in the TB diagnostics market. According to WHO, as of 30 June 2014, a cumulative number of 3269 GeneXpert instruments (comprising 15 846 modules) and 7.53 million Xpert® MTB/RIF cartridges had been procured in the public sector in 108 of the 145 countries eligible for concessional pricing (Figure 31), with South Africa accounting for over half of all the cartridges procured to date. These numbers include a recent shipment of 774 GeneXpert systems to China. Along with previously purchased systems, this increased the cumulative total of GeneXpert systems to more than 970 in China. Cepheid also reported the recent purchase of almost 170 000 Xpert® MTB/RIF cartridges by China.[234]

Figure 32. WHO monitoring of Xpert® MTB/RIF scale-up



Notes: As of 30 June 2014, a total of 3269 GeneXpert instruments (comprising 15 846 modules) and 7 531 360 Xpert® MTB/RIF cartridges had been procured in the public sector in 108 of the 145 countries eligible for concessional pricing.

Source: WHO monitoring of Xpert rollout, based on data provided by FIND, published regularly at http://www.who.int/tb/laboratory/mtbrifrollout/en/.

Several international donors are actively supporting Xpert scale-up, including UNITAID, PEPFAR, USAID, the World Bank, the Global Fund, and Foreign Affairs, Trade and Development Canada.

Experiences of implementers have been presented in various meetings, including the annual Global Laboratory Initiative meeting, and in peer-reviewed publications. A large study in India tested over 40 000 people with suspected TB in the public sector and reported that under routine conditions, Xpert® MTB/RIF provided 99.1% valid results in TB suspects with low overall failure rates (7.2% initial failure, 0.9% final failure). Installation required minimal infrastructure modifications. However, high modular replacement (32%) and inter-lot cartridge performance variation were reported as sources of concern. [235]

Implementation experience from TB REACH projects in nine countries was also published recently.[236] The projects placed 65 machines (196 modules) in a variety of facilities and employed diverse case-finding strategies and algorithms. Over 47 000 cartridges were used. Of valid tests, 17% was positive for MTB, and of these positives, 14% was RIF resistant. Of all tests conducted, 10.6% failed. The need for continuous power supply was noted by all projects and module failure rate was 42%, but variable across countries.

An investigation conducted by Cepheid showed that dust was a major cause of the high module failure rate seen in some settings.[237] Dust can enter the modules and form a fine layer on all the components and reduce the sensitivity of the optics. Cepheid is taking action to remedy the situation, but is clear that module replacement and calibration/maintenance costs must be considered by all purchasers and donors, and should also be incorporated into cost-effectiveness analyses. Module failure and other such operational issues have been discussed in detail in the recently published WHO Xpert® MTB/RIF implementation manual on technical, operational and practical considerations.[203] While a large number of studies have confirmed the high accuracy of Xpert® MTB/RIF,[238] new studies are starting to address the issue of how the test impacts patient outcomes. The first randomized controlled trial of Xpert® MTB/RIF was published in October 2013.[239] While Xpert® MTB/RIF was found to be more accurate than smears, reduce time to treatment and result in more patients starting same-day treatment, these short-term benefits did not translate into lower TB-related morbidity in the longer term, partly because of high levels of empiric treatment at the African sites in the study. Similar results were reported by the XTEND cluster randomized trial in South Africa.[240] Xpert, compared to microscopy, increased the proportion test positive by 50%. However, Xpert did not reduce rates of initial loss-to-follow-up and there were no differences in rates of mortality or proportion starting TB treatment between the two study arms. Results of a stepped-wedge trial from Brazil are expected by the end of Q4 2014. A third randomized trial from Zimbabwe assigned patients to Xpert or fluorescence SSM at antiretroviral treatment (ART) initiation.[241] The primary end point was a composite of three-month mortality and ART-associated TB. Among HIV-infected individuals initiating ART, centralized TB screening with Xpert did not reduce the rate of ART-associated TB and mortality, as compared to fluorescence microscopy.

A common theme in these trials is the need to strengthen health systems to ensure that test results are linked with rapid and appropriate treatment for TB and co-morbid conditions such as HIV infection. In addition, Theron G et al. have discussed emerging data on how empiric treatment is often the same day, and might still be the predominant form of treatment in high-burden settings, even after Xpert implementation.[242] Thus, in such settings, Xpert might displace so-called true-positive, rather than false-positive, empiric treatment.

While mathematical modelling studies suggest that Xpert (and similar new diagnostics) can potentially save lives and help reduce transmission, it is becoming clear that the impact of Xpert may depend on whether:

- NTPs choose to implement Xpert only as a DST or as a diagnostic tool among all patients with suspected TB—i.e. restricted versus broader use;
- Xpert results are actually used in a manner that reduces empiric TB treatment;
- NTPs implement Xpert in centralized and reference laboratories, rather than decentralized subdistrict-level settings;
- Xpert can reach the level of most microscopy centres where the majority of TB testing is currently happening;
- Xpert is deployed in the best-performing laboratories/areas versus in underperforming areas where even routine diagnostic capacity is limited;
- Xpert is used in POC testing programmes to make rapid treatment decisions in the same visit (or day), and whether Xpert results are adequately linked to correct TB treatment and follow-up to ensure adherence;
- Xpert is accessible or affordable to first-contact providers (informal/private) who often see patients first and could shorten diagnostic delays.

A new survey of 22 HBCs suggests that while a majority have a policy or algorithm with Xpert, current implementation is mostly donor funded, largely dependent on testing in centralized laboratories and primarily used on patients with presumed drug resistance or HIV infection, and the test is not yet widely used as a rapid TB diagnostic tool outside of South Africa.[14] A new transmission modelling study underscored the need to implement Xpert on a broader scale, going beyond its role as a DST and outside of the NTP, for impact to be meaningful in India.[243] If Xpert is mostly restricted to the public sector, and used mainly as a DST tool, then the population-level impact on reducing incidence and mortality is limited. In contrast, private/informal provider engagement, adequate referral systems, improved treatment quality and increased resources can have a transformative impact. In fact, efforts are under way to enhance uptake of the Xpert technology in the private sector in HBCs such as Bangladesh, India, Indonesia and Pakistan. Currently, the private sector in high-TB burden countries is excluded from the negotiated pricing agreement and the US$ 9.98 price does not apply.

With donor support, Interactive Research and Development (http://irdresearch.org/) and partners are expanding access to Xpert® MTB/RIF, a WHO-endorsed test, in the private sector in Dhaka, Jakarta and Karachi, through mass verbal screening in private clinic waiting rooms and referrals for computer-aided digital X-ray diagnosis. This model includes screening and management of co-morbid conditions such as diabetes and chronic obstructive pulmonary disease to generate revenue for this social enterprise. In India, the Initiative for Promoting Affordable, Quality Tests (IPAQT) initiative (www.ipaqt.org), coordinated by the Clinton Health Access Initiative (CHAI), brought together a group of private laboratories into a partnership for promoting use of WHO-approved TB tests in the highly fragmented private sector. CHAI facilitated an agreement between the participating laboratories and negotiated with suppliers/distributors of WHO-approved tests (Xpert® MTB/RIF, LPA and liquid cultures). The laboratories that are part of IPAQT sign a charter and are eligible to access lower negotiated prices for these tests in exchange for meeting certain guiding principles laid down in the charter that, inter alia, include, case notification, affordable and agreed-upon ceiling pricing to patients and non-use of banned serological tests. Since its launch in 2013, IPAQT has grown to include 70 member laboratories across India. These member laboratories collectively account for over 3500 collection centres, covering approximately 80% of the districts in India. The number of Xpert® MTB/RIF tests and Hain Lifescience Genotype MTBDRplus used in the private sector in IPAQT's first 18 months (2013–2014) is over 60 000 tests, up from less than 2000 tests during the whole of 2012.

Regardless of whether TB patients seek care in the public or the private sector, it is important to ensure that they receive quality care that is accessible and affordable.[244] Models such as those used by Interactive Research and Development and IPAQT are among many such approaches being tried out in various settings. Continued innovation in the development of scalable, sustainable and replicable business models to provide complete, patient-centric solutions is, therefore, crucial.

6.2. Unmet needs and high-priority TPPs for new diagnostics

While the Xpert® MTB/RIF assay is a much-needed breakthrough, it was not designed to reach lower tiers of the health-care system, and not intended to meet all needs (e.g. it cannot detect latent TB or resistance against multiple drugs). High cost is also a hurdle for underfunded NTPs.

A recent study of various stakeholders helped establish the most important unmet needs, and helped identify TPPs that are of highest importance. Kik SV et al. conducted a priority-setting exercise to identify the highest priority tests for TPP development and investment in research and development. For each of the potential TPPs, 10 criteria were used to set priorities, including prioritization by key stakeholders (e.g. NTP managers), potential impact of the test on TB transmission, morbidity and mortality, market potential and implementation and scalability of the test.[1]

As shown in Table 6, a rapid, sputum-based, molecular test for microscopy centres (with the option of add-on DST cartridge) was ranked highest, followed by a rapid biomarker-based, instrument-free test for non-sputum samples (which can also detect childhood and EPTB).

Table 6. Prioritization of target product profiles (TPPs) for new diagnostics according to 10 criteria, by various stakeholders

		Prioritization by key stakeholders				Impact		Market		Implementation and scalability		Score	Priority rank
	Target product profiles for potential new TB diagnostic tests	Patients and community advocates	National tuberculosis programmes	Field practitioners	Researchers	Potential to reduce TB incidence	Potential to reduce TB morbidity and mortality	Potential (global) market size	Potential to reach the market in the next 5 years	Potential use as a point-of-care test	Potential to get scaled-up		
	TRIAGE, RULE OUT AND SYSTEMATIC SCREENING TEST												
A	Triage test for those seeking care	high	high	high	medium	high	medium	high	low	high	high	26	3
B	An HIV/ART clinic-based test to rule out active TB	high	high	high	high	low	high	medium	medium	high	high	26	3
C	Systematic screening test for active case finding	high	high	medium-high	medium	high	medium	medium	low	high	high	24.5	5
	RAPID TB DIAGNOSIS TEST (WITH OPTIONAL DRUG SUSCEPTIBILITY TESTING)												
D	Rapid, sputum-based, cartridge-based, molecular test for microscopy centers (with the option of add-on drug susceptibility testing cartridge)	medium-high	high	high	high	high	high	high	high	high	high	29.5	1
E	Rapid biomarker-based instrument-free test for non-sputum samples (which can also detect childhood and extrapulmonary TB)	high	high	high	high	high	high	high	low	high	high	28	2
F	Multiplexed test for TB and other infectious diseases	high	medium-high	low	medium	medium	medium-high	medium-high	low	high	medium	19	8
	NEXT-GENERATION DRUG SUSCEPTIBILITY TEST												
G	Centralized, high-throughput, drug susceptibility test (incorporating new drugs to support the roll out of new TB treatment regimens post 2014)	medium	high	medium	medium	low	medium	low	high	low	medium	18	9
	TREATMENT MONITORING TEST												
H	Treatment monitoring test (test for cure)	high	high	medium	medium	low	medium	low-medium	low	low	high	19.5	7
	PREDICTIVE TEST FOR LATENT TB INFECTION												
I	Predictive test for latent TB infection at high risk of active TB	high	high	medium	high	high	high	high	low	low	low	23	6

Notes: Colours in the table reflect the number of points attributed for each answer option, where a darker colour shade corresponds to more points. Five different expert groups rated one or two criteria in their field of expertise as high, medium or low priority. The final priority rank was based on the total score for all 10 criteria, where respectively 3, 2, 1, 2.5 and 1.5 points were attributed for consensus answers that were "high", "medium", "low", "medium-high" or "low-medium". The TPP ranked first received the highest score and was, therefore, judged as highest priority.

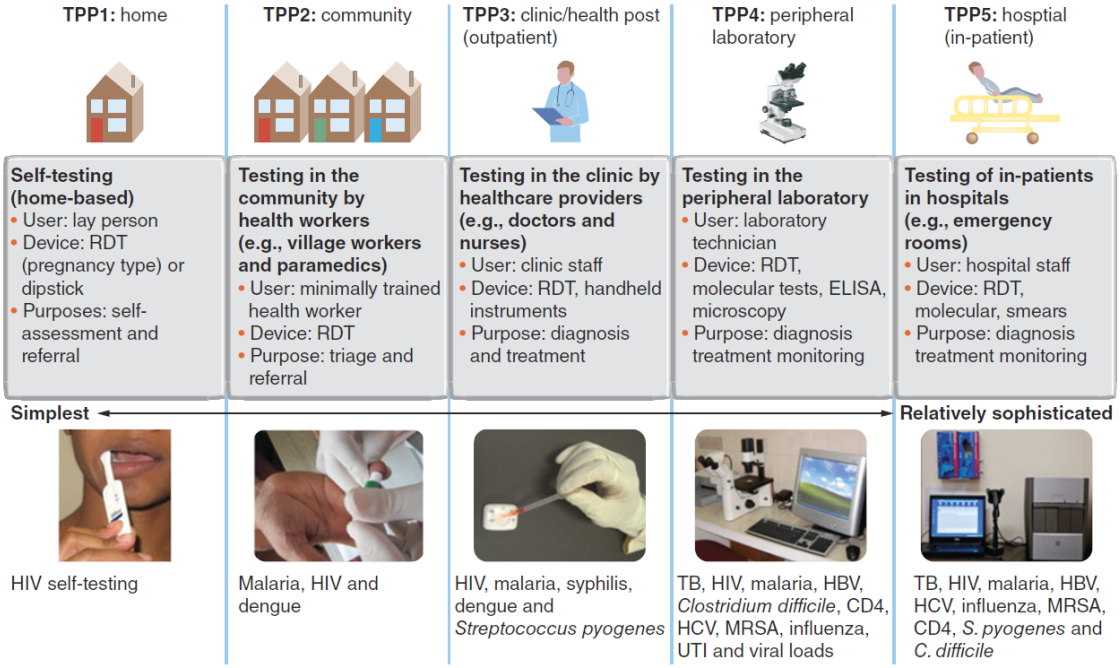
Source: reproduced with permission from Kik SV et al.1

Given the variety of felt needs, and diversity of sites where testing can occur (Figure 32), it is important for product developers to have access to: (i) a clearly identified list of diagnostics that are considered high priority by the TB community; (ii) well-developed, detailed TPPs for priority diagnostics; and (iii) up-to-date market size estimates for the priority TPPs. These issues are being addressed by ongoing activities, supported by the BMGF, FIND, UNITAID and NDWG.

Detailed TPPs have been developed for the rapid sputum-based molecular test and a biomarker-based assay (ranked first and second), as well as for a triage test (ranked third). In parallel, a TPP for a rapid DST for new drug regimens has been developed under the aegis of FIND and CPTR.

All TPPs underwent an expert Delphi process to reach consensus, and were reviewed at a Consensus Meeting on High Priority Target Product Profiles for TB diagnostics, convened by WHO in April 2014, in conjunction with the Global Laboratory Initiative and NDWG. The final consensus report, with revised TPPs, will be published by Q3/Q4 2014.

Figure 33. Diversity of potential TPPs across the spectrum of POC testing



Notes : HBV: hepatitis B virus; HCV: hepatitis C virus; MRSA: methicillin-resistant *Staphylococcus aureus*; UTI : urinary tract infection. *Source:* Reproduced with permission from Kik SV et al.[3]

6.3. Ongoing efforts to estimate market for new TB diagnostics

Efforts are also ongoing to quantify the potential market value around the various priority TPPs. For the highest ranked TPP (smear replacement molecular test), the market value estimate was published recently by Kik SV et al., who surveyed experts in each of the 22 TB HBCs in order to estimate the current SSM market and its potential replacement market for the diagnosis of PTB.[2]

The survey showed that 22 HBCs performed a total of 77.6 million sputum smears annually at a value of US$ 137 million in 42 827 microscopy centres. Of these, 61 % was done in the BRICS countries. The annual SSM market size for the initial diagnosis of TB in the 22 HBCs was estimated to consist of 61.7 million smears (79 % of 77.6 million) at an expenditure of US$ 109 million (range based on IQR of smear costs; US$ 79–166 million). After accounting for the assumptions about single replacement tests at a cost of US$ 5, the authors estimated the potential market size for a replacement test to be 30.8 million tests, with a potential market value of US$ 154 million per year. This analysis did not account for the private-sector smear market and thus may have underestimated the market potential.

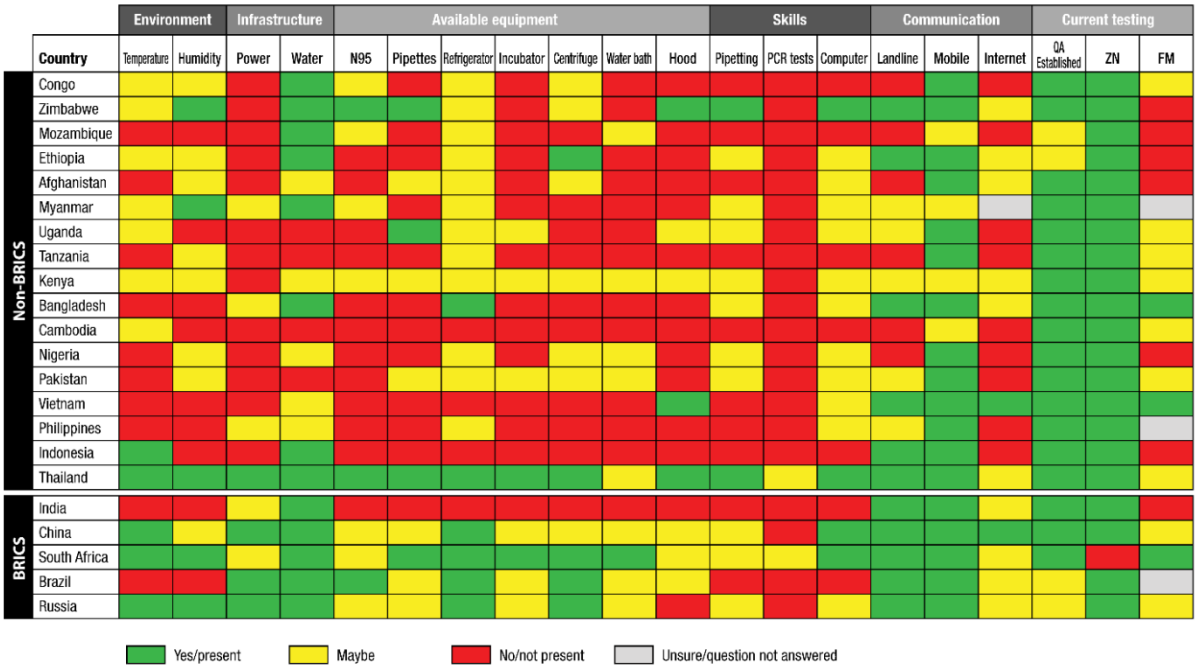
Similar efforts are now under way to estimate the potential market for a triage test, a biomarker-based test and a DST TPP for new drug regimens. This builds on a parallel initiative to estimate the current served available TB diagnostics market in Brazil, China, India, and South Africa led by the McGill International TB Centre and supported by FIND, the BMGF, UNITAID and NDWG.

Results of the assessment of the served available market in Brazil were recently published.[245] During 2012, an estimated total of 2.4 million TB diagnostic tests were done in Brazil, resulting in an estimated overall market value of US$ 17.2 million. The public sector accounted for 91 % of the test volumes and 88 % of the market value. Smear microscopy was the most commonly used test (n = 1.3 million; 55 % of the total) at an estimated value of US$ 3.7 million. Culture overall (n = 302 761) represented 13 % of test volumes and 40 % (US$ 6.9 million) of the market value. On average, US$ 208 was spent on TB diagnostics for every notified TB patient in Brazil in 2012. Results of similar market assessments in China, India and South Africa will be available by late 2014.

In addition to estimating the potential replacement market for SSM, efforts have been made to summarize the current state of peripheral microscopy centres where most smears are done, and where next-generation molecular tests are being aimed at. Because microscopy centres are usually embedded in or attached to primary health centres, they are closer to patients than district or subdistrict-level hospitals and laboratories. This suggests that TB can be diagnosed earlier at the microscopy centre level.

Denkinger CM et al. have published a survey of microscopy centres in 22 highest TB burden countries.[246] They surveyed multiple respondents from each country and asked them to complete a simple questionnaire, keeping in mind a typical, peripheral microscopy centre. The results of the survey are summarized in the "heat map" (Figure 33), which highlights scarcity of infrastructure (e.g. temperature control; uninterrupted power), lack of basic equipment (e.g. biosafety hood; centrifuge) and limited skills at the level of peripheral microscopy centres in all HBCs, although BRICS countries fare better than the others.

Figure 34. Heat map showing characteristics of peripheral microscopy centres in 22 high-TB burden countries



Notes: Questions related to environmental conditions (Is temperature or humidity not a concern?), infrastructure (Is stable power supply, clean water supply present?), presence of equipment (Are N95 respirator, micropipettes, refrigerator, incubator, centrifuge, hot water bath or biosafety hood present?) and skills (Are staff able to operate a micropipette or computer or perform a PCR test?) and the presence of means of communication (Is landline, mobile network or internet present?). Additional questions asked about whether quality assurance (QA) measures were established and which smear methods were currently used. Countries are sorted by increasing purchasing power parity. BRICS countries are Brazil, Russian Federation, India, China and South Africa. Congo refers to the Democratic Republic of the Congo; ZN, Ziehl Neelsen; and FM, fluorescence microscopy.

Source: Adapted with permission from Denkinger CM et al.[246]

6.4. Market shortcomings

The ongoing rollout of Xpert® MTB/RIF has had a positive influence on the TB diagnostics landscape, and has attracted new investments, product developers and a robust pipeline of promising technologies. It has also paved the way for wider access to molecular tests and universal DST and prepared the ground for the next wave of innovative technologies. Lessons learnt from Xpert implementation will be invaluable for scaling up next-generation technologies.

The unprecedented scale-up of Xpert® MTB/RIF has thus, in many ways, reinvigorated the market for TB diagnostics. As described in section 5, the technology landscape offers a robust pipeline of commercially available products and new technologies in late-stage development—including smaller, simpler and more robust options that may be more amenable to use at the POC, or address unmet and evolving diagnostic needs.

Despite progress, however, challenges persist. High cost of Xpert, dependence on a single-source supplier, exclusion of the private sector in HBCs from negotiated pricing agreements and difficulties in implementing this test in lower tiers of the health-care delivery system (i.e. primary care centres and peripheral microscopy labs) are important concerns. Also, emerging data suggest that the impact of Xpert on TB transmission and mortality may be limited because of widespread empiric therapy, weak health systems and lack of adequate linkages between diagnosis and treatment/follow-up. Models suggest that implementation of this technology as a DST tool in the public sector will probably have limited impact on TB incidence, especially in settings where patients often seek care from private and informal sectors.

The market has many shortcomings, in the public (NTP) sector as well as the private (non-NTP) sector. These shortcomings, and some of the reasons for them, include:

Availability: There is no true, simple, inexpensive POC TB diagnostic test: GeneXpert still requires basic laboratory infrastructure. Furthermore, while newer NAATs designed for microscopy centres have emerged, few have been adequately validated for policy and scale-up.

Reasons: Significant technical challenges (e.g. biomarker discovery) hinder development of a true POC product. Insufficient or inappropriate field evaluation deters wider application of newer NAATs. Unclear potential market and lack of clarity on available market share after GeneXpert scale-up reduce developers' willingness to invest in research. Laboratory capacity for existing culture, DST and molecular testing is suboptimal in most HBCs, and while Xpert can rapidly increase access to DST, numbers of tests performed remain low in most HBCs, with the exception of South Africa. Lack of resources for expensive second-line TB drugs (which are expensive) and effective programmatic management of MDR TB can deter commitment to universal DST (that is, NTPs can be reluctant to move towards universal DST without ensuring capacity to manage MDR TB).

Acceptability/adaptability: Current diagnostics are not adapted for specific patient groups or decentralized health-care settings. For example: (i) limited DST ability; (ii) no ability to perform multiple different tests (multiplatform functionality); (iii) not suited for children (the tests require sputum, which is hard to collect from children); and (iv) not suited for populations with low levels of mycobacteria in sputum (children, HIV co-infected patients, cases of extrapulmonary disease).

Reasons: Technical difficulty of developing technologies to address specimen collection and other challenges presented by specific patient groups. Although biomarker discovery is an active area and several potential products (e.g. antigen or antibody detection tests; VOCs, enzymatic detection) are under development for non-sputum-based testing, no test under development is likely to be on the market with policy endorsements within the next three to five years.

Affordability: New technologies are expensive: the GeneXpert machine costs US$ 17 500 (4-module), and each cartridge costs about US$ 10 to preferred buyers, or considerably more in the private sector (typical retail cost: US$ 60).

Reasons: Monopolistic supplier. High complexity of incorporating multiple reagents into a robust cartridge. Pricing agreements (e.g. buy-down pricing) are not accessible to private sector purchasers, even in low-income countries. In addition, import duties, markups by distributors and intermediaries, referral fees and incentives to providers result in significantly inflated pricing to patients in the private sector.

Quality: No information on quality of diagnostics to guide procurement. Continued use of inappropriate tests, particularly in the private sector. Insufficient regulation of TB tests and in vitro diagnostics (IVD) in general often results in suboptimal tests being easily available on the market.

Reasons: Limited global quality assurance processes for TB diagnostics; current reliance on ad hoc recommendations from the WHO STAG-TB committee. Limited in-country regulation of laboratories (e.g. few laboratories with accreditation or quality assurance) and of IVD. Underutilization of WHO-endorsed tests in favour of cheaper suboptimal tests (e.g. TB serology).

Delivery: Supply constraints affecting delivery of GeneXpert cartridges, and concerns about high rates of module failure in some settings.

Reasons: Monopolistic market with limited production capacity. No alternative suppliers for purchasers to use. Dust and environmental conditions causing Xpert module failure.

Delivery: Barriers to adoption of novel innovative technologies hinder uptake.

Reasons: Novel product types require extensive training and integration into diagnostic and clinical algorithms.

Delivery: Currently, most NTPs do not offer universal DST, resulting in less than one in four cases of MDR TB being detected. Xpert is often reserved for patients at risk of MDR or HIV, and not as a tool for early case detection in all patients with presumed TB.

Reasons: Public sector reliance on SSM that cannot detect drug resistance (i.e. only patients who fail to respond to standard treatment, or have recurrence of TB, are screened for MDR TB, resulting in morbidity, and continued transmission). Due to limited budgets, many NTPs have limited capacity to scale up new diagnostics (especially DST) without external donor support.

Delivery: Poor adherence to standards and guidelines and low quality of care, especially in the private sector—in turn resulting in widespread empiric treatment that is not supported by any diagnostic (i.e. underuse of good tests).

Reasons: Perverse incentives to use inappropriate tests and non-standard treatments in the private sector. Poor linkages between the private sector and NTP in many countries.

6.5. Potential opportunities for market intervention

As noted in section 5, the 2014 TB diagnostics technology landscape looks promising, with many product developers and a robust pipeline of technologies. Emerging technologies include smaller, simpler, more robust and portable options to address needs for more decentralized testing—including competitive alternatives to Xpert® MTB/RIF that may be better positioned to replace or complement smear microscopy in the most decentralized settings. However, lack of evidence in intended settings remains a market access barrier for most next-generation molecular tests (Eiken Loopamp™ MTBC Detection Kit being a possible exception), with WHO endorsement unlikely in the next two to three years.

With the recent US FDA approval of BED, EMA approval of delamanid and the likely introduction of new TB drug regimens, new technologies are needed to detect the emergence of novel alleles associated with drug resistance. NGS is expected to become more affordable and accessible and may address the need for new DST tools; NGS will lead the identification of these new alleles so that developers can subsequently create assays to detect them. These companion diagnostics will then be used to detect resistance to emerging drug regimens, including new and existing TB medicines, and could ensure a coherent, seamless approach to the test and treat strategy.

In the longer term, the need for a biomarker-based, low-cost, non-sputum-based test remains a key priority. Such a test could potentially be implemented at points of first contact in the community—not only to diagnose TB, but also potentially to help triage people who require confirmatory testing. Although biomarker discovery is an active area, no test under development is likely to be on the market and policy endorsed within the next five years.

The engagement of several new product developers in the TB space is a promising development and it is important to sustain and support their interest. Recently developed priority TPPs offer a starting point and provide guidance for targeted development efforts by manufacturers interested in entering the TB diagnostic area. In addition, there have been recent efforts to quantify or estimate the market size, reflecting the most important unmet needs and potential for commercial developers.

UNITAID recognizes that first-contact TB care providers are often those in the informal or private sector, particularly in some HBCs such as Bangladesh, India and Pakistan. As described earlier in section 6, engagement of these private-sector care providers can significantly improve TB diagnosis and care. Increasing private-sector care providers' access to appropriate diagnostic tools could improve diagnostic accuracy and shorten delays in initiating effective TB treatment. However, private sector care providers may respond to different incentives and drivers of behaviour than those in the public sector. Continued innovation in the development of scalable, sustainable and replicable business models to provide complete, patient-centric solutions is, therefore, crucial.

In summary, potential market-based interventions related to TB diagnostics may include efforts to:

- *Accelerate market entry for innovative POC TB diagnostics*, especially those positioned to replace smear microscopy in the most decentralized settings. Where critical for access, consider supporting capacity to scale up manufacturing and/or appropriate field evaluation of newer tests to generate performance data needed to inform NTP policies.
- *Sustain and support manufacturers' engagement in development of new TB diagnostics that address unmet needs* (e.g. evolving DST capability; use of specimens other than sputum). Support efforts to describe priority TPPs and quantify potential markets for these diagnostics.
- *Develop or refine novel approaches to engage private sector care providers, including innovative business models that leverage market-based incentives for appropriate TB diagnosis*.

As noted in the previous (2nd) edition of the UNITAID TB diagnostics technology and market landscape,[174] additional potential interventions may include efforts to:

- *Support global efforts to develop quality assurance policies and systems for TB diagnostics*.
- *Facilitate development of open platforms or generic competition and TB diagnostics for use in underserved patient groups*, including EPTB, children and PLHIV.

7. References

1. Kik SV, Denkinger CM, Casenghi M, Vadnais C, Pai M. Tuberculosis diagnostics: which target product profiles should be prioritised? Eur Respir J. 2014.
2. Kik SV, Denkinger CM, Chedore P, Pai M. Replacing smear microscopy for the diagnosis of tuberculosis: what is the market potential? Eur Respir J. 2014.
3. Kik SV, Qin ZZ, Pai M. Optimal diagnosis: how early and improved diagnosis can help prevent TB transmission. Clinical Insights: Tuberculosis Prevention. Future Medicine Ltd. 2014;7–32.
4. Global tuberculosis report 2013. Geneva: World Health Organization; 2013 (http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf?ua = 1, accessed 25 May 2014).
5. Getahun H, Gunneberg C, Granich R, Nunn P. HIV infection-associated tuberculosis: the epidemiology and the response. Clin Infect Dis. 2010;50(Suppl. 3):S201–S207.
6. Light RW. Update on tuberculous pleural effusion. Respirology. 2010;15(3):451–8.
7. Dodd PJ, Gardiner E, Coglan R, Seddon JA. Burden of childhood tuberculosis in 22 high-burden countries: a mathematical modelling study. Lancet Global Health. 29 July 2014;2(8):e453–e459..
8. Nicol MP, Zar HJ. New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects. Paediatr Respir Rev. 2011;12(1):16–21.
9. Swingler GH, du TG, Andronikou S, van der ML, Zar HJ. Diagnostic accuracy of chest radiography in detecting mediastinal lymphadenopathy in suspected pulmonary tuberculosis. Arch Dis Child. 2005;90(11):1153–6.
10. Global strategy and targets for tuberculosis prevention, care and control after 2015. Geneva: World Health Organization; 2014.
11. Blok L, Creswell J, Stevens R et al. A pragmatic approach to measuring, monitoring and evaluating interventions for improved tuberculosis case detection. Int Health. 2014.
12. Sreeramareddy CT, Panduru KV, Menten J, Van den EJ. Time delays in diagnosis of pulmonary tuberculosis: a systematic review of literature. BMC Infect Dis. 2009;9:91.
13. Sreeramareddy CT, Qin ZZ, Satyanarayana S, Subbaraman R, Pai M. Delays in diagnosis and treatment of pulmonary tuberculosis in India: a systematic review. Int J Tuberc Lung Dis. 2014;18(3):255–66.
14. Qin ZZ. National policies and practices for use of Xpert MTB/RIF in 22 high-tuberculosis-burden countries. Montreal: McGill University; 9 August 2014.
15. Eldred LJ et al. Isoniazid preventive therapy for HIV-infected people: evidence to support implementation. AIDS. 2010;24(Suppl.)5:S1–S3.
16. Getahun H et al. Development of a standardized screening rule for tuberculosis in people living with HIV in resource-constrained settings: individual participant data meta-analysis of observational studies. PLoS Med. 2011;8(1):e1000391.
17. Ou X et al. Diagnostic accuracy of the PURE-LAMP Test for Pulmonary Tuberculosis at the county-level laboratory in China. PLoS One. 2014;9(5):e94544.
18. Ou X et al. A multicenter study of cross-priming amplification for tuberculosis diagnosis at peripheral level in China. Tuberculosis (Edinb). 2014.
19. Weyer K et al. Rapid molecular TB diagnosis: evidence, policy making and global implementation of Xpert MTB/RIF. Eur Respir J. 2013;42(1):252–71.
20. Commercial Serodiagnostic tests for diagnosis of tuberculosis: policy statement. Geneva: World Health Organization; 2011.

21. Use of Interferon-γ Release Assays (IGRAs) In Tuberculosis Control in Low- and Middle-Income Settings. Expert Group Meeting Report, Geneva: World Health Organization; 2011 (http://whqlibdoc.who.int/hq/2011/WHO_HTM_TB_2011.17_eng.pdf?ua=1, accessed: 6 January 2014).

22. The Use of a Commercial Loop-Mediated Isothermal Amplification Assay (TB-LAMP) For The Detection of Tuberculosis. Expert Group Meeting Report, Geneva: World Health Organization; 2013.

23. The Use of Molecular Line Probe Assay For The Detection of Resistance to Second-Line Anti-Tuberculosis Drugs. Expert Group Meeting Report, Geneva: World Health Organization; 2013 (http://apps.who.int/iris/bitstream/10665/78099/1/WHO_HTM_TB_2013.01.eng.pdf, accessed: 5 May 2014).

24. Technical Guide: Sputum Examination for Tuberculosis by Direct Microscopy in Low Income Countries, 5th edition. International Union Against Tuberculosis and Lung Disease; 2000 (http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/IUATLD_afb%20microscopy_guide.pdf, accessed: 1 May 2014).

25. Standards for TB Care in India. Geneva: World Health Organization Country Office for India; 2014 (http://www.tbcindia.nic.in/pdfs/STCI%20Book_Final%20%20060514.pdf, accessed: 4 June 2014.

26. Fang R et al. Cross-priming amplification for rapid detection of Mycobacterium tuberculosis in sputum specimens. J Clin Microbiol. 2009;47(3):845–7.

27. Mitarai S et al. Evaluation of a simple loop-mediated isothermal amplification test kit for the diagnosis of tuberculosis. Int J Tuberc Lung Dis. 2011;15(9):1211–7,i.

28. Castan P et al. Point-of-care system for detection of Mycobacterium tuberculosis and rifampin resistance in sputum samples. J Clin Microbiol. 2014;52(2):502–7.

29. Nikam C et al. Rapid diagnosis of Mycobacterium tuberculosis with Truenat MTB: a near-care approach. PLoS One. 2013;8(1):e51121.

30. Hofmann-Thiel S, Hoffmann H. Evaluation of Fluorotype MTB for detection of Mycobacterium tuberculosis complex DNA in clinical specimens from a low-incidence country. BMC Infect Dis. 2014;14:59.

31. Palmieri F et al. Pulmonary tuberculosis in HIV-infected patients presenting with normal chest radiograph and negative sputum smear. Infection. 2002;30(2):68–74.

32. Pepper T et al. Normal chest radiography in pulmonary tuberculosis: implications for obtaining respiratory specimen cultures. Int J Tuberc Lung Dis. 2008;12(4):397–403.

33. Tuberculosis prevalence surveys: a handbook, 2nd edition. Geneva: World Health Organization; 2011.

34. van't Hoog AH et al. Screening strategies for tuberculosis prevalence surveys: the value of chest radiography and symptoms. PLoS One. 2012;7(7):e38691.

35. Kisembo HN et al. Chest radiographic findings of pulmonary tuberculosis in severely immunocompromised patients with the human immunodeficiency virus. Br J Radiol. 2012;85(1014):e130–e139.

36. Wei X et al. Changes in pulmonary tuberculosis prevalence: evidence from the 2010 population survey in a populous province of China. BMC Infect Dis. 2014;14:21.

37. Theron G et al. Do adjunct tuberculosis tests, when combined with Xpert MTB/RIF, improve accuracy and the cost of diagnosis in a resource-poor setting? Eur Respir J. 2012;40(1):161–8.

38. Muto H et al. Filmless versus film-based systems in radiographic examination costs: an activity-based costing method. BMC Health Serv Res. 2011;11:246.

39. Jaeger S et al. Automatic tuberculosis screening using chest radiographs. IEEE Trans Med Imaging. 2014;33(2):233–45.

40. Maduskar P, Muyoyeta M, Ayles H, Hogeweg L, Peters-Bax L, van Ginneken B. Detection of tuberculosis using digital chest radiography: automated reading vs. interpretation by clinical officers. Int J Tuberc Lung Dis. 2013;17(12):1613–20.

41. Muyoyeta M et al. The sensitivity and specificity of using a computer aided diagnosis program for automatically scoring chest X-rays of presumptive TB patients compared with Xpert MTB/RIF in Lusaka, Zambia. PLoS One. 2014;9(4):e93757.

42. Fujita A, Murata K, Takamori M. Novel method for sputum induction using the Lung Flute in patients with suspected pulmonary tuberculosis. Respirology. 2009;14(6):899–902.

43. Holmberg RC et al. High-throughput, automated extraction of DNA and RNA from clinical samples using TruTip technology on common liquid handling robots. J Vis Exp. 2013(76):e50356.

44. Daum LT et al. A clinical specimen collection and transport medium for molecular diagnostic and genomic applications. Epidemiol Infect. 2011;139(11):1764–73.

45. Daum LT, Choi Y, Worthy SA, Rodriguez JD, Chambers JP, Fischer GW. A molecular transport medium for collection, inactivation, transport, and detection of Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 2014;18(7):847–9.

46. Liu J et al. Increased case finding of tuberculosis from sputum and sputum deposits after magnetic bead concentration of mycobacteria. J Microbiol Methods. 2013;93(2):144–7.

47. Wang X et al. Bead capture increases the sensitivity of sputum microscopy for the diagnosis of tuberculosis in Beijing, China. Trans R Soc Trop Med Hyg. 2013;107(11):741–3.

48. Ghodbane R, Drancourt M. Magnetic bead protocol for culturing Mycobacterium tuberculosis from sputum specimens. J Clin Microbiol. 2013;51(5):1578–9.

49. Mitarai S et al. TRICORE, a novel bead-based specimen concentration method for the culturing of Mycobacterium tuberculosis. J Microbiol Methods. 2012;90(3):152–5.

50. Mase SR et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. Int J Tuberc Lung Dis. 2007;11(5):485–95.

51. Cuevas LE et al. A multi-country non-inferiority cluster randomized trial of frontloaded smear microscopy for the diagnosis of pulmonary tuberculosis. PLoS Med. 2011;8(7):e1000443.

52. Davis JL, Cattamanchi A, Cuevas LE, Hopewell PC, Steingart KR. Diagnostic accuracy of same-day microscopy versus standard microscopy for pulmonary tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis. 2013;13(2):147–54.

53. Anagaw B et al. Improved detection of acid-fast bacilli in sputum by the bleach-concentration technique at Gondar University Teaching Hospital, northwest Ethiopia. Ethiop Med J. 2012;50(4):349–54.

54. Mindolli PB, Salmani MP, Parandekar PK. Improved diagnosis of pulmonary tuberculosis using bleach microscopy method. J Clin Diagn Res. 2013;7(7):1336–8.

55. Thammavong C et al. Bleach treatment of sputum samples aids pulmonary tuberculosis screening among HIV-infected patients in Laos. Int J Tuberc Lung Dis. 2011;15(10):1353–8.

56. Albert H et al. Operational implementation of LED fluorescence microscopy in screening tuberculosis suspects in an urban HIV clinic in Uganda. PLoS One. 2013;8(9):e72556.

57. Trusov A et al. Comparison of Lumin LED fluorescent attachment, fluorescent microscopy and Ziehl-Neelsen for AFB diagnosis. Int J Tuberc Lung Dis. 2009;13(7):836–41.

58. Xia H et al. Multicentre evaluation of Ziehl-Neelsen and light-emitting diode fluorescence microscopy in China. Int J Tuberc Lung Dis. 2013;17(1):107–12.

59. Andrew AB, Wilson W, Stuart RD. The application of fluorescent microscopy to the detection of tubercle bacilli in public health bacteriology. Glasgow Med J. 1947;28(8):220–3.

60. Fluorescent light emitting diode microscopy for diagnosis of tuberculosis, 2010: policy statement. Geneva: World Health Organization; 2010 (http://www.stoptb.org/wg/gli/assets/documents/WHO%20Policy%20Statement%20on%20LED%20Microscopy%20FINAL%20March%202010.pdf, accessed: 1 May 2014).

61. Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis: policy statement. Geneva: World Health Organization; 2011 (http://whqlibdoc.who.int/publications/2011/9789241501613_eng.pdf, accessed: 1 May 2014.

62. Turnbull ER et al. An evaluation of the performance and acceptability of three LED fluorescent microscopes in Zambia: lessons learnt for scale-up. PLoS One. 2011;6(11):e27125.

63. Cuevas LE et al. LED fluorescence microscopy for the diagnosis of pulmonary tuberculosis: a multi-country cross-sectional evaluation. PLoS Med. 2011;8(7):e1001057.

64. Mugusi F, Villamor E, Urassa W, Saathoff E, Bosch RJ, Fawzi WW. HIV co-infection, CD4 cell counts and clinical correlates of bacillary density in pulmonary tuberculosis. Int J Tuberc Lung Dis. 2006;10(6):663–9.

65. Behr MA et al. Transmission of Mycobacterium tuberculosis from patients smear-negative for acid-fast bacilli. Lancet. 1999;353(9151):444–9.

66. Kam KM, Yip CW, Tang HS, Van DA. Bulk acid-fast staining of sputum smears: time to end a taboo. Int J Tuberc Lung Dis. 2009;13(9):1119–23.

67. Khutlang R, Krishnan S, Whitelaw A, Douglas TS. Automated detection of tuberculosis in Ziehl-Neelsen-stained sputum smears using two one-class classifiers. J Microsc. 2010;237(1):96–102.

68. Mateos-Perez JM et al. Comparative evaluation of autofocus algorithms for a real-time system for automatic detection of Mycobacterium tuberculosis. Cytometry A. 2012;81(3):213–21.

69. Osibote OA, Dendere R, Krishnan S, Douglas TS. Automated focusing in bright-field microscopy for tuberculosis detection. J Microsc. 2010;240(2):155–63.

70. Patel B, Douglas TS. Creating a virtual slide map of sputum smears by auto-stitching. Conf Proc IEEE Eng Med Biol Soc. 2011;5088–91.

71. Patel B, Douglas TS. Creating a virtual slide map from sputum smear images for region-of-interest localisation in automated microscopy. Comput Methods Programs Biomed. 2012;108(1):38–52.

72. Sadaphal P, Rao J, Comstock GW, Beg MF. Image processing techniques for identifying Mycobacterium tuberculosis in Ziehl-Neelsen stains. Int J Tuberc Lung Dis. 2008;12(5):579–82.

73. Veropoulos K, Learmonth G, Campbell C, Knight B, Simpson J. Automated identification of tubercle bacilli in sputum: a preliminary investigation. Anal Quant Cytol Histol. 1999;21(4):277–82.

74. Lewis JJ et al. "Proof-of-concept" evaluation of an automated sputum smear microscopy system for tuberculosis diagnosis. PLoS One. 2012;7(11):e50173.

75. Chang J et al. Automated tuberculosis diagnosis using fluorescence images from a mobile microscope. Med Image Comput Comput Assist Interv. 2012;15(Part 3):345–52.

76. Tapley A et al. Mobile digital fluorescence microscopy for diagnosis of tuberculosis. J Clin Microbiol. 2013;51(6):1774–8.

77. Zwirglmaier K, Ludwig W, Schleifer KH. Recognition of individual genes in a single bacterial cell by fluorescence in situ hybridization-RING-FISH. Mol Microbiol. 2004;51(1):89–96.

78. Stender H, Mollerup TA, Lund K, Petersen KH, Hongmanee P, Godtfredsen SE. Direct detection and identification of Mycobacterium tuberculosis in smear-positive sputum samples by fluorescence in situ hybridization (FISH) using peptide nucleic acid (PNA) probes. Int J Tuberc Lung Dis. 1999;3(9):830–7.

79. Albert H et al. Performance of three LED-based fluorescence microscopy systems for detection of tuberculosis in Uganda. PLoS One. 2010;5(12):e15206.

80. Hobby GL, Holman AP, Iseman MD, Jones JM. Enumeration of tubercle bacilli in sputum of patients with pulmonary tuberculosis. Antimicrob Agents Chemother. 1973;4(2):94–104.

81. Yeager H, Jr, Lacy J, Smith LR, LeMaistre CA. Quantitative studies of mycobacterial populations in sputum and saliva. Am Rev Respir Dis. 1967;95(6):998–1004.

82. Tuberculosis laboratory biosafety manual. Geneva: World Health Organization; 2012 (http://www.who.int/tb/publications/2012/tb_biosafety/en/index.html, accessed: 5 May 2014).

83. Chatterjee M, Bhattacharya S, Karak K, Dastidar SG. Effects of different methods of decontamination for successful cultivation of Mycobacterium tuberculosis. Indian J Med Res. 2013;138(4):541–8.

84. Buijtels PC, Petit PL. Comparison of NaOH-N-acetyl cysteine and sulfuric acid decontamination methods for recovery of mycobacteria from clinical specimens. J Microbiol Methods. 2005;62(1):83–8.

85. Peres RL et al. Comparison of two concentrations of NALC-NaOH for decontamination of sputum for mycobacterial culture. Int J Tuberc Lung Dis. 2009;13(12):1572–5.

86. Cernoch PL, Enni RK, Saubolle MA, Wallace AS, Weissfeld AS, editors. Laboratory diagnosis of the mycobacterioses; cumulative techniques and procedures in clinical microbiology. Washington DC: American Society of Microbiology; 1994.

87. Drancourt M, Raoult D. Cost-effectiveness of blood agar for isolation of mycobacteria. PLoS Negl Trop Dis. 2007;1(2).

88. Cohn ML, Waggoner RF, McClatchy JK. The 7H11 medium for the cultivation of mycobacteria. Am Rev Resp Dis. 1968;98:295.

89. Kidenya BR et al. Early and efficient detection of Mycobacterium tuberculosis in sputum by microscopic observation of broth cultures. PLoS One. 2013;8(2).

90. Lazarus RP, Kalaiselvan S, John KR, Michael JS. Evaluation of the microscopic observational drug susceptibility assay for rapid and efficient diagnosis of multi-drug resistant tuberculosis. Indian J Med Microbiol. 2012;30(1):64–8.

91. Use of liquid TB culture and drug susceptibilty testing (DST) in low and medium income settings. Summary report of the Expert Group Meeting on the Use of Liquid Culture Media, Geneva: World Health Organization; 27 March 2007.

92. Kocagoz T et al. Efficiency of the TK Culture System in the diagnosis of tuberculosis. Diagn Microbiol Infect Dis. 2012;72(4):350–7.

93. Kocagöz T. Culture medium for rapid detection of mycobacterial growth by color change. Inventor: US Patent No. 7,754,485 B2; 13 July 2010.

94. Ciftci IH, Karakece E. Comparative evaluation of TK SLC-L, a rapid liquid mycobacterial culture medium, with the MGIT system. BMC Infect Dis. 2014;14:130.

95. Noncommercial Culture and Drug-Susceptibility Testing Methods for Screening Patients at Risk for Multidrug-Resistant Tuberculosis: policy statement. Geneva: World Health Organization; 2011 (http://whqlibdoc.who.int/publications/2011/9789241501620_eng.pdf?ua=1, accessed: 5 September 2014).

96. Caviedes L et al. Rapid, efficient detection and drug susceptibility testing of Mycobacterium tuberculosis in sputum by microscopic observation of broth cultures. The Tuberculosis Working Group in Peru. J Clin Microbiol. 2000;38(3):1203–8.

97. Caviedes L, Moore DA. Introducing MODS: a low-cost, low-tech tool for high-performance detection of tuberculosis and multidrug resistant tuberculosis. Indian J Med Microbiol. 2007;25(2):87–8.

98. Moore DA et al. Microscopic observation drug susceptibility assay, a rapid, reliable diagnostic test for multidrug-resistant tuberculosis suitable for use in resource-poor settings. J Clin Microbiol. 2004;42(10):4432–7.

99. Rastogi N, Goh KS, David HL. Selective inhibition of the Mycobacterium tuberculosis complex by p-nitro-alpha-acetylamino-beta-hydroxypropio phenone (NAP) and p-nitrobenzoic acid (PNB) used in 7H11 agar medium. Res Microbiol. 1989;140(6):419–23.

100. Shah NS et al. Rapid diagnosis of tuberculosis and multidrug resistance by the microscopic-observation drug-susceptibility assay. Am J Respir Crit Care Med. 2011;183(10):1427–33.

101. Nishiyama H et al. Optimization of the microscopic observation drug susceptibility assay for four first-line drugs using Mycobacterium tuberculosis reference strains and clinical isolates. J Microbiol Methods. 2014;101C:44–8.

102. Minion J, Leung E, Menzies D, Pai M. Microscopic-observation drug susceptibility and thin layer agar assays for the detection of drug resistant tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis. 2010;10(10):688–98.

103. Martin L et al. A field evaluation of the Hardy TB MODS Kit™ for the rapid phenotypic diagnosis of TB and MDRTB. PloS One. In press.

104. Singh S, Kumar P, Sharma S, Mumbowa F, Martin A, Durier N. Rapid identification and drug susceptibility testing of Mycobacterium tuberculosis: standard operating procedure for non-commercial assays: Part 1: Microscopic Observation Drug Susceptibility Assay v2.4.12. J Lab Physicians. 2012;4(2):101–11.

105. Warren NG, Body BA, Dalton HP. An improved reagent for mycobacterial nitrate reductase tests. J Clin Microbiol. 1983;18(3):546–9.

106. Ramos E et al. Optimizing tuberculosis testing for basic laboratories. Am J Trop Med Hyg. 2010;83(4):896–901.

107. Martin A, Portaels F, Palomino JC. Colorimetric redox-indicator methods for the rapid detection of multidrug resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis. J Antimicrob Chemother. 2007;59(2):175–83.

108. Singh S, Kumar P, Sharma S, Mumbowa F, Martin A, Durier N. Rapid identification and drug susceptibility testing of Mycobacterium tuberculosis: standard operating procedure for non-commercial assays: Part 3: Colorimetric Redox Indicator Assay v1.3.12. J Lab Physicians. 2012;4(2):120–6.

109. Singh S, Kumar P, Sharma S, Mumbowa F, Martin A, Durier N. Rapid identification and drug susceptibility testing of Mycobacterium tuberculosis: standard operating procedure for non-commercial assays: Part 2: Nitrate Reductase Assay v1.3.12. J Lab Physicians. 2012;4(2):112–19.

110. Martin A, Palomino JC. Thin layer agar (TLA): microcolony detection. Antwerp: Institute of Tropical Medicine; 2009.

111. Mejia GI, Castrillon L, Trujillo H, Robledo JA. Microcolony detection in 7H11 thin layer culture is an alternative for rapid diagnosis of Mycobacterium tuberculosis infection. Int J Tuberc Lung Dis. 1999;3(2):138–42.

112. Satti L, Ikram A, Abbasi S, Malik N, Mirza IA, Martin A. Evaluation of thin-layer agar 7H11 for the isolation of Mycobacterium tuberculosis complex. Int J Tuberc Lung Dis. 2010;14(10):1354–6.

113. Roger JV, Choi JW. Preliminary evaluation of Mycobacterium tuberculosis detection in culture and artificial sputum using a BioNanoPore Membrane and Real-time PCR. Microbial & Biochemical Technology. 2012;4(7):147–51.

114. Toit K et al. The colour test for drug susceptibility testing of Mycobacterium tuberculosis strains. Int J Tuberc Lung Dis. 2012;16(8):1113–18.

115. Devasia RA et al. Fluoroquinolone resistance in Mycobacterium tuberculosis: an assessment of MGIT 960, MODS and nitrate reductase assay and fluoroquinolone cross-resistance. J Antimicrob Chemother. 2009;63(6):1173–8.

116. Lee S et al. Evaluation of a modified antimycobacterial susceptibility test using Middlebrook 7H10 agar containing 2,3-diphenyl-5-thienyl-(2)-tetrazolium chloride. J Microbiol Methods. 2006;66(3):548–51.

117. Rodrigues C, Jani J, Shenai S, Thakkar P, Siddiqi S, Mehta A. Drug susceptibility testing of Mycobacterium tuberculosis against second-line drugs using the Bactec MGIT 960 System. Int J Tuberc Lung Dis. 2008;12(12):1449–55.

118. Martin A et al. Prospective multicentre evaluation of the direct nitrate reductase assay for the rapid detection of extensively drug-resistant tuberculosis. J Antimicrob Chemother. 2014;69(2):441–4.

119. Huang ZK, Luo Q, Xiong GL, Li WT, Xu XM, Li JM. Evaluation of indirect drug susceptibility testing using the MODS assay for the detection of XDR-TB in China. Int J Tuberc Lung Dis. 2014;18(4):461–5.

120. Martin A et al. Multicentre laboratory validation of the colorimetric redox indicator (CRI) assay for the rapid detection of extensively drug-resistant (XDR) Mycobacterium tuberculosis. J Antimicrob Chemother. 2011;66(4):827–33.

121. Mpagama SG et al. Application of quantitative second-line drug susceptibility testing at a multidrug-resistant tuberculosis hospital in Tanzania. BMC Infect Dis. 2013;13:432.

122. Mulvey MC, Sacksteder KA, Einck L, Nacy CA. Generation of a novel nucleic acid-based reporter system to detect phenotypic susceptibility to antibiotics in Mycobacterium tuberculosis. MBio. 2012;3(2).

123. Horsburgh CR Jr. Epidemiology of disease caused by nontuberculous mycobacteria. Semin Respir Infect. 1996;11(4):244–51.

124. Johnson MM, Odell JA. Nontuberculous mycobacterial pulmonary infections. J Thorac Dis. 2014;6(3):210–20.

125. Maiga M et al. Failure to recognize nontuberculous mycobacteria leads to misdiagnosis of chronic pulmonary tuberculosis. PLoS One. 2012;7(5):e36902.

126. Miguez-Burbano MJ et al. Non-tuberculous mycobacteria disease as a cause of hospitalization in HIV-infected subjects. Int J Infect Dis. 2006;10(1):47–55.

127. Chikamatsu K et al. Comparative evaluation of three immunochromatographic identification tests for culture confirmation of Mycobacterium tuberculosis complex. BMC Infect Dis. 2014;14:54.

128. Global Laboratory Initiative. SRLN evaluation protocol for the laboratory evaluation of commercial assays for the rapid species identification of M. tuberculosis from cultured isolates in both solid and liquid culture systems. Geneva: World Health Organization; 2014.

129. Steingart KR et al. Commercial serological tests for the diagnosis of active pulmonary and extrapulmonary tuberculosis: an updated systematic review and meta-analysis. PLoS Med. 2011;8(8):e1001062.

130. Pinto LM, Grenier J, Schumacher SG, Denkinger CM, Steingart KR, Pai M. Immunodiagnosis of tuberculosis: state of the art. Med Princ Pract. 2012;21(1):4–13.

131. Dowdy DW, Steingart KR, Pai M. Serological testing versus other strategies for diagnosis of active tuberculosis in India: a cost-effectiveness analysis. PLoS Med. 2011;8(8):e1001074.

132. Pai M et al. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. Clin Microbiol Rev. 2014;27(1):3–20.

133. Kunnath-Velayudhan S et al. Dynamic antibody responses to the Mycobacterium tuberculosis proteome. Proc Natl Acad Sci USA. 2010;107(33):14703–8.

134. Boyle DS, Hawkins KR, Steele MS, Singhal M, Cheng X. Emerging technologies for point-of-care CD4 T-lymphocyte counting. Trends Biotechnol. 2012;30(1):45–54.

135. Shen G, Behera D, Bhalla M, Nadas A, Laal S. Peptide-based antibody detection for tuberculosis diagnosis. Clin Vaccine Immunol. 2009;16(1):49–54.

136. Houghton RL et al. Use of multiepitope polyproteins in serodiagnosis of active tuberculosis. Clin Diagn Lab Immunol. 2002;9(4):883–91.

137. Wanchu A et al. Biomarkers for clinical and incipient tuberculosis: performance in a TB-endemic country. PLoS One. 2008;3(4):e2071.

138. Achkar JM et al. Antibodies against immunodominant antigens of Mycobacterium tuberculosis in subjects with suspected tuberculosis in the United States compared by HIV status. Clin Vaccine Immunol. 2010;17(3):384–92.

139. Singh KK et al. Peptides of a novel Mycobacterium tuberculosis-specific cell wall protein for immunodiagnosis of tuberculosis. J Infect Dis. 2009;200(4):571–81.

140. Fukuda T et al. Critical roles for lipomannan and lipoarabinomannan in cell wall integrity of mycobacteria and pathogenesis of tuberculosis. MBio. 2013;4(1):e00472–12.

141. Minion J, Leung E, Talbot E, Dheda K, Pai M, Menzies D. Diagnosing tuberculosis with urine lipoarabinomannan: systematic review and meta-analysis. Eur Respir J. 2011;38(6):1398–1405.

142. Lawn SD, Kerkhoff AD, Vogt M, Wood R. Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study. Lancet Infect Dis. 2012;12(3):201–9.

143. Shah M et al. Comparative performance of urinary lipoarabinomannan assays and Xpert MTB/RIF in HIV-infected individuals with suspected tuberculosis in Uganda. AIDS. 2014.

144. Nicol MP et al. Urine lipoarabinomannan testing for diagnosis of pulmonary tuberculosis in children: a prospective study. Lancet Glob Health. 2014;2(5):e278–e284.

145. Pollock NR et al. Validation of Mycobacterium tuberculosis Rv1681 protein as a diagnostic marker of active pulmonary tuberculosis. J Clin Microbiol. 2013;51(5):1367–73.

146. Young BL et al. The identification of tuberculosis biomarkers in human urine samples. Eur Respir J. 2014;43(6):1719–29.

147. Gray JM, Cohn DL. Tuberculosis and HIV coinfection. Semin Respir Crit Care Med. 2013;34(1):32–43.

148. Kingkaew N et al. HIV-associated extrapulmonary tuberculosis in Thailand: epidemiology and risk factors for death. Int J Infect Dis. 2009;13(6):722–9.

149. Dheda K et al. Clinical utility of a commercial LAM-ELISA assay for TB diagnosis in HIV-infected patients using urine and sputum samples. PLoS One. 2010;5(3):e9848.

150. Kroidl I et al. Reasons for false-positive lipoarabinomannan ELISA results in a Tanzanian population. Scand J Infect Dis. 2014;46(2):144–8.

151. Kong Y, Cirillo JD. Reporter enzyme fluorescence (REF) imaging and quantification of tuberculosis in live animals. Virulence. 2010;1(6):558–62.

152. Wang F, Cassidy C, Sacchettini JC. Crystal structure and activity studies of the Mycobacterium tuberculosis beta-lactamase reveal its critical role in resistance to beta-lactam antibiotics. Antimicrob Agents Chemother. 2006;50(8):2762–71.

153. Xie H et al. Rapid point-of-care detection of the tuberculosis pathogen using a BlaC-specific fluorogenic probe. Nat Chem. 2012;4(10):802–9.

154. Cheng Y et al. Fluorogenic probes with substitutions at the 2 and 7 positions of cephalosporin are highly BlaC-specific for rapid Mycobacterium tuberculosis detection. Angew Chem Int Ed Engl. 2014.

155. Christopherson RI et al. Classification of AML using a monoclonal antibody microarray. Methods Mol Med. 2006;125:241–51.

156. Barber N, Gez S, Belov L, Mulligan SP, Woolfson A, Christopherson RI. Profiling CD antigens on leukaemias with an antibody microarray. FEBS Lett. 2009;583(11):1785–91.

157. Woolfson A, Ellmark P, Chrisp JS, Scott A, Christopherson RI. The application of CD antigen proteomics to pharmacogenomics. Pharmacogenomics. 2006;7(5):759–71.

158. Jiang J et al. Reduced CD27 expression on antigen-specific CD4 + T cells correlates with persistent active tuberculosis. J Clin Immunol. 2010;30(4):566–73.

159. Streitz M et al. The phenotypic distribution and functional profile of tuberculin-specific CD4 T-cells characterizes different stages of TB infection. Cytometry B Clin Cytom. 2012;82(6):360–8.

160. Portevin D et al. Assessment of the novel T-cell activation marker-tuberculosis assay for diagnosis of active tuberculosis in children: a prospective proof-of-concept study. Lancet Infect Dis. 2014.

161. Phillips M, Basa-Dalay V, Bothamley G, et al. Breath biomarkers of active pulmonary tuberculosis. *Tuberculosis (Edinb).* 2010;90(2):145–151.

162. Syhre M, Manning L, Phuanukoonnon S, Harino P, Chambers ST. The scent of Mycobacterium tuberculosis: Part II breath. Tuberculosis (Edinb). 2009;89(4):263–6.

163. Mahapatra S et al. A metabolic biosignature of early response to anti-tuberculosis treatment. BMC Infect Dis. 2014;14:53.

164. Mgode GF et al. Mycobacterium tuberculosis volatiles for diagnosis of tuberculosis by Cricetomys rats. Tuberculosis (Edinb). 2012;92(6):535–42.

165. Mgode GF et al. Ability of Cricetomys rats to detect Mycobacterium tuberculosis and discriminate it from other microorganisms. Tuberculosis (Edinb). 2012;92(2):182–6.

166. Phillips M et al. Point-of-care breath test for biomarkers of active pulmonary tuberculosis. Tuberculosis (Edinb). 2012;92(4):314–20.

167. Bruins M, Rahim Z, Bos A, van de Sande WW, Endtz HP, van Belkum A. Diagnosis of active tuberculosis by e-nose analysis of exhaled air. Tuberculosis (Edinb). 2013;93(2):232–38.

168. McNerney R, Wondafrash BA, Amena K, Tesfaye A, McCash EM, Murray NJ. Field test of a novel detection device for Mycobacterium tuberculosis antigen in cough. BMC Infect Dis. 2010;10:161.

169. Meldau R et al. Comparison of same day diagnostic tools including Gene Xpert and unstimulated IFN-gamma for the evaluation of pleural tuberculosis: a prospective cohort study. BMC Pulm Med. 2014;14:58.

170. Pandie S et al. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon-gamma in a high burden setting: a prospective study. BMC Med. 2014;12:101.

171. Niemz A, Ferguson TM, Boyle DS. Point-of-care nucleic acid testing for infectious diseases. Trends Biotechnol. 2011;29(5):240–50.

172. Flores LL, Pai M, Colford JM, Jr., Riley LW. In-house nucleic acid amplification tests for the detection of Mycobacterium tuberculosis in sputum specimens: meta-analysis and meta-regression. BMC Microbiol. 2005;5:55.

173. UNITAID. Tuberculosis diagnostics technology and market landscape, 1st edition. Geneva: World Health Organization; 2012.

174. UNITAID. Tuberculosis diagnostics technology and market landscape, 2nd edition. Geneva: World Health Organization; 2013.

175. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Geneva: World Health Organization; 2008.

176. Barnard M, Gey van Pittius NC, van Helden PD, Bosman M, Coetzee G, Warren RM. The diagnostic performance of the GenoType MTBDRplus version 2 line probe assay is equivalent to that of the Xpert MTB/RIF assay. J Clin Microbiol. 2012;50(11):3712–16.

177. Kaswa MK et al. Pseudo-outbreak of pre-extensively drug-resistant (pre-XDR) tuberculosis in Kinshasa: collateral damage caused by false detection of fluoroquinolone resistance by GenoType MTBDRsl. J Clin Microbiol. 2014;52(8):2876–80.

178. Gamboa F et al. Comparative evaluation of initial and new versions of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for direct detection of Mycobacterium tuberculosis in respiratory and nonrespiratory specimens. J Clin Microbiol. 1998;36(3):684–9.

179. Little MC et al. Strand displacement amplification and homogeneous real-time detection incorporated in a second-generation DNA probe system, BDProbeTecET. Clin Chem. 1999;45(6 Part 1):777–84.

180. Mazzarelli G, Rindi L, Piccoli P, Scarparo C, Garzelli C, Tortoli E. Evaluation of the BDProbeTec ET system for direct detection of Mycobacterium tuberculosis in pulmonary and extrapulmonary samples: a multicenter study. J Clin Microbiol. 2003;41(4):1779–82.

181. Bergmann JS, Keating WE, Woods GL. Clinical evaluation of the BDProbeTec ET system for rapid detection of Mycobacterium tuberculosis. J Clin Microbiol. 2000;38(2):863–65.

182. Ling DI, Flores LL, Riley LW, Pai M. Commercial nucleic-acid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens: meta-analysis and meta-regression. PLoS One. 2008;3(2):e1536.

183. Bloemberg GV, Voit A, Ritter C, Deggim V, Bottger EC. Evaluation of Cobas TaqMan MTB for direct detection of the Mycobacterium tuberculosis complex in comparison with Cobas Amplicor MTB. J Clin Microbiol. 2013;51(7):2112–17.

184. Lee MR et al. Evaluation of the Cobas TaqMan MTB real-time PCR assay for direct detection of Mycobacterium tuberculosis in respiratory specimens. J Med Microbiol. 2013;62(Partt 8):1160–4.

185. Antonenka U et al. Comparison of Xpert MTB/RIF with ProbeTec ET DTB and COBAS TaqMan MTB for direct detection of M. tuberculosis complex in respiratory specimens. BMC Infect Dis. 2013;13:280.

186. Park KS et al. Comparison of the Xpert MTB/RIF and Cobas TaqMan MTB assays for detection of Mycobacterium tuberculosis in respiratory specimens. J Clin Microbiol. 2013;51(10):3225–7.

187. Lee SH et al. Rapid detection of Mycobacterium tuberculosis using a novel Ultra-Fast Chip-Type Real-Time PCR System. Chest. 2014.

188. Drouillon V et al. Multicenter evaluation of a transcription-reverse transcription concerted assay for rapid detection of Mycobacterium tuberculosis complex in clinical specimens. J Clin Microbiol. 2009;47(11):3461–5.

189. Takakura S et al. Rapid detection of Mycobacterium tuberculosis in respiratory samples by transcription-reverse transcription concerted reaction with an automated system. J Clin Microbiol. 2005;43(11):5435–9.

190. Tanaka H, Hirose H, Kato Y, Kida S, Miyajima E. Clinical evaluation of TRCRapid M.TB for detection of Mycobacterium tuberculosis complex in respiratory and nonrespiratory specimens. J Clin Microbiol. 2010;48(5):1536–41.

191. Perry MD, Lewis White P, Ruddy M. The Seegene Anyplex™ MTB/NTM real-time detection assay: potential for use in a regional reference laboratory. J Clin Microbiol. Ahead of Print. 4 June 2014.

192. Hu S et al. Rapid detection of isoniazid resistance in Mycobacterium tuberculosis isolates by use of real-time-PCR-based melting curve Mycobacterium tuberculosis isolates by analysis. J Clin Microbiol. 52(5):1644–2014.

193. Vairavan R. AutoGenomics, Inc. Pharmacogenomics. 2004;5(5):585–8.

194. Liu J et al. Performance assessment of the CapitalBio Mycobacterium identification array system for identification of mycobacteria. J Clin Microbiol. 2012;50(1):76–80.

195. Guo Y et al. Rapid, accurate determination of multidrug resistance in M. tuberculosis isolates and sputum using a biochip system. Int J Tuberc Lung Dis. 2009;13(7):914–20.

196. Linger Y et al. Demonstrating a multi-drug resistant Mycobacterium tuberculosis amplification microarray. J Vis Exp. 2014(86).

197. Linger Y et al. Simplified microarray system for simultaneously detecting rifampin, isoniazid, ethambutol, and streptomycin resistance markers in Mycobacterium tuberculosis. J Clin Microbiol. 2014;52(6):2100–7.

198. Wang C et al. High throughput sequencing reveals a complex pattern of dynamic interrelationships among human T cell subsets. Proc Natl Acad Sci USA. 2010;107(4):1518–23.

199. Boehme CC et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet. 2011;377(9776):1495–1505.

200. Chakravorty S et al. Rapid, high-throughput detection of rifampin resistance and heteroresistance in Mycobacterium tuberculosis by use of sloppy molecular beacon melting temperature coding. J Clin Microbiol. 2012;50(7):2194–2202.

201. Chakravorty S et al. Rapid detection of fluoroquinolone-resistant and heteroresistant Mycobacterium tuberculosis by use of sloppy molecular beacons and dual melting-temperature codes in a real-time PCR assay. J Clin Microbiol. 2011;49(3):932–40.

202. 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. Policy update. Geneva: World Health Organization; 2013.

203. Xpert MTB/RIF implementation manual: technical and operational "how-to": practical considerations. Geneva: World Health Organization; 2014 (http://apps.who.int/iris/bitstream/10665/112469/1/9789241506700_eng.pdf, accessed: 1 June 2014).

204. Lee MA, Siddle AL, Page RH. ResonSense®: simple linear fluorescent probes for quantitative homogeneous rapid polymerase chain reaction. Analytica Chimica Acta. 2002;457:61–70.

205. UNITAID. Tuberculosis diagnostics technology and market landscape: semi-annual update. Geneva: World Health Organization; 2012.

206. Yoshida H et al. Comparative study of the efficacy of the COBAS TaqMan and LAMP assay for the rapid diagnosis of tuberculosis. Kekkaku. 2013;88(11):727–33.

207. Xu G et al. Cross priming amplification: mechanism and optimization for isothermal DNA amplification. Sci Rep. 2012;2:246.

208. Jani IV et al. Accurate early infant HIV diagnosis in primary health clinics using a point-of-care nucleic acid test. J Acquir Immune Defic Syndr. 2014.

209. Ullrich T, Ermantraut E, Schulz T, Steinmetzer K. Competitive reporter monitored amplification (CMA): quantification of molecular targets by real time monitoring of competitive reporter hybridization. PLoS One. 2012;7(4):e35438.

210. Sanchez JA, Pierce KE, Rice JE, Wangh LJ. Linear-after-the-exponential (LATE)-PCR: an advanced method of asymmetric PCR and its uses in quantitative real-time analysis. Proc Natl Acad Sci USA. 2004;101(7):1933–8.

211. Carver-Brown RK, Reis AH Jr, Rice LM, Czajka JW, Wangh LJ. Design and construction of a single-tube, LATE-PCR, Multiplex Endpoint Assay with Lights-On/Lights-Off Probes for the detection of pathogens associated with sepsis. J Pathog. 2012;2012: Article ID 424808.

212. Rice JE, Reis AH Jr, Rice LM, Carver-Brown RK, Wangh LJ. Fluorescent signatures for variable DNA sequences. Nucleic Acids Res. 2012;40(21):e164.

213. Vandeventer PE et al. Mechanical disruption of lysis-resistant bacterial cells by use of a miniature, low-power, disposable device. J Clin Microbiol. 2011;49(7):2533–9.

214. Roskos K et al. Simple system for isothermal DNA amplification coupled to lateral flow detection. PLoS One. 2013;8(7):e69355.

215. Jenison R, Jaeckel H, Klonoski J, Latorra D, Wiens J. Rapid amplification/detection of nucleic acid targets utilizing a HDA/thin film biosensor. Analyst. 2014.

216. Ao W et al. Rapid detection of rpoB gene mutations conferring rifampin resistance in Mycobacterium tuberculosis. J Clin Microbiol. 2012;50(7):2433–40.

217. Ao W, Jenison R. Detection of rpoB gene mutations using helicase-dependent amplification. Methods Mol Biol. 2013;1039:89–98.

218. Boyle DS et al. Rapid Detection of Mycobacterium tuberculosis by Recombinase Polymerase Amplification. *PLoS One.* 2014;9(8):e103091.

219. Boyle DS et al. Rapid detection of HIV-1 proviral DNA for early infant diagnosis using recombinase polymerase amplification. MBio. 2013;4(2).

220. Niemann S et al. Genomic diversity among drug sensitive and multidrug resistant isolates of Mycobacterium tuberculosis with identical DNA fingerprints. PLoS One. 2009;4(10):e7407.

221. Roetzer A et al. Whole genome sequencing versus traditional genotyping for investigation of a Mycobacterium tuberculosis outbreak: a longitudinal molecular epidemiological study. PLoS Med. 2013;10(2):e1001387.

222. Daum LT et al. Next-generation ion torrent sequencing of drug resistance mutations in Mycobacterium tuberculosis strains. J Clin Microbiol. 2012;50(12):3831–7.

223. Telenti A et al. Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. Lancet. 1993;341(8846):647–50.

224. Merker M et al. Whole genome sequencing reveals complex evolution patterns of multidrug-resistant Mycobacterium tuberculosis Beijing strains in patients. PLoS One. 2013;8(12):e82551.

225. Farhat MR et al. Genomic analysis identifies targets of convergent positive selection in drug-resistant Mycobacterium tuberculosis. Nat Genet. 2013;45(10):1183–9.

226. Zhang H et al. Genome sequencing of 161 Mycobacterium tuberculosis isolates from China identifies genes and intergenic regions associated with drug resistance. Nat Genet. 2013;45(10):1255–60.

227. Bryant JM et al. Whole-genome sequencing to establish relapse or re-infection with Mycobacterium tuberculosis: a retrospective observational study. Lancet Respir Med. 2013;1(10):786–92.

228. Liu L et al. Comparison of next-generation sequencing systems. J Biomed Biotechnol. 2012; 251–64.

229. Stein LD. The case for cloud computing in genome informatics. Genome Biol. 2010;11(5):207.

230. Chang YJ, Chen CC, Chen CL, Ho JM. A de novo next generation genomic sequence assembler based on string graph and MapReduce cloud computing framework. BMC Genomics. 2012;13(Suppl. 7):S28.

231. Edwards DJ, Holt KE. Beginner's guide to comparative bacterial genome analysis using next-generation sequence data. Microb Inform Exp. 2013;3(1):2.

232. STOP TB Partnership New Diagnostics Working Group. NDWG workshop on sequencing data sharing, London, 3 February 2014.

233. Wells WA et al. Alignment of new tuberculosis drug regimens and drug susceptibility testing: a framework for action. Lancet Infect Dis. 2013;13(5):449–58.

234. Cepheid reports China installed base close to 1,000 GeneXpert® systems, 9 August. Sunnyvale, California: Cepheid Inc.; 2014.

235. Raizada N et al. Feasibility of decentralised deployment of Xpert MTB/RIF test at lower level of health system in India. PLoS One. 2014;9(2):e89301.

236. Creswell J et al. Results from early programmatic implementation of Xpert MTB/RIF testing in nine countries. BMC Infect Dis. 2014;14:2.

237. Colla M. Module issue investigation. Presented at the World Health Organization, Geneva, 5 January 2014.

238. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst. Rev. 2014;1:CD009593.

239. Theron G et al. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. Lancet. 2014;383(9915):424–35.

240. Effect of Xpert MTB/RIF on early mortality of adults with suspected TB: a pragmatic randomized trial. CROI, 2014.

241. Mupfumi L, Makamure B, Sagonda T, Zinyowera S, Metcalfe JZ, Mutetwa R. Impact of Xpert MTB/RIF on antiretroviral therapy-associated tuberculosis and mortality: a pragmatic randomized controlled trial. Open Forum Infect Dis. Oxford University Press; 2014; Advanced Access:1–20.

242. Theron G, Peter J, Dowdy D, Langley I, Squire SB, Dheda K. Do high rates of empirical treatment undermine the potential effect of new diagnostic tests for tuberculosis in high-burden settings? Lancet Infect Dis. 2014;14(6):527–32.

243. Salje H et al. The importance of implementation strategy in scaling-up of Xpert MTB/RIF in the Indian healthcare system; a transmission mode. PLoS Medicine. 2014;11(7):e10011674.

244. Pai M, Yadav P, Anupindi R. Tuberculosis control needs a complete and patient-centric solution. Lancet. 2014;2:e189–e190.

245. Market assessment of tuberculosis diagnostics in Brazil in 2012. PLoS One. 2014;9(8):e104105.

246. Denkinger CM, Kik SV, Pai M. Robust, reliable and resilient: designing molecular tuberculosis tests for microscopy centers in developing countries. Expert Rev Mol Diagn. 2013;13(8):763–7.

Appendix: NAAT product attributes

Table A 1. NAATs described in this report, highlighting core test technology, detection method, ability to genotype drug resistance, intended laboratory setting and time to market with current regulatory status

Manufacturer	Test name	Amplification, detection technology	Integrated	Dedicated instrument	Multiplexed	Throughput	Genotyping for drug resistance	Lowest intended use setting	Market release	Regulatory status
Abbott	m2000 RealTime MTB assay	Real-time PCR	No	Yes	No	96 tests per day	No	Reference	2013	CE-IVD Q3 2014
AID	TB Resistance Module INH/RIF	PCR, LPA	No	No*	Yes	96 tests per day	RIF, INH	Intermediate	2013	CE-IVD
AID	TB Resistance Module FLQ	PCR, LPA	No	No*	Yes	96 tests per day	FLQ	Intermediate	2013	CE-IVD
AID	TB Resistance Module AMG	PCR, LPA	No	No*	Yes	96 tests per day	AMG	Intermediate	2013	CE-IVD
Akonni Biosystems	TruArray® MDR-TB	PCR, microarray	No	Yes	Yes	Unkn	RIF, INH	Reference	Unkn	None
Autogenomics	BioFilmChip® MDR TB	PCR, microarray	No	Yes	Yes	48 tests in 6.5 hours	RIF, INH	Reference	2011	None
Autogenomics	MTBC-OCTA	PCR, microarray	No	Yes	Yes	384 tests in 6.5 hours	No	Reference	2014	None
Alere	Alere™ q TB assay	NEAR, fluorescence	Yes	Yes	Unkn	Unkn	No#	Peripheral	Unkn	None
BD	BD ProbeTec™ ET Direct TB Assay	SDA, fluorescence	No	Yes	Yes	150 tests, 8 hours	No	Reference	1999	CE-IVD
CapitalBio Corp.	Drug Resistance Detection Kit	PCR, microarray	No	Yes	Yes	24–48 tests, 8 hours	MDR	Reference	2009	CE-IVD, CFDA
Cepheid	Xpert® MTB/RIF assay	Real-time PCR	Yes	Yes	Yes	2 hours per module	RIF	Intermediate	2009	WHO, CE-IVD, US FDA, CFDA, Health Canada
Eiken	Loopamp™ MTBC detection kit	LAMP, fluorescence	No	Yes	No	42 tests per day	No	Peripheral	2011	WHO‡, CE-IVD, Japan

Manufacturer	Test name	Amplification, detection technology	Integrated	Dedicated instrument	Multiplexed	Throughput	Genotyping for drug resistance	Lowest intended use setting	Market release	Regulatory approvals
Enigma	Enigma ML	Real-time PCR	Yes	Yes	Unkn	Unkn	Yes, Unkn	Intermediate	Unkn	N/A
Epistem	Genedrive® Mycobacterium iD® Test-kit	Real-time PCR	No	No*	No	1.5 hours per test device	RIF	Peripheral	2013	CE-IVD
Fuji-Rebio Europe	INNO-LiPA Mycobacteria v2	PCR, LPA	No	No*	Yes	96 tests per day	No	Intermediate	N/A	CE-IVD
Fuji-Rebio Europe	INNO-LiPA Rif.TB	PCR, LPA	No	No*	Yes	96 tests per day	RIF, INH	Intermediate	N/A	CE-IVD
Hain Lifescience	GenoType® MTBDRplus (v2.0)‡	PCR, LPA	No	No*	Yes	96 tests per day	MDR	Intermediate	2012	WHO‡, CE-IVD
Hain Lifescience	GenoType® MTBDRsl	PCR, LPA	No	No*	Yes	96 tests per day	FLQ, AMG, EMB	Intermediate	2009	WHO‡, CE-IVD
Hain Lifescience	GenoQuick® MTB	PCR, LPA	No	No*	Yes	96 tests per day	No	Intermediate	2010	CE-IVD
Hain Lifescience	GenoType® Mycobacterium CM	PCR, LPA	No	No*	Yes	96 tests per day	No	Intermediate	2004	CE-IVD
Hain Lifescience	GenoType® Mycobacterium AS	PCR, LPA	No	No*	Yes	96 tests per day	No	Intermediate	2004	CE-IVD
Hain Lifescience	FluoroType® MTB	Real-time PCR	No	Yes	No	10 tests in 3 hours per machine	No	Intermediate	2012	CE-IVD
Hain Lifescience	FluoroType® MTB RNA	Real-time RT PCR	No	Yes	No	11 tests in 3 hours per machine	No	Intermediate	2014	CE-IVD (Q4 2104)
Hologic Genprobe®	AMTD test	TMA, luminescence	No	Yes	No	50 tests, 5.5 hours	No	Reference	1993	US FDA, CE-IVD
iCubate	iC2.0-MYCO assay	PCR, microarray	Yes	Yes	Yes	Unkn	RIF, INH, EMB, FLQ, AMG, BED	Intermediate	2012	None
Insilixa Inc.	HYDRA	PCR, microarray	No	Yes	Yes	8 tests per device per day	RIF, INH, PZA, EMB, FLQ, AMG	Peripheral	2015	N/A
KGI	TBDx System	LAMP/CPA, lateral flow strip	Yes	Yes	No	8 tests per device per day	No	Peripheral	2018	N/A

Manufacturer	Test name	Amplification, detection technology	Integrated	Dedicated instrument	Multiplexed	Throughput	Genotyping for drug resistance	Lowest intended use setting	Market release	Regulatory approvals
LG Life sciences	AdvanSure Mycobacteria GenoBlot Assay	PCR, LPA	No	No*	Yes	96 tests per day	no	Intermediate	N/A	Unkn
LG Life sciences	AdvanSure MDR TB GenoBlot Assay	PCR, LPA	No	No*	Yes	96 tests per day	RIF/INH	Intermediate	N/A	Unkn
MolBio	Truelab™ TB Assay	Real-time PCR	No	Yes	No	12 tests per test device in 8 hours	No	Peripheral	2013	CE-IVD, Indian DCGI
NIPRO Co.	NTM/MDR TB	PCR, LPA	No	No*	Yes	96 tests per day	RIF/INH	Intermediate	2012	WHO‡, Unkn
NIPRO Co.	INH	PCR, LPA	No	No*	Yes	96 tests per day	INH	Intermediate	2012	Unkn
NIPRO Co.	PZA	PCR, LPA	No	No*	Yes	96 tests per day	PZA	Intermediate	N/A	Unkn
NIPRO Co.	FLQ	PCR, LPA	No	No*	Yes	96 tests per day	FLQ	Intermediate	N/A	Unkn
NWGHF	Savanna	Real-time PCR	Yes	Yes	Unkn	13 tests, 8 hours	No#	Peripheral	2016	Unkn
Roche	Cobas® TaqMan® MTB Test	Real-time PCR	No	Yes	Yes	44 tests, 3 hours	No	Reference	2009	CE-IVD, Health Canada
Seegene	Anyplex II™ MTB/ MDR/XDR	Real-time PCR	No	No*	Yes	96 tests, 6 hours	RIF, INH, FLQ, AMG	Reference	2012	CE-IVD, Korean FDA
Seegene	Anyplex™ MTB/ NTM	Real-time PCR	No	No*	Yes	96 tests, 6 hours	No	Reference	2012	CE-IVD, Korean FDA
Seegene	Anyplex™ plus MTB/NTM/MDR TB	Real-time PCR	No	No*	Yes	96 tests, 6 hours	RIF, INH	Reference	2012	CE-IVD, Korean FDA
Seegene	Magicplex™ MTB	Real-time PCR	No	No*	No	96 tests, 6 hours	No	Reference	2012	CE-IVD, Korean FDA
Tosoh Bioscience	TRC Rapid® M.TB	TRC	No	Yes	No	N/A	No	Reference	Unkn	N/A
Ustar	EasyNAT™ TB	CPA, lateral flow strip	No	No	No	30 tests per day	No	Peripheral	2013	CE-IVD, CFDA

Manufacturer	Test name	Amplification, detection technology	Integrated	Dedicated instrument	Multiplexed	Throughput	Genotyping for drug resistance	Lowest intended use setting	Market release	Regulatory approvals
Veredus Laboratories Pte Ltd	VereMTBTM Detection Kit	PCR, microarray	No	Yes	Yes	16 tests in 8 hours	RIF, INH	Intermediate	2012	Health Sciences Authority (Singapore),, CE IVD, Russian Federation Q3 2014, CFDA Q4 2015
Vircell	SPEED-OLIGO® DIRECT MTB	PCR, LPA	No	No	No	96 tests per day	No	Intermediate	Unkn	Unkn
Vircell	SPEED-OLIGO® DIRECT Mycobacteria	PCR, LPA	No	No	No	96 tests per day	No	Intermediate	Unkn	Unkn
Wave 80	EOSCAPE-TB System	NASBA, Fluorescence	No	Yes	No	50 tests per day	No#	Peripheral	2016	N/A
YD Diagnostics	MolecuTech REBA Myco-ID	PCR, LPA	No	No*	Yes	96 tests per day	No	Intermediate	Unkn	Unkn
YD Diagnostics	MolecuTech REBA MTB-MDR	PCR, LPA	No	No*	Yes	96 tests per day	RIF/INH	Intermediate	Unkn	WHO‡, Unkn
YD Diagnostics	MolecuTech REBA MTB FQ	PCR, LPA	No	No*	Yes	96 tests per day	FLQ	Intermediate	Unkn	Unkn
YD Diagnostics	MolecuTech REBA MTB KM	PCR, LPA	No	No*	Yes	96 tests per day	AMG	Intermediate	Unkn	Unkn
YD Diagnostics	MolecuTech REBA MTB XDR	PCR, LPA	No	No*	Yes	96 tests per day	FLQ/AMG	Intermediate	Unkn	Unkn
Zeesan Biotech	MeltPro® RIF	Real-time PCR	No	No*	Yes	48 samples per day	RIF	Reference	Q4 2014	CFDA
Zeesan Biotech	MeltPro® INH	Real-time PCR	No	No*	Yes	48 samples per day	INH	Reference	Q4 2014	CFDA
Zeesan Biotech	MeltPro® FLQ	Real-time PCR	No	No*	Yes	48 samples per day	FLQ	Reference	Q4 2014	CFDA
Zeesan Biotech	MeltPro® AMG	Real-time PCR	No	No*	Yes	48 samples per day	AMG	Reference	Q4 2014	CFDA

Unkn, unknown; N/A, not available; DCGI, Drug Controller General of *India*. For other abbreviations, please see the list at the start of this report.

* While dedicated equipment is not specifically required, many of these products can use generic equipment (e.g. most real time PCR machines) or have dedicated hybridization and strip reading platforms in the case of LPAs for higher-throughput testing.

The genotyping of drug resistance alleles is possible via a second reflex assay.

‡ Demonstration studies of these technologies are currently under way for WHO Expert Group review for WHO endorsement.