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TUBERCULOSIS

**Diagnostics Technology
and Market Landscape**

4TH EDITION

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Abbreviations

AIDS	acquired immune deficiency syndrome	HBC	high-burden country
AMG	aminoglycoside	HIV	human immunodeficiency virus
BCG	bacillus Calmette-Guérin	IFN-γ	interferon-gamma
°C	degrees Celsius	IGRA	interferon-gamma release assay
CAD	computer-aided diagnosis	INH	isoniazid
CE-IVD	European conformity (Conformité Européenne)-in vitro diagnostic	IPAQT	Initiative for Promoting Affordable, Quality Tests
CFDA	China Food and Drug Administration	IPT	isoniazid preventive therapy
cfu	colony forming unit	IVD	in vitro diagnostics
CHAI	Clinton Health Access Initiative	JMHLW	Japanese Ministry of Health, Labour and Welfare
CMOS	complementary metal-oxide semiconductor	KGI	Keck Graduate Institute
CPA	cross-priming amplification	L	litre
CPTR	Critical Path to Tuberculosis Drug Regimens	LAM	lipoarabinomannan
CXR	chest X-ray	LAMP	loop-mediated amplification
DCS	dried culture spot	LATE	linear-after-the-exponential
DNA	deoxyribonucleic acid	LCD	liquid-crystal display
DPO™	Dual Priming Oligonucleotides	LED	light emitting diode
DST	drug susceptibility testing	L-J	Löwenstein-Jensen
DCXR	digital chest X-rays	LOD	limit of detection
ELISA	enzyme linked immunosorbent assay	LPA	line probe assay
EMB	ethambutol	LTBI	latent TB infection
EPTB	extrapulmonary TB	MDR	multidrug resistant
EQA	external quality assurance	MIRU	mycobacterial interspersed repeating units
FIND	Foundation for Innovative New Diagnostics	μL	microlitre
FLQ	fluoroquinolone	mL	millilitre
FM	fluorescence microscopy	ML	mini laboratory
Global Fund	Global Fund to Fight AIDS, Tuberculosis and Malaria	MTB	Mycobacterium tuberculosis
GLI	Global Laboratory Initiative	MTBC	Mycobacterium tuberculosis complex
GPS	global positioning system	NAAT	nucleic acid amplification test
GPRS	general packet radio service	NALC	N-acetyl cysteine
GSM	Global System for Mobile Communication	NaOH	sodium hydroxide
		NDWG	New Diagnostics Working Group
		NEAR	nicking enzyme amplification reaction
		NGS	next-generation sequencing

NHLS	National Health Laboratory Services	STR	Streptomycin
NIH	National Institutes of Health (USA)	T-cell	T-lymphocyte
NTM	non-tuberculous mycobacteria	TAM-TB	T-cell activation marker–tuberculosis
NTP	national tuberculosis programme	TB	Tuberculosis
PACS	Picture Archive and Communication System	TOP	totally optimized PCR
PaMZ	PA-824/moxifloxacin/pyrazinamide	TPP	target product profile
PCR	polymerase chain reaction	TU	TB programme unit
PLHIV	people living with HIV/AIDS	UK	United Kingdom
POC	point of care	US	United States
PON	point of need	US CDC	United States Centers for Disease Control and Prevention
PTB	pulmonary TB	US FDA	United States Food and Drug Administration
PZA	Pyrazinamide	USA	United States of America
QRDR	quinolone resistance determining region	USAID	United States Agency for International Development
Q1, 2, 3, 4	Quarter 1, 2, 3, 4	USB	universal serial bus
RIF	rifampicin or rifampin	VOC	volatile organic compound
RNA	ribonucleic acid	VNTR	variable-number tandem repeats
rRNA	ribosomal RNA	WGS	whole genome sequencing
S4S	Support for Success	WHO	World Health Organization
SM	single molecule	XCR	extreme chain reaction
SSM	sputum smear microscopy	XDR	extensively drug resistant
STAG-TB	Strategic and Technical Advisory Group for Tuberculosis		

Foreword

Tuberculosis (TB), despite being a curable disease, continues to be a major public health threat: the World Health Organization (WHO) estimated that 1.5 million people died of the disease in 2013. Rapid, accurate diagnosis is critical for timely initiation of treatment and, ultimately, control of the disease. But of the nine million people who developed TB in 2013, over one third were not diagnosed or notified. 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 many countries still rely on basic tools such as smear microscopy, 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 unmet needs identified and articulated in target product profiles (TPPs), and a technology pipeline promising new products to address these needs.

This updated report reviews current and potential future technologies, as well as critical market issues, to identify market-based approaches to improve access to better TB diagnostics. For example, potential opportunities may include efforts to accelerate market entry or scale-up of 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 2013 alone, 9.0 million people fell ill with tuberculosis (TB) and 1.5 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 2013, only 6.1 million TB cases were notified to national TB programmes (NTPs). The remaining 3 million cases were either not diagnosed, or not notified to NTPs. In 2013, 58% of the 4.9 million pulmonary TB (PTB) patients notified globally were bacteriologically confirmed via a WHO-recommended test, including rapid tests such as Cepheid Inc.'s GeneXpert® MTB/RIF (Xpert® MTB/RIF). The rest were likely managed on the basis of clinical suspicion or non-specific tests (e.g. chest x-rays [CXR]).

Access to diagnosis is particularly challenging in people with multidrug-resistant (MDR) TB and in children with TB. Globally, in 2013, WHO estimated that 480 000 people developed MDR TB. It is estimated that 136 000 of the estimated 300 000 MDR TB patients who could have been detected were diagnosed and notified. This was equivalent to almost one in two (as compared to one in six in 2009). Some of this progress in the detection of drug-resistant TB has been attributed to the use of rapid molecular diagnostics such as line probe assays (LPAs) and Xpert® MTB/RIF. Recognizing the critical importance of drug susceptibility testing (DST), the *End TB Strategy*, published in 2015, includes universal DST as a key component of its first pillar: integrated, patient-centred TB care and prevention.

In 2013, an estimated 550 000 children became ill with TB, but the true case burden of childhood 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

Although TB diagnosis in many countries is still reliant on older tools, new diagnostics are changing the landscape. Stimulated, in part, by the success and rollout of Xpert® MTB/RIF, there is now considerable interest in new technologies. The landscape looks promising with a robust pipeline of new tools, particularly molecular diagnostics or nucleic acid amplification test (NAAT) technologies, and well over 50 companies actively engaged in product development. However, new diagnostics are yet to reach scale, and there needs to be greater convergence between diagnostics development and development of shorter TB drug regimens. While the pipeline is robust for NAATs, the pipeline is less robust for other products, especially biomarker-based tests for cure, triage and latent TB progression. Another concern is the relative absence of non-sputum-based diagnostics in the pipeline for children.

Although a range of validation studies has been undertaken for emerging molecular tools, the primary barrier to the introduction of new molecular tools continues to be the lack of evidence provided by unbiased field evaluations. Many of the developers are small companies that do not have the resources to promote or support the use of their technology beyond their intended market entry point (e.g. China

or India). In addition, it is assumed that developers also face challenges in scaling the manufacturing of their products as they move from product development to production and marketing. As an example, of recent new tools, only the Eiken Chemical Co. Ltd Loopamp™ Mycobacterium tuberculosis complex (MTBC) assay has been evaluated with sufficient rigour. Data from 14 independent field evaluations of this technology have been compiled by the Foundation for Innovative New diagnostics (FIND) and were presented to the WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) in June 2015 for expert review. In the past year, there has been very limited evidence presented on other NAAT products now being marketed as potential replacements for sputum smear microscopy (SSM). Of the other NAATs in development, several are in late-stage development with prototypes estimated to be ready in the next one or two years. Large diagnostic companies have entered the NAAT space by offering polymerase chain reaction (PCR) assays for Mycobacterium tuberculosis (MTB) that can be applied to existing technology platforms for other diseases (e.g. HIV viral load). Abbott Molecular; Becton, Dickinson and Company; and Roche Diagnostics now offer or are developing assays for their existing molecular platforms. These products are focused on high-throughput testing at the reference laboratory level and are not intended for use at peripheral laboratories.

While many manufacturers remain interested in developing biomarker-based assays for point-of-care (POC) testing or triaging (screening) patients for TB infection, they face significant challenges with identifying optimal biomarkers and specimen types and establishing cut-off values. A rapid diagnostic test for lipoarabinomannan (LAM) in urine, the Determine™ assay (Alere Inc., USA), underwent STAG-TB review in June 2015, but its use is limited to people living with HIV (PLHIV) who have a low CD4 count (i.e. < 50 CD4 cells per µL of whole blood). The detection of volatile organic compounds is an area of increasing interest with several developers claiming to have products at late-stage development, but again, independent evidence on performance is still lacking.

Several initiatives, described in this landscape report, have been launched to further stimulate product development and policy, including assessment of needs and priorities, development of target product profiles (TPPs), compilation of data on resistance-associated mutations, and assessment of market size and potential for new diagnostics. Advocacy is needed to increase funding for TB research and development, and governments in high-burden countries (HBCs) must invest more in TB control, to meet the *End TB Strategy* targets.

Market landscape

Globally, the scale-up of Xpert® MTB/RIF continues to be the most important, measurable shift in the TB diagnostics market, with over 10 million Xpert® MTB/RIF cartridges procured in the public sector in 116 of the 145 countries eligible for concessional pricing. However, data suggest that most NTPs use Xpert® MTB/RIF 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 SSM as the primary, and often, sole diagnostic test. A recent study 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.

While a large number of studies have confirmed the high accuracy of Xpert® MTB/RIF, new studies are starting to address the issue of how the test impacts patient outcomes, with mixed results. And although 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. 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 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. TPPs are now published, and efforts have also been made to estimate the current, served available market for TB tests in priority countries, and to quantify the potential market value for each priority TPP.

Market shortcomings related to TB diagnostics

Market shortcomings related to TB diagnostics include issues of availability, quality, affordability, demand and adoption, delivery and innovation. Current diagnostics are not adapted for specific patient groups or decentralized health-care settings, and emerging innovations still do not meet all needs. 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, and integration in country programmes has been challenging. 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. 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.

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 countries, 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. Previous editions of this landscape report are available at <http://www.unitaid.eu/en/resources/publications/technical-reports>.

This landscape report, the fourth edition, 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 (section 3);
- presents a comprehensive overview of TB diagnostic technologies that are commercially available or close to market (section 4);
- analyses the market landscape, including high-priority target product profiles (TPPs) for new TB tests, data on market size and potential, and efforts to improve access to World Health Organization (WHO)-endorsed TB tests in the private sector) (section 5);
- describes major market shortcomings related to TB diagnostics (section 5.4) and presents opportunities for market-based intervention to address these shortcomings (section 5.5).

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 *UNITAID Tuberculosis Diagnostics Technology and Market Landscape, 4th edition (2015)* 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 landscape report 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 assess 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 report further increased the scope and number of developers approached for product/market information. With the dissemination of UNITAID landscape reports since 2012, diagnostics developers also now approach the authors with product information to be included in the reports.

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.

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- High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Geneva: World Health Organization; 2014. WHO/HTM/TB/2014.18 (http://www.who.int/tb/publications/tpp_report/en/).
- Denkinger CM, Schito M, Pai M, editors. Tuberculosis diagnostics in 2015: landscape, priorities, needs, and prospects. *J Inf Dis*. 2015 April;211(Suppl. 2) (http://jid.oxfordjournals.org/content/211/suppl_2.toc).

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. Industry and FIND contributions were technical in nature; the section on potential opportunities for market intervention was developed independently by authors David Boyle and Madhukar Pai with support from UNITAID.

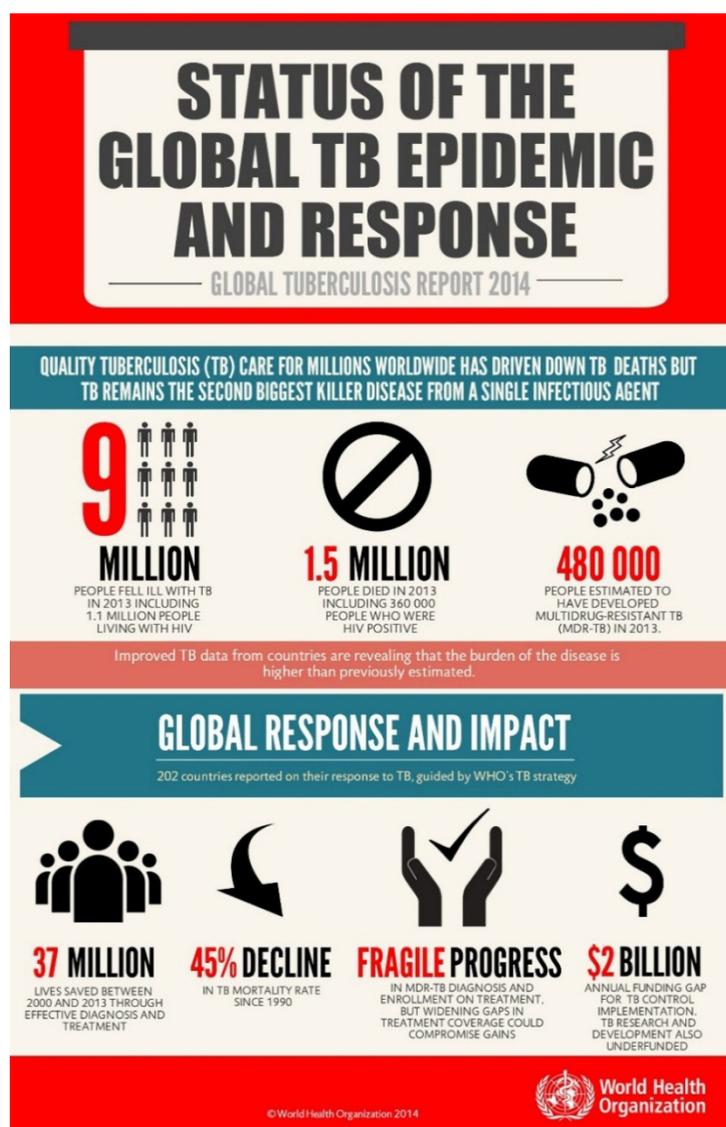
David Boyle is a consultant to the Keck Graduate Institute (KGI) TBDx System project (R01AI111477). In addition, he receives funding from two grants unrelated to TB in which Ustar Biotechnologies (China) and TwistDx Ltd (United Kingdom; a subsidiary of Alere Inc.) are collaborators. He has no other commercial/financial conflicts pertaining to information described in this landscape report.

Madhukar Pai has no commercial/financial conflicts. He has received grant funding for TB diagnostics market research from the Bill & Melinda Gates Foundation. 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 the Access Advisory Committee of the TB Alliance, and as a consultant for the Bill & Melinda Gates Foundation.

3. Public health problem and commodity access issues relating to diagnostics

WHO estimates that, in 2013 alone, 9.0 million people fell ill with TB and 1.5 million died from the disease (Figure 1). Rapid, accurate diagnosis is critical for timely initiation of treatment, but many people with TB do not have access to adequate initial diagnosis. In 2013, only 6.1 million TB cases were notified to national tuberculosis 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. In 2013, 58% of the 4.9 million pulmonary TB (PTB) patients notified globally were bacteriologically confirmed via a WHO-recommended test.

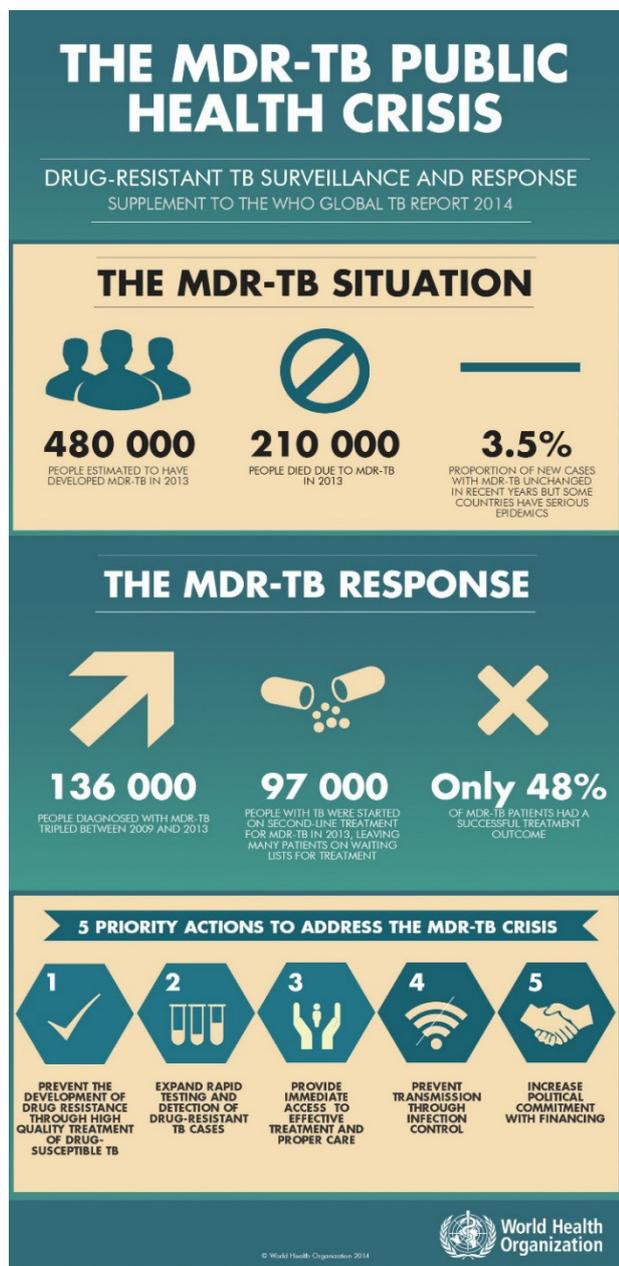
Figure 1. Infographic on the TB burden in 2013



Source: WHO, Geneva (http://www.who.int/tb/features_archive/TB_Situation.jpg?ua=1).

Access issues in people with multidrug-resistant (MDR) TB and in children are even more pronounced. Globally, in 2013, WHO estimated that 480 000 people developed MDR TB (Figure 2). It is estimated that 136 000 of the estimated 300 000 MDR TB patients who could have been detected were diagnosed and notified.¹ This was equivalent to almost one in two (as compared to one in six in 2009). Some of this progress in the detection of drug-resistant TB has been attributed to the use of rapid molecular diagnostics such as line probe assays (LPAs) and the GeneXpert® MTB/RIF (Xpert® MTB/RIF).

Figure 2. Burden of MDR TB in 2013



Source: WHO, Geneva (http://www.who.int/entity/tb/features_archive/MDR_TB_CRISIS.jpg?ua=1).

Also, in 2013, an estimated 550 000 children became ill with TB, but the true case burden of childhood TB is likely higher than estimated. A model-based estimate suggests that the number is closer to one million children in 2010.² Childhood TB is very difficult to diagnose, and most conventional TB tests perform poorly in this high-risk population.

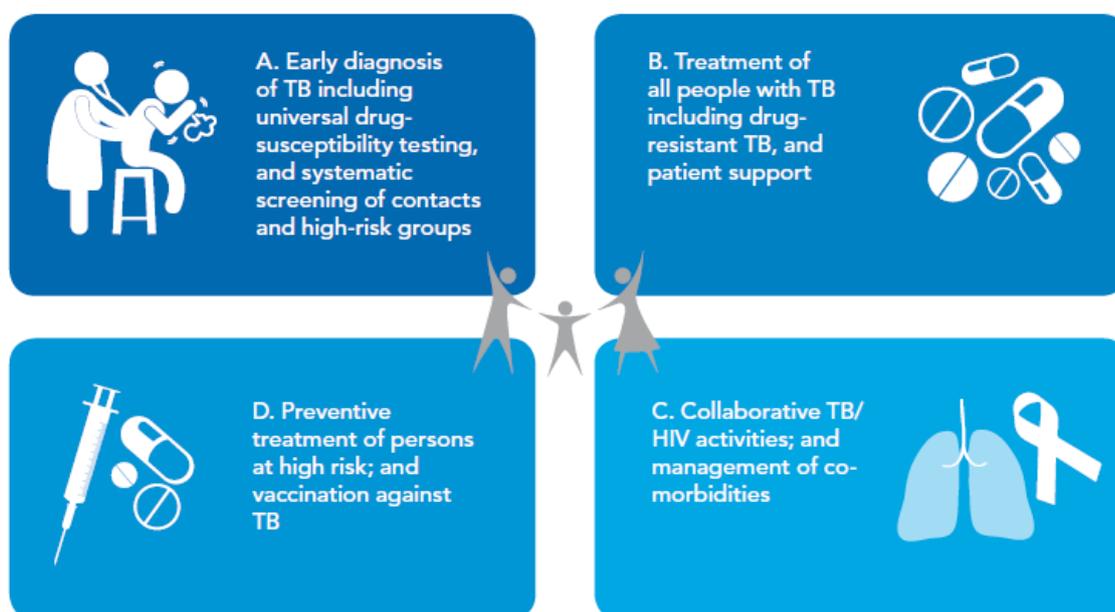
When appropriately diagnosed, TB is largely curable with currently available medicines. But initiation of appropriate TB drug regimens is impossible without timely access to the right diagnostic tools to diagnose both TB disease and drug resistance. A variety of barriers can prolong a patient’s pathway to TB diagnosis. 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.³

3. Public health problem and commodity access issues relating to diagnostics

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. Without drug susceptibility testing (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.

Recognizing the critical importance of early diagnosis and access to DST, the 2015 *End TB Strategy* includes early diagnosis of TB and universal access to DST as a key component of its first pillar: integrated, patient-centred TB care and prevention (Figure 3; see also http://www.who.int/tb/EndTBadvocacy_brochure/en/).

Figure 3. End TB Strategy: first pillar on integrated, patient-centred care and prevention



Source: WHO, Geneva.

Although rapid DST tests (e.g. 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 two 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 – for example, it cannot detect resistance against multiple 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 high-burden countries (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.⁴

Thus, a majority of the high-TB burden countries still relies 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. In 2014, WHO published its new guidelines on LTBI management (<http://www.who.int/tb/publications/latent-tuberculosis-infection/en/>). WHO recommends that only selected risk groups should be evaluated for LTBI. These include: people living with HIV (PLHIV); adult and child contacts of PTB cases; patients initiating anti-tumour necrosis factor-alpha (TNF- α) treatment; patients with end-stage renal failure on dialysis; patients preparing for organ or haematologic transplantation; and patients with silicosis. In 2013, WHO estimated that only 21 % of countries globally and 14 of the 41 high-TB/HIV burden countries reported provision of isoniazid preventive therapy (IPT) to PLHIV. 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.

4. Technology landscape

4.1 Overview of the technology landscape and approach

This landscape report is designed to serve as an update to the comprehensive 2014 edition, which detailed the primary methods for diagnosis of PTB and many of the technologies and products associated with these methods. This report has the latest updates from developers with a focus on newer products, regulatory news and reports on the unbiased validation of technologies that are now on the market or close to market release. In particular, this landscape report features key milestones in the development of nucleic acid amplification test (NAAT) technologies, of interest for their potential to replace SSM and/or offer faster, more effective diagnosis of PTB.

A series of key documents were released in 2014 to aid developers in understanding the size and value of the current market for smear microscopy and in identifying key attributes to address in product development. An expert consensus group created a series of evidence-based TPPs that specifically cover a variety of different diagnostic modalities.⁵⁻¹⁰ These market estimates and TPPs are also described in this landscape report.

Data for this landscape report were derived from contact with over 100 technology developers working within the TB diagnostics market, ranging from established multinational diagnostic companies to startups and academic groups. Further information on product development and test validations was acquired via press releases, online technology updates, the peer-reviewed literature, accessing clinical study websites and via FIND.

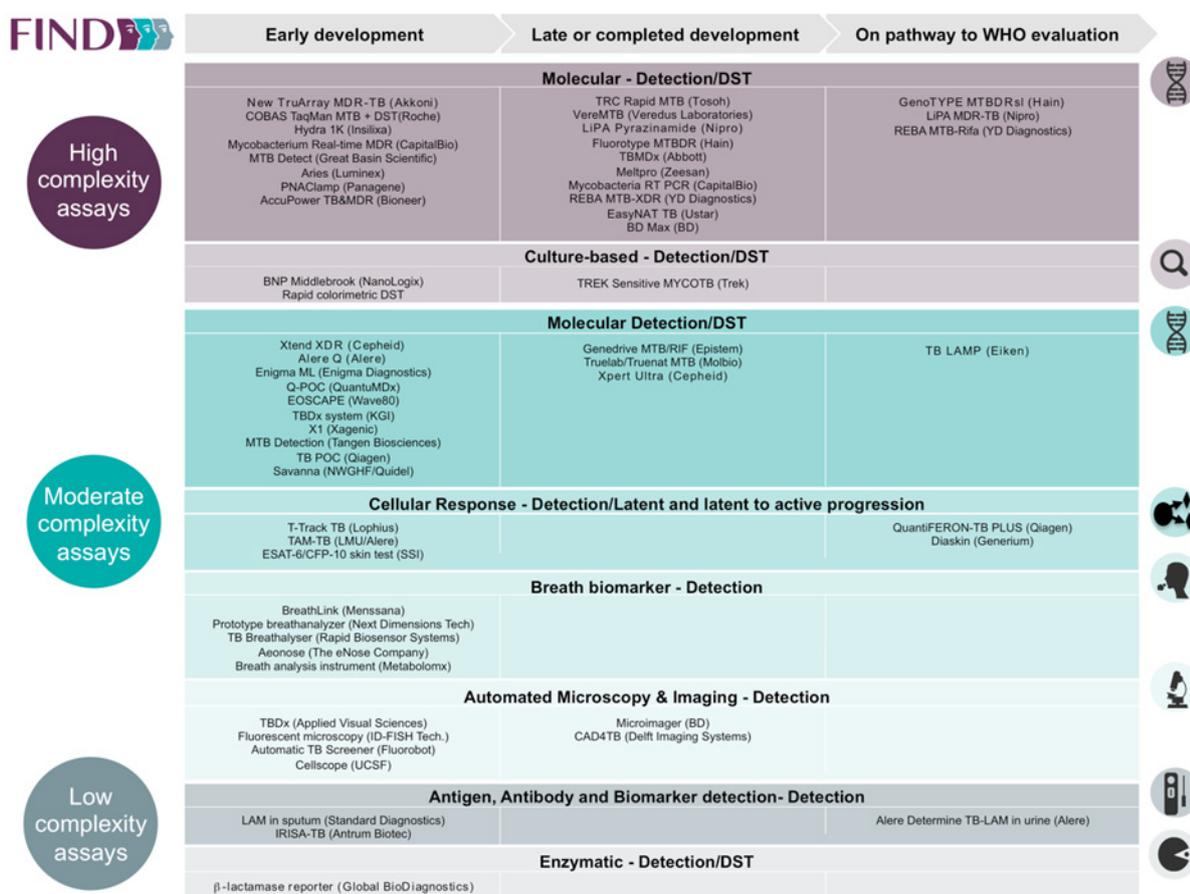
This landscape report highlights the development of extant NAATs assays in terms of product release and regulatory approvals. One point that has been stressed in every report to date is that the route to market of several NAATs is generally stalled due to a lack of independent validation studies to inform on the performance of the products in their intended use-case settings. It is now noted that several significant validation studies are under way or have already been completed. These will play an important role in helping the TB community assess if their performance can match expectations and how these new technologies may best be applied for TB control. The validations also start the evidence base for WHO endorsement and creating the potential for donor driven investments to expand the use of these tools into routine use at the country level. This will ultimately create a more competitive market for the Xpert® MTB/RIF technology, which remains the only NAAT endorsed by WHO since 2010.

The search for effective biomarkers in non-sputum-based tests to indicate active TB infection and the development of test technologies that have the ability to adopt these remain very active areas of upstream research interest and investment. While there are still few clear candidates at this point, the broad scope of biomarker types being assessed makes it likely that rapid triaging tests using biomarkers will become available in the longer term (5+ years) and that they will significantly improve initial screening of suspect cases prior to confirmatory diagnosis. The perceived outcomes will include more rapid diagnosis of active cases of TB and, therefore, reduce the spread of TB in the community, while also saving costs on unnecessary diagnostic testing.

The Cepheid Inc. (USA) Xpert® MTB/RIF still remains the only integrated NAAT for the diagnosis of MDR TB, but there have been several developers manufacturing LPAs that can discriminate drug resistance alleles to first-line and a variety of second-line drugs. Again, there is great interest and investment being made in this area, especially in developing the appropriate diagnostic tool to augment the implementation of new anti-TB drugs or regimens (e.g. bedaquiline; delamanid; PA-824/moxifloxacin/pyrazinamide [PaMZ]). In addition to the need to link diagnostic and drug resistance tests with new treatment, the current diagnosis of MDR TB is still inadequate. New or improved tools are still urgently needed to better diagnose MDR TB and improve treatment options and limit their spread within exposed communities and, in particular, to highly vulnerable individuals such as the immunocompromised where outcomes are often poor due to delay in appropriate diagnosis. Many of the NAATs in development are including genotypic DST to first- and second-line drugs.

The 2014 pipeline was intended to give a fully comprehensive analysis of the landscape of products in use or intended for the diagnosis of PTB or relating more generally to active or LTBI. The eight categories that comprised the product landscape included: chest X-rays (CXRs) and computer-aided diagnosis (CAD); sputum collection and sample processing tools; microscopic diagnosis of MTB; culture-based tools for the diagnosis of TB and DST; biomarkers to detect active TB or indicate LTBI; serodiagnostic assays for detection of MTB antigens or immune response to Mycobacterium tuberculosis (MTB); volatile organic compounds (VOCs); and NAATs and sequencing methods for determination of drug resistance. Figure 4 shows the updated overall diagnostics pipeline, including all types of technologies.

Figure 4. Current FIND TB diagnostics pipeline listing the development phases and the types of technologies in development or evaluation



Source: FIND, Geneva.

This landscape report does not expand upon smear microscopy and culture techniques, instead it focuses primarily on the pipeline of NAATs in development for replacement of smear microscopy – still the most active area of product development. With these new tools, rapid diagnosis of PTB could be significantly improved upon by supplanting or augmenting SSM. This landscape report highlights developers’ information on product development, peer-reviewed evidence of technology performance and regulatory milestones including approvals.

Key updates in NAAT development include cartridge-based assays being developed for the Cepheid Inc. Xpert® to either increase test sensitivity or expand testing for drug resistance to second-line drugs. Cepheid recently announced the development of a new product intended for release in 2016: the Omni, a small, battery powered platform designed to host Xpert® test cartridges in resource-limited settings such as microscopy centres. Also included are new developers, including Qiagen (Germany), Tangenbio and Scanogen

(both USA). Other developers are increasing the utility of their platforms by improving upon sample preparation (Molbio Diagnostics [India] and Epistem ([UK]) or by developing new platforms with increased test capacity (Molbio Diagnostics). Both Molbio Diagnostics and Ustar Biotechnologies (China) have also started to develop fully integrated platforms. In the markets targeting intermediate or reference laboratories, new products are also in development or have received further regulatory approval. For example, Becton, Dickinson and Company (hereinafter Becton Dickinson; USA) is developing a TB assay for integration into its BD Max™ product, an automated extraction and amplification platform. In 2014, Abbott Molecular (USA) announced European conformity (Conformité Européenne)-in vitro diagnostic (CE-IVD) marking for its TB assay for use on its automated *m2000* molecular diagnostic platform and CE-IVD marking for a reflex assay to detect both rifampicin (RIF) and isoniazid (INH) resistance in TB positive samples. In 2015, Epistem announced that its Genedrive® platform and MTB assay had received Indian approval for an import license. A key regulatory achievement was made by Cepheid Inc. in 2015 when its Xpert® MTB/RIF assay received United States Food and Drug Administration (US FDA) approval, making this TB diagnostic tool the most comprehensively endorsed product in terms of national and international regulatory agency approvals. However, the China Food and Drug Administration (CFDA) approval of this assay in 2014 notes that the Xpert® MTB/RIF assay may be used only on sputum-smear-positive samples.

4.2. Currently available and pipeline technologies

Introduction: As noted earlier, the primary focus of products presented in this landscape report details the NAAT product landscape. In the *2014 Global Tuberculosis Report*, WHO noted that of the estimated nine million cases of TB in 2013, only 64% was notified as newly diagnosed, leaving a remaining three million people who were left not diagnosed or diagnosed but not reported to NTPs.¹ Clearly, improved diagnosis of TB is urgently needed. Within the population of active cases of TB, there are an estimated 300 000 MDR TB patients, of which only 45% was diagnosed and on treatment. WHO notes that this represents a significant improvement from 2009 data where one in six (17%) was diagnosed, but it also notes that of the five priority actions – from prevention to cure – one is the expansion of rapid testing and detection of MDR TB cases.¹

The range of technologies that is either available or in development for the improved diagnosis of TB continues to complement and/or offer improvements over existing methods or technologies. Developers still include many smaller companies, but larger companies such as Becton Dickinson and Qiagen are devoting significant resources to develop technologies in this space. Several of the NAATs technologies on the market and many in development offer the capacity to perform DST via an integrated companion assay to TB diagnosis or as a reflexive test once TB diagnosis has been confirmed. Triaging tools can identify those most at risk and/or rule out people who appear symptomatic, but do not have PTB. CXRs, a longstanding tool in the traditional TB diagnostic algorithm, are now being offered with much greater utility through more robust, user-friendly equipment and the development of automated algorithms to score abnormalities from digital CXR (DCXR) images. Together, these components can enable active case finding in high-prevalence areas and direct fewer patients for high-quality tests (e.g. Xpert® MTB/RIF) with a reduction of more expensive tests, but with a greater degree of confidence that patients undergoing further testing are more likely to have PTB. Alternatively, the burden of specimens in microscopy centres could be reduced.

Relative to SSM, MTB biomarker-specific technologies may present a yet lower cost and more rapid and effective screening tool for MTB infection. LAM assays can improve suspicion of TB infection in late-staging HIV patients and the use of VOCs or other TB-specific biomarkers may rule out TB infection in a rapid 5-minute automated test. In contrast to the triaging tools, it is unlikely that NAAT-based testing alone can match the price point or rapid turnaround time required to make a test result that is offered by using CXRs or PTB biomarkers. Therefore, more validation studies of triaging tools and their impact on case rate detection and cost savings offered by more specific testing with higher-quality NAATs are necessary to understand what and where incremental improvements can be made, and at what cost.

Other available or emerging technologies can offer incremental improvements to diagnose PTB by optimizing specimen collection or by better preserving the specimen integrity prior to analysis. This approach

may offer better access to diagnosis with existing tools, including SSM, NAAT and culture. Similarly, automated microscopy systems may improve on diagnosis by alleviating challenges with throughput, efficiency or resource requirements (skilled staff and quality assurance).

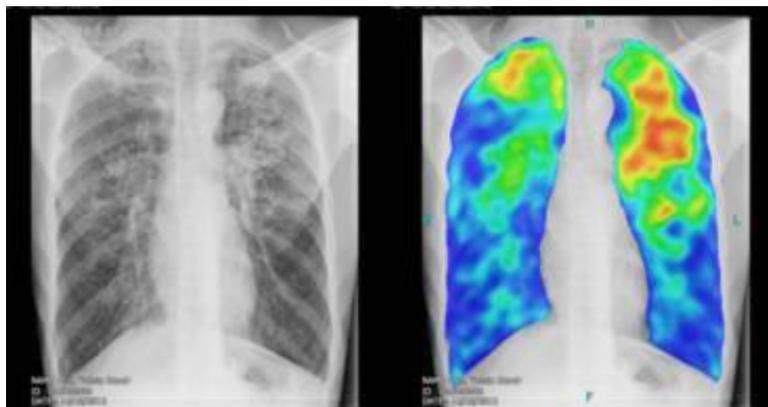
The pipeline of NAAT-based assays is the primary focus of this landscape report and is a dynamic and evolving space in terms of new technologies that are being developed for use at all laboratory levels. There is significant completion in terms of the products on offer and in development. In the upper-tier facilities, high-throughput systems are available, and Becton Dickinson and Qiagen offer new automated technologies. The Becton Dickinson system leverages off its extant BD Max™ diagnostic platform, while Qiagen offers an automated genotyping system to type *Mycobacterium tuberculosis complex* (MTBC) strains via mycobacterial interspersed repetitive unit (MIRU) variable-number tandem repeats (VNTR). The range of LPAs is significant, and several companies offer specific MTB diagnostic and drug-resistance tests to expedite DST testing, as well as platforms to improve throughput of tests and automate scoring. A range of microarray developers intends to offer potentially greater precision by virtue of screening for greater allelic variance and, in several instances, via reduced complexity of testing. The current challenge faced by many of these technologies is sufficient evidence of their performance in the intended use settings.

Xpert® technology remains the dominant technology for use in mid-tier facilities. Cepheid Inc. is also releasing a new cartridge-based assay to improve the sensitivity of its current MTB/RIF assay as well as adding a reflexive test cartridge to confirm MDR TB and indicate extensively drug resistant (XDR) TB. In addition to these, Cepheid is developing a small processing unit, the Omni, which is intended to be placed in more peripheral facilities than the current GeneXpert® machines. Other groups are developing platforms to challenge Xpert® market dominance via higher-throughput or similar modular systems at competitive prices. The range of technologies to directly compete with or replace SSM has expanded. The first generation of NAATs in this space has all of the regulatory requirements to access the primary intended markets. The technology space has also expanded in terms of the next generation of tools, which all host similar product features that include full integration of test processes that are mains electricity independent with onboard storage of test reagents that can withstand elevated extremes of temperature. User input is designed to be minimal. These tests may incorporate DST genotyping as a reflexive test.

The primary challenge to the vast majority of current NAATs tests offered and others in development is the garnering of sufficient performance data to better inform TB programmes and donor support as to their performance in their intended use settings. It is encouraging to note that the Epistem and Molbio Diagnostics technologies are undergoing focused validation studies and the Eiken Chemical Co. Ltd (hereinafter Eiken; Japan) Loopamp™ MTBC assay is under WHO review for endorsement. However, many technologies still lack sufficient validation by independent groups to provide full information on their true performance and potential to improve diagnosis of PTB. Even if a technology is proven to be potentially high-impact, scaling production can be a further challenge – especially since many of the developers are small companies that may face a short period of unprecedented demand. In the next five years, many technologies will enter the market or will have been fully validated. While the current range of available technologies remains small, therefore, the product mix available to HBCs is expected to change markedly in the coming years.

4.2.1. DCXRs

The potential of portable DCXRs as a tool for TB screening in low-resource settings was first introduced in the 2014 landscape report (third edition), which should be referred to for a complete review of the history, benefits and limitations of CXRs in identifying lung abnormalities indicative of current or previous PTB infection. For the identification of TB suspects, WHO has recommended X-ray screening as a very efficient triage/referral test. DCXR has potential use in both active and passive case finding of TB and can integrate with other diagnostic tests such as Xpert® MTB/RIF for rapid identification of active TB. DCXR is fast, has low variable cost and has a good sensitivity/specificity score of around 90/80 in populations with low HIV prevalence and 80/75 for medium/high HIV prevalence.

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

Source: Maduskar et al.¹¹ Reproduced with permission from the *International Journal of Tuberculosis and Lung Diseases*.

Enhancements to X-ray technology and image interpretation software have led to the creation of DCXR systems that are not hindered by a need for key infrastructure and highly trained staff. The advances in technology mean that there are systems that are small yet powerful and robust enough to be transited either in a vehicle or protective flight cases for setup at field sites. A key challenge to more widespread use of DCXR as a screening tool for indication of PTB infection is there are insufficient expert staff trained to read the images. In order to use these advantages and overcome the need for an onsite trained radiologist or clinician to diagnose each chest image, Delft Imaging Systems (the Netherlands) invested in the development of software that automatically scores each CXR image within one minute (Figure 5). The research for CAD was provided by Radboud University in the Netherlands and the Lung Institute in Cape Town. In October 2014, Delft Imaging Systems released the fourth version of CAD4TB, which was also CE-certified in Q2 2015 for use with any DCXR platform. CAD4TB now surpasses the performance of a trained reader in the field or hospital and can be used for passive and active TB case finding as well as in prevalence surveys.¹¹⁻¹³ The CAD4TB software automatically analyses the chest image on abnormalities consistent with TB and scores it between 0 and 100 within one minute.

Figure 6. Components from the EasyPortable DCXR that highlight its portability for use in non-hospital settings

Notes: Collapsed DCXR unit in protective flight case (A); battery pack powering the DXR unit (B); CAD4TB system in operation (C); highlights the work in Zimbabwe with the incorporation of the EasyPortable DCXR as a mobile unit housed in a shipping container mounted on a truck (D).
Source: Images reproduced with permission from Delft Imaging Systems.

Delft Imaging Systems has released the EasyPortable DCXR as a complete and truly portable DCXR system for medical use in low-resource settings (Figure 6). The company notes a high daily throughput (> 250 patients per day) for screening patient images with CAD4TB. The system comes in a sturdy flight case for safe transportation and has built-in roller wheels. Included in this package are an EasyPortable stand, a

lightweight X-ray module, a state-of-the-art digital X-ray detector (Canon CXDI-55G), CAD4TB software and the option of the Picture Archive and Communication System (PACS) that can hold approximately 50 000 DCXR images and operates from a laptop computer. A dedicated medical image viewer is part of the system and allows for fast and easy diagnostic review of the images, only seconds after the image has been made on the X-ray unit. For central archiving or further image review, a teleradiology system is included to allow for compacted images (250 kB) to be sent over the Internet or mobile phone network to the optional central archive or reading room. The use of the tool in resource-limited settings is further enhanced by the addition of a battery power pack that allows the complete X-ray unit and PACS system to operate for four hours without the need for external grid (mains) power or a power generator.

This system is especially developed for use in areas with high ambient temperatures and elevated humidity such as Africa or South-East Asia. The system comes with a powerful (8kW) and portable X-ray unit allowing for high-quality X-ray images at the lowest possible patient dose. The design facilitates ease of transport (Figure 6D) due to its ability to be folded, low weight, flexible positioning of the X-ray and detector, and simple setup in under 10 minutes. The Canon imager was developed with robustness in mind with the expectation of rough handling afforded by routine portage and setup/breakdown in the field (Figure 6B). The cost of the EasyPortable DCXR system is listed at US\$ 79 000. Additional costs are incurred with the use of CAD4TB with a payment structure starting at US\$ 1.75 per image analysed by CAD4TB. However, the manufacturer notes this price can rapidly decrease with economies of scale (to approximately US\$ 1/image).

The effectiveness of CAD4TB software in identifying active PTB has undergone evaluation in a variety of settings, including Zambia,^{11,13} South Africa,¹² the United Kingdom,^{12,14} Pakistan^{15,16} and Tanzania¹⁷ for a variety of applications, including passive and active case finding, in addition to use in screening at-risk populations such as prison inmates and the homeless. The application of the system is ongoing and the company has noted that, in 2015, donor-sponsored projects are under way in Bangladesh, Ghana, Libya, Pakistan, South Africa, Zambia and Zimbabwe.

Advenio (Chandigarh, India) is also developing automated image analysis software and has a TB-specific product, riView-TB. This is similar to CAD4TB in that DCXRs can be automatically scored for lung abnormalities without the need for a trained radiographer or clinician to interpret the images. Advenio notes that it is developing algorithms that will also screen for pneumonia and silicosis to augment PTB diagnosis within the same software package. Unlike Delft Imaging Systems, Advenio is not developing any hardware. Currently, there is limited information on the riView-TB product or descriptions of its application in screening patients for PTB.

4.2.2. Specimen collection and manipulation

A persistent problem with the collection of an adequate sputum specimen from suspect cases of PTB is when the patient cannot provide sufficient volume of specimen for diagnostic testing. A suboptimal specimen, in terms of limited overall volume or the composition of test material or compromised sample (e.g. a gross excess of saliva), makes any diagnostic test more likely to fail and is exacerbated in cases where the patient cannot physically provide an adequate sputum sample by the conventional expectoration. In such cases, the patient needs to have sputum induction via a saline spray. This represents additional effort that may not be easily provided in low-resource settings, increases costs and adds an increased risk of acquired infection to medical personnel administering the procedure. A further challenge is that PLHIV typically produce paucibacillary specimens, which means that an adequate specimen is important.

An assumption based on their cost and complexity is that high-throughput NAAT systems will be located in centralized facilities and, therefore, specimens must be shipped to the facility prior to testing. To optimize such a strategy, specimens must not only be acquired correctly, but also be stable, ideally inactivated to prevent further infection risk or limit contamination by commensal microflora so that each specimen is uncompromised prior to testing. Therefore, two products that can improve specimen collection are noted.

The first product is the Lung Flute from Medical Acoustics (USA) that permits the individual to collect their own sputum specimen when they cannot expectorate a sufficient volume for diagnostic testing.¹⁸

Traditionally, saline spray has been used to induce collection, but this method requires medical assistance and adds risk of acquired infection to the health-care worker. The Lung Flute is non-invasive and the user holds the small plastic device to their lips and exhales into it. This creates vibrations in the lungs that help to loosen and liquefy sputum in the alveolar cavities and, therefore, the person may produce more sputum. The product received regulatory approval by the US FDA in 2011 and is also CE-IVD marked. Since the 2014 landscape report, the Japan Anti-Tuberculosis Association and local partners have assessed the performance of the Lung Flute in Japan on a small cohort of patients with hypertonic saline-induced collection as the control method. The sensitivity of hypertonic saline and the Lung Flute was 78.4% and 84.3%, respectively, and there was no statistical difference between these methods. The data will be presented in a peer-reviewed manuscript in 2015. A further study is underway in Bangladesh. Current cost per device is US\$ 10.

Deton Corp. (USA) takes an alternative technological approach to collecting specimens that is in the mid-development stage. Rather than collecting sputum, its technology takes advantage of the physical properties of microdroplets that may contain MTB bacilli. The patient coughs into a 1L bag and the microdroplets remain suspended in the airspace of the collection bag due to their very small size. The collected air is then forced through a device engineered to impact the particles onto a collection surface. This is then removed and used for diagnostic testing. Currently, Deton Corp. is solely investigating the sampling with downstream analysis by PCR. Its current development timeline indicates a CE-IVD marked product for release in Q4 2016 with HBCs in the developing world being the intended market.

Once an acceptable specimen is acquired, the next step is to present it for diagnostic testing, ideally in optimal condition. Certain tests are only available in larger facilities (e.g. culture or LPAs) and so improvements to specimen transport are important. Several companies are making products that retain the viability of TB cells in specimens and/or by nucleic acid stabilization prior to NAAT without cold chain.

DNA Genotek Inc. (Ottawa, Canada; a wholly owned subsidiary of Orasure Technologies Inc.; USA) has developed two products to process sputum. OMNIgene® SPUTUM is intended to liquefy and decontaminate the sample, allowing it to be transported without cold chain to a laboratory for diagnostic testing. Treated samples can be used for microscopy, culture (including the mycobacterial growth indicator tube [MGIT™] from Becton Dickinson), Xpert® MTB/RIF and other molecular detection methods. An evaluation of OMNIgene® SPUTUM by GENETUP (Nepal) showed transport of samples up to eight days without cold chain and demonstrated significant reduction in culture contamination (from 13% to 0%) when tested by Löwenstein-Jensen (L-J) culture and an improvement in the average time to positive result. The company's prepIT® MAX technology is intended to be a high-performance chemical lysis method to release nucleic acids from MTBC cells without the need for a mechanical approach, such as bead beating. Samples first treated with OMNIgene® SPUTUM can be subsequently processed with this kit to obtain nucleic acids for further analysis. The current method relies on ethanol precipitation of DNA that is compatible with NAATs and whole genome sequencing. A direct-to-PCR method and direct integration into automated extraction systems are under development. In the past year, the company began rigorous product evaluations and clinical testing in over 18 countries, including key partners such as the National Health laboratory (NHLS, South Africa), GENETUP, the TB National Reference Laboratory (Moldova) and the TB Supranational Reference Laboratory (Italy), to demonstrate claims and impacts on downstream diagnostics. It is anticipated that both products will receive CE-IVD marking by Q4 2015. DNA Genotek Inc. is working with STOP-TB, WHO, FIND and the Global Laboratory Initiative (GLI) to determine appropriate paths forward for technical evaluations and to facilitate adoption by countries receiving donor funds. The costs of these products are currently not available.

Longhorn Vaccines & Diagnostics (USA) takes a solely NAAT-focused approach with its products being used to inactivate, lyse and stabilize MTB DNA without cold chain via the PrimeStore Molecular Transport Medium® (PS-MTM®). PS-MTM® contains chemical denaturants that permit lysis of MTB cells with subsequent stabilization of MTB DNA without cold chain. It is intended to aid microscopy centres to send specimens to higher-tier laboratories for further NAAT analysis.^{19,20} Approximately 0.5 mL of raw sputum is added to the liquid via the PrimeSwab™, a polyester flocked swab. A proposed additional benefit of the PS-MTM® product is that not all of the genetic material is used for a single test thus there is extra material

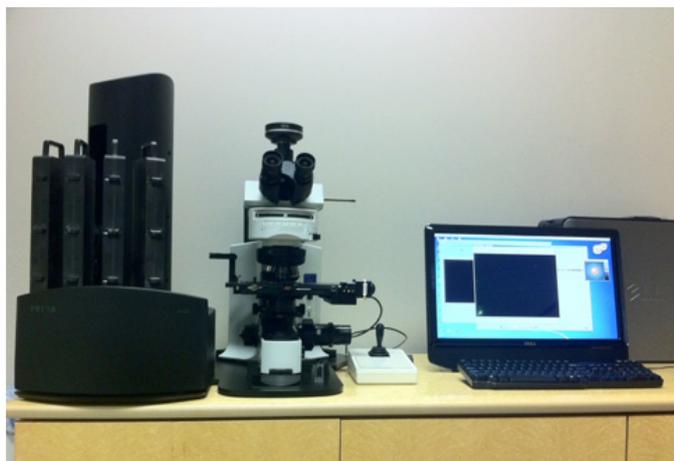
for secondary testing as required (e.g. MDR screening on TB positive samples). One study demonstrated that the DNA from PS-MTM[®] was suitable for polymerase chain reaction (PCR) amplification <7days after collection.²¹ PrimeStore has expanded its product range incorporated into a kit, the PrimeSuite TB[™] to include a sample preparation (PrimeSwab[™] and PS-MTM[®]), DNA extraction (PrimeExtract[™]) and a real time PCR assay (PrimeMix[®]). The company is identifying which real time PCR machines may be used, but in principle many could host the PrimeMix[®]-based reactions. In addition, a DNA sequencing kit for screening of drug resistance to 10 drugs, PrimeSeq[®], is in development.^{21,22} To increase production capacity, the company has partnered with a European manufacturer for scaled production of its kit and other products and envisage CE-IVD marking in Q4 2015.

Although the products listed in this section are for the storage and extraction of MTB DNA for sputum, there is also a product that permits the extraction of MTB DNA from up to 10 mL of whole blood or other liquid specimens. The number of MTB cells in a liquid specimen is often low and, therefore, processing a larger sample volume permits greater opportunity to capture more DNA from MTB cells. In addition to inhibitory compounds in blood,²³ an excess of genomic DNA from the nuclear cells can also be inhibitory within an amplification reaction and so its removal may increase the diagnostic sensitivity of MTB-directed NAATs. To address this issue, Molzym GmbH & Co. KG (hereinafter Molzym) launched the MTB-DNA Blood kit in 2014, which removes excess human genomic DNA and, in addition, extracts and concentrates MTB DNA. This comes as a kit (25 or 50 reactions) that is intended for use in higher-tier laboratories. The method takes two hours if manually performed and can be completed in a 1-hour process using the SelectNA[™] or SelctNA[™]plus semi- and fully automated platforms from Molzym. The cost per extraction is €11.80 and €12 500 or €24 500 for the instruments listed above.

4.2.3. Automated microscopy

The direct visual examination for MTB cells via SSM remains the most common rapid test performed for the diagnosis of PTB infection in low- and middle-income countries. While it is a simple test, it suffers from low throughput, requires quality assurance programmes to maintain user performance and has variability of test results and poor sensitivity, especially with paucibacillary specimens. A recent analysis of the number of tests to initially confirm TB infection performed in 22 HBCs was estimated to be 61.7 million smears at a median cost of US\$ 109 million.⁹ There are only two products to report upon.

Figure 7. TBDx system showing the automated slide loader (left), FM with digital camera and automated stage (centre), and laptop to operate the reader and employ the scoring algorithm (right)



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

The first is the TBDx system from Signature Mapping Medical Sciences Inc. (a wholly owned subsidiary of Applied Visual Sciences Inc.; USA) that reported further advances in the validation of its product in 2014. The TBDx system utilizes novel software algorithms to scan high-resolution digital images of fluorescent smears to automatically score the fluorescent bodies therein. The system integrates a high-quality fluorescent microscope and the software analyses digital images of each field. Stained sputum smear slides are prepared manually and then can be automatically loaded for imaging and interrogation (Figure 7). The current system can either process one or four slides consecutively on the automated slide stage or use a robotic slide loader that can automatically process from 50 to 200 slides. The entire process takes approximately five minutes to analyse one slide, 1 hour to analyse 10–12 slides, 8 hours to analyse 100 slides or 16 hours to analyse 200 slides. The company notes that the TBDx algorithm may be adjusted for highly positive slides (e.g. 3+ or 2+) in order to expedite testing.

A recent evaluation in South Africa compared the performance of the TBDx to two 40-year veterans of smear microscopy at the Centre for Tuberculosis, using culture as the reference standard.²⁴ The TBDx was 98% sensitive on SSM+/C+, and 40% on SSM-/C+. The TBDx technology had an overall sensitivity of 79.8% and specificity of 78.9%. When Scanty 1 cases were treated as Normal, the sensitivity declined to 73.4% and specificity rose to 95.7%. The researchers concluded that, “as a standalone diagnostic system, it proved to be comparable to highly experienced microscopists and offered a diagnostic solution that could provide quality-assured microscopy in settings where trained microscopists are difficult to find”. It has the potential to reduce the test burden and cost of the Xpert® by its use of a triage tool to rule out strong TB positive and negative specimens.

A second element of the study was to understand the impact of a screening-confirmation algorithm using a combination of the TBDx and Xpert®. A total of 1009 prospective samples from patients with presumed TB were processed in parallel with conventional smear microscopy, TBDx microscopy and liquid culture.²⁴ The TBDx positive samples were also processed by Xpert®. The primary outcome of this study was that using the TBDx to screen specimens prior to using Xpert® could detect 90% of patients with Xpert® positive TB, while reducing the number of Xpert® tests required by 73%. As noted by the researchers, “In either algorithm, the use of the TBDx automated microscopy has the potential to improve upon the existing diagnostic standard of care”.²⁴

Further validation studies on the TBDx platform have been performed in Nigeria under the direction of the Liverpool School of Tropical Hygiene and Medicine, and in Peru and Viet Nam under the direction of FIND. Data from these evaluations are not yet available. An economic model measuring the impact of combining the TBDx and Xpert® in South Africa has been created by Johns Hopkins University and the results are pending release. The system is available for purchase with the microscope and computer offered at US\$ 23 000 with the (optional) 200-slide robotic loader at US\$ 21 000. The cost of 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\$ 1–2 depending upon the length of license period.

Figure 8. Automated smear microscopy reader in development by Becton Dickinson



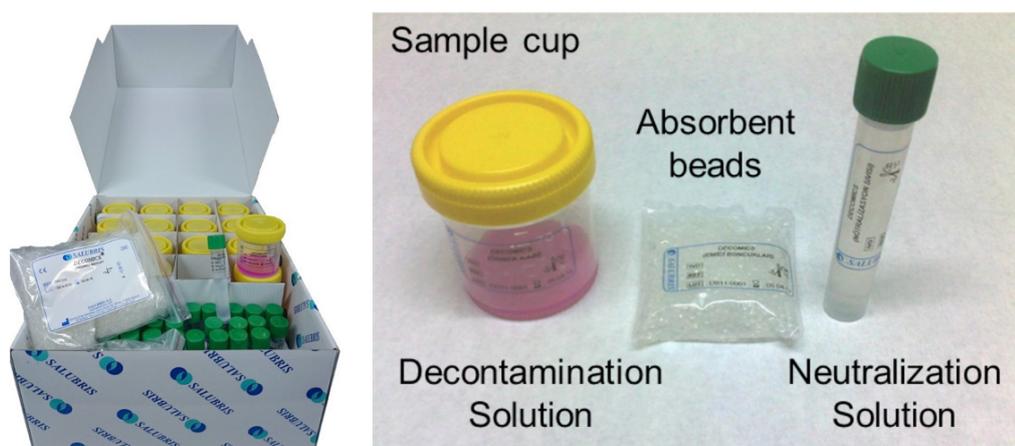
Source: Image courtesy of Becton Dickinson. Reprinted with permission.

Becton Dickinson is currently developing a fully automated instrument that also incorporates the staining procedure for detection of acid fast bacteria in sputum (Figure 8). The device is being developed for use in microscopy centres or lower-tier facilities. The system standardizes specimen preparation using a single-use disposable cartridge that houses unitized reagents, including quality control. A slide-reading algorithm scores the reading of slides, eliminating the subjectivity associated with manual examination. The sensitivity of the prototype is similar to that of LED-FM performed by a skilled microscopist. The system is designed for ease of use so as to suit relatively unskilled workers. Reagents are intended for storage at temperatures up to 45°C. Data input and operation are via a touchscreen interface. Sample preparation takes about five minutes hands-on time and up to 40 slides per day can be batch-processed by a battery-powered machine that has a small footprint. The company anticipates that this product will be commercially available in 18–22 months. Costs are unknown.

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

There is little to report in terms of new product information from developers regarding culture-based diagnosis of TB. Salubris Inc. (USA) no longer supplies the biphasic TK MEDIUM® SLC for use in its MYCOLOR TK® automated culture system. The company now offers the TK MEDIUM® SLC-L, a liquid media that is now housed in a plastic tube to limit risks of mishandling. A consistent issue with the conventional decontamination method of sodium hydroxide and N-acetyl cysteine (NaOH-NALC) is the risk of incomplete decontamination or overexposure to reagents killing the MTB cells. The NaOH-NALC method also requires centrifugation that can limit processivity rate and incorrect use of this can result in infection risks via aerosolisation. The Decomics® kit (Figure 9) uses absorbent beads and reagents to liquefy, decontaminate and neutralize samples in less than 25 minutes without requiring a centrifuge or NaOH-NALC.

Figure 9. Decomics® kit from Salubris Inc.: individual components including sample cup, decontamination solution, beads and neutralization solution



Source: Image reproduced with permission from Salubris Inc.

A recent evaluation of the TK MEDIUM® SLC-L and Decomics® beads was performed in India with 500 specimens with comparison to NaOH-NALC processing and L-J culture. The authors noted an improved performance of MTB diagnosis from the liquid media versus L-J (130 MTB vs 110 MTB) and with an earlier median time to detection (12 days vs 30). Rates of contaminated culture were reduced from 7% observed with the NaOH-NALC method to only 2% with Decomics®. This kit also reduced the median time for specimen processing to approximately 23 minutes as compared to NaOH-NALC, which took 45 minutes. The Decomics® product is CE-IVD marked and US FDA (510k) cleared. Current pricing of this product is unknown.

4.2.5. Biomarkers to detect active TB

A direct and rapid method for the diagnosis of all active infections of TB (including extrapulmonary TB [EPTB] and paediatric TB) using non-invasive or minimally invasive specimens (e.g. breath; urine; finger-stick) would be of huge benefit for the early diagnosis of MTB or screening for possible MTB infection in minimal health-care settings. This landscape report notes that a significant amount of product development continues in this area and many of these technologies are in early or mid-stage development. However, there is some important product information regarding specific development in this relatively broad field with updates on the LAM immunologic strip assay offered by Alere Inc. (USA).

Figure 10. Determine™ TB LAM Ag rapid assay, with strip ready for use shown at right



Source: Image reproduced with permission from Alere Inc.

Alere Inc. markets the Determine™ TB LAM Ag rapid assay (Figure 10), an immunochromatographic strip that targets the LAM antigen in urine via an antibody capture and detection method on a nitrocellulose strip. LAM, a 19 kDa glycolipid, is a key component of the mycobacterial cell wall and is produced by both growing cells and the degradation of the cell wall.²⁵ As such, it should be noted that this assay is not only specific for MTBC, but will also detect the presence of other non-tuberculous mycobacteria (NTMs). LAM is released into the blood and is ultimately expelled from the body in urine and, therefore, the detection of LAM in this sample matrix can indicate all sites of mycobacterial infection and not just PTB. The test requires only a single 60 µL aliquot of urine and no other materials are required. The test result is visually noted after 25 minutes incubation. The simple format of the assay needs only minimal training for the user and the product is stable for 15 months at 30°C.

Since its release in 2012, the Determine™ TB LAM Ag assay has undergone significant independent evaluation of its potential use as a rapid screening tool for TB infection. Lawn et al. first noted that the test's performance improves with increasing severity of illness and more advanced immunosuppression, thus offering the potential utility for rapid suspicion of TB coinfection in hospitalized HIV-infected patients.²⁶ Their findings noted that the sensitivity of the assay incrementally improved from 4% to 76% as the CD4 count decreased from >200 to <50 cells/µL in whole blood. Rapid diagnosis of TB in PLHIV is very challenging using either conventional tests or NAATs, and mortality rates are high if treatment is delayed. In the past three years, more groups have assessed the utility of the Determine™ TB LAM Ag assay as a rapid test within this highly vulnerable population. Validation studies have been performed in high-income countries and HBCs in Africa, Asia and South America. The wealth of evidence provided by these validations is sufficient that a dossier of evidence was prepared for review by WHO STAG-TB in June 2015. This group will review the diagnostic accuracy and other performance attributes of the Determine™ TB LAM Ag rapid assay and make a recommendation to WHO as to its application in TB control. Alere Inc. has identified the primary markets for sale of the Determine™ TB LAM Ag rapid assay as Africa, Asia Pacific and Latin America and the projected cost is US\$ 3.50.

4.2.6. VOCs

Assessing VOCs via a simple breath test is another area of increasing interest for the rapid screening of TB infection for point-of-care (POC) diagnosis of PTB or as a triage tool. The potential of this technology has been proven by giant pouched African cane rats that can quite accurately predict active PTB infection via sniffing sputum specimens.^{27,28} A recent study has noted their potential ability to detect different genotypes of MTB.²⁹ A variety of technological developments are ongoing in this space as noted in the 2014 landscape report with different core technologies to identify a variety of chemicals associated with active PTB. The developers include Menssana Research Inc. (USA; gas chromatography), the eNose Company and Nanosynth (the Netherlands and USA; metal-oxide sensors), Rapid Biosensor Systems Ltd (United Kingdom; antigen detection), Metabolomx (USA; metabolite detection by chemical reaction) and Next Dimension Technologies (USA; electric sensors). Of these developers, eNose has reported significant progress in the development of its eNose device. Scaled production is anticipated to begin in mid-2015 and CE regulatory marking soon thereafter. The tool is currently undergoing validation studies in Bangladesh, Indonesia, Kenya, Paraguay, South Africa and Venezuela, will continue until late 2015 or Q1 2016. eNose intends to work with WHO to define market release of its technology.

Figure 11. TB Breathalyser from Rapid Biosensor Systems Ltd: cough collector (left and centre) and sensor to detect the presence of MTBC-specific antigens (right)



Source: Image reproduced with permission from Rapid Biosensor Systems Ltd.

Rapid Biosensor Systems Ltd has developed the TB Breathalyser (Figure 11) and is currently looking to license the technology. The TB Breathalyzer is designed to detect active TB bacilli in cough samples in less than four minutes. The test is simple to perform. The patient coughs into a cough collector, which is then placed into the portable optical reader that interrogates the cough sample via MTB specific antigen detection. Start and stop buttons operate the reader and the software outputs a TB positive or TB negative result on the liquid-crystal display (LCD) display. For patients that require saline induction in order to produce a cough, Rapid Biosensor Systems Ltd has a cough collector that can accommodate for excess moisture in the sample. The instrument is battery operated and intended for use by a low-skilled user. One study with a cohort of 60 participants in Ethiopia found that the TB Breathalyser had a specificity of 79% and the developer claims that additional improvements have been made to significantly increase both the sensitivity and specificity.³⁰ They intend that the more recent studies with over 1000 participants from Ethiopia and India will be published shortly in peer-reviewed journals. FIND is also reviewing these data. The estimated cost per test is < US\$ 8 and the developers estimate scale-up to manufacturing will take one year once the technology is licensed.

There are two new technologies that are being developed to detect TB biomarkers in breath. One is from Siemens (Germany) that underwent a small clinical evaluation in Germany in 2013. The other is in development by Nanosynth. Early validation of a Nanosynth prototype device is currently being performed in Uganda.

4.2.7. Immune response-based tests for LTBI

Immune response-based tests are laboratory-based tests that require incubation of T-lymphocytes (T-cells) that are harvested from whole blood. Interferon-gamma release assays (IGRAs) are not intended as a direct diagnostic tool for active TB.³¹ Instead, they can identify both latent and active TB infection. These are in vitro blood tests of cell-mediated immune response and they measure the T-cell release of interferon-gamma (IFN- γ) following stimulation by MTB specific antigens; the early secreted antigenic target 6 (ESAT-6), culture filtrate protein 10 (CFP-10) and the TB7.7 peptide antigen (only the QuantiFERON-TB Gold assay [QFT]). IGRAs have higher specificity than tuberculin skin testing, have less cross-reactivity with the bacillus Calmette-Guérin (BCG) than the TST and correlate well with MTB exposure.

There are currently at least five IGRAs available on the market and one in development. The assays are: the QFT (Cellestis/Qiagen; Australia); T-SPOT[®] TB assay (Oxford Immunotec; United Kingdom); the Immuchek TB Platinum (Immunoshop India Pvt Ltd; India); the TB-IGRA, (Beijing Wantai Biological Pharmacy Enterprise Co. Ltd (China); and the ASACIR TB (Haikou VTI Biological Institute (China). A further test, the Fluorospot TB assay is currently under development by Autoimmun Diagnostika (Germany) and was recently described in the peer-reviewed literature.³² Of these assays, only the QFT plus and T-SPOT[®] TB assay have CE-IVD marking and US FDA approval and other country-specific regulatory approvals; the QFT is approved by Health Canada, the Japanese Ministry of Health, Labour and Welfare (JMHLW), the Russian Federation and Taiwan, whereas the T-SPOT[®] assay is approved by the CFDA and JMHLW.

Figure 12. QuantiFERON-TB Gold Plus kit from Qiagen: reagents and ELISA plates (left) and collection materials (right)



Source: Images reproduced with permission from Qiagen.

In January 2015, Qiagen announced the release of its third generation IGRA product, the QuantiFERON-TB Gold Plus (Figure 12). The company claims that this assay has greater sensitivity (95.3%) and specificity (97.6%) than other IGRAs. Improvements to the assay include a single-tube blood collection option as well as the ongoing use of the unique “assay in collection tube” design that allows for immediate stimulation of the blood sample, which ensures no loss of T-cell activity that is critical for obtaining accurate and reliable results. The product has CE-IVD marking, but as yet is not US FDA approved. Qiagen notes a target price in the range of US\$ 15-25 per test and US\$ 35 000–100 000 for automated instrumentation. The assay is available in Europe and other regions. Currently, there is no peer-reviewed evidence of the performance of the QFT plus product.

4.2.8. Other TB biomarker assays

The T-cell activation marker-tuberculosis (TAM-TB) assay was first reported in 2014.³³ The assay measures the down regulation of a cell surface protein, CD127 when TB antigen-specific CD4 T-cells are challenged with MTB antigens.^{34,35} The TAM-TB assay uses flow cytometry to detect fluorescently stained TB antigen-specific CD4 T-cells that are counterstained with an antibody to CD127. Exposure to TB infection is identified by measuring the CD127 levels within the population of TB antigen challenged CD4 cells. This is a laboratory-based test requiring a centrifuge, incubator and flow cytometer as critical equipment and the reagents currently require cold chain. One worker can process < 15 samples per day. In the original study, the authors noted a sensitivity of 83% for TB infection with a specificity of 96.6%.³³ Current unpublished data include a sensitivity of 88.9% for TB infection with a specificity of 96.6% for paediatrics, and in adults a sensitivity of 83.3% for TB infection with a specificity of 83.7%. This work is a collaboration between the Ludwig Maximilian University of Munich (Germany) and the Tropical and Public Health Institute (Switzerland). A commercial version of the test in partnership with Alere Inc. is under development with key improvements to include a more rapid time to result and temperature-stable reagents. It is estimated to be ready for market in 2019 at a projected cost of €10 per test.

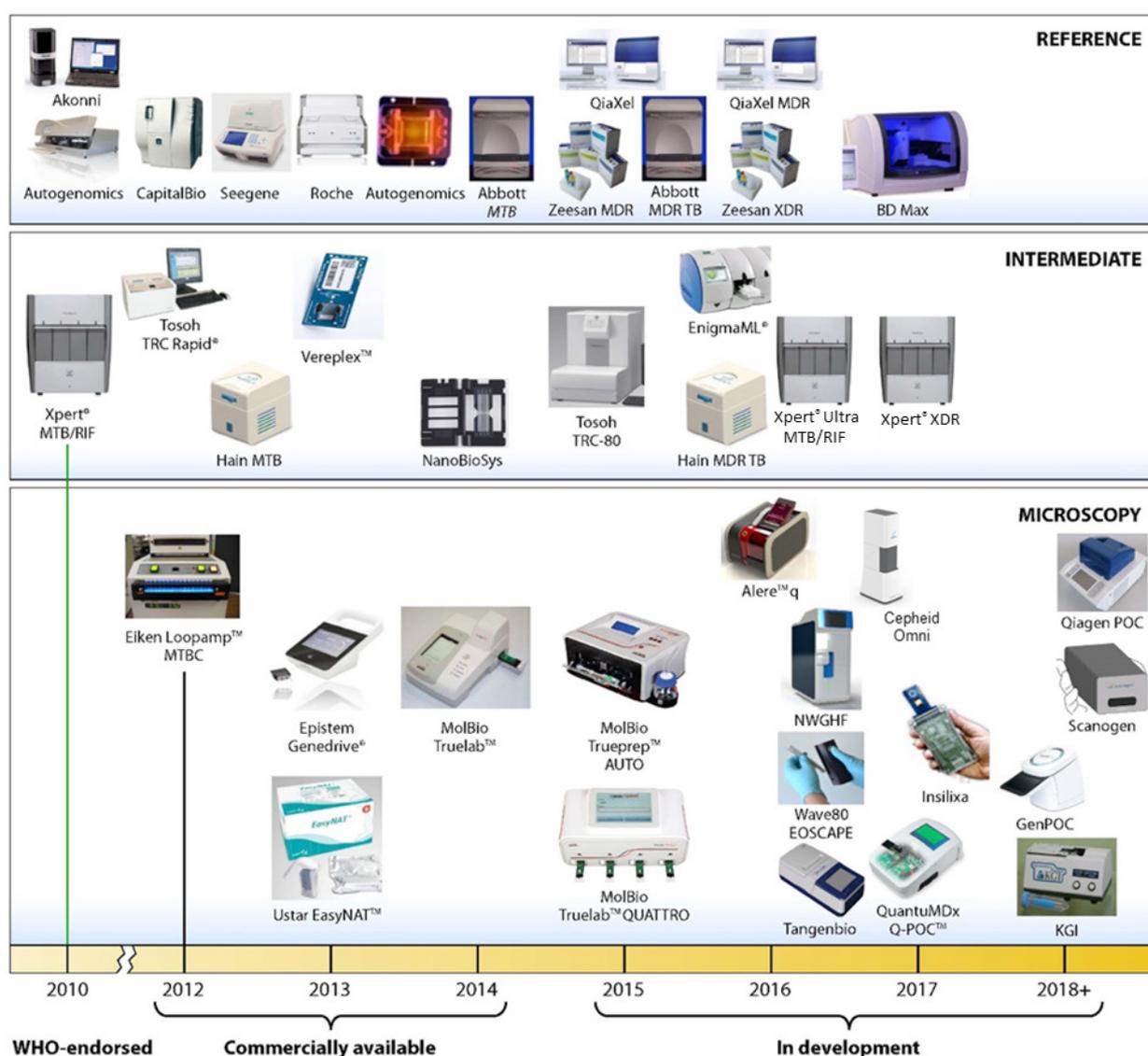
4.2.9 NAAT tools for TB diagnosis, drug resistance screening and genotyping

The application of NAATs has revolutionized rapid and accurate diagnostic testing for most infectious diseases in terms of time to result and general high performance when compared to extant methods such as microscopy, serologic, immunologic or culture-based methods and combinations thereof. Highly sensitive NAAT-based diagnosis of PTB is challenging in terms of the specimen type, the relatively low number of bacilli present and the waxy cell wall physiology of MTB cells being difficult to lyse. The requirement that NAATs must be performed in minimally resourced facilities with relatively unskilled users must also be applied if the rapid diagnosis of TB is to be achieved at the required scale.^{5,6} While a broad range of technologies is presented in this section, the area of greatest need (and interest from technology developers and TB programmes) is the development of superior products to replace or augment SSM. Recent reports inform and detail the logistical and infrastructural challenges presented by placing a high-complexity test and host technology into microscopy centres in HBCs and the development of TPPs to identify the key attributes necessary for NAATs to be functional and impactful in peripheral facilities.^{7,10,36,37} The last comprehensive market analysis of TB diagnostics was released in 2006,³⁸ and new and revised market estimates have been performed to help developers understand the current global market value of SSM. This is very relevant as cost remains a key issue for the NAATS envisaged for use in peripheral facilities and acceptable prices for TB programmes and donors have yet to be accurately established.^{39,40}

While culture-based methods remain the gold standard for TB diagnosis as well as phenotypic DST, the extensive validation work of the Xpert® MTB/RIF questions the diagnostic performance of culture; for example, the validity of a positive Xpert® result is overruled as a negative by culture data. A fully integrated methodology is considered lesser to a highly variable manual process. The Ultra, detailed later in this landscape report, is a new highly sensitive TB NAAT assay in development by Cepheid Inc. that has the potential to provide a rapid NAAT with equal or better sensitivity to MTB culture, but hosted in an integrated system that is largely free of test-to-test variability in processing or test reagents.

The number of NAAT products on the market or envisaged to enter within the next four years continues to grow. Of the 50 products noted in the NAAT pipeline presented in 2014, 48 are thought to be still on the market or in development, and a further 6 new products are noted in this landscape report. The product development pipeline for NAAT-based TB tests other than LPAs and next generation sequencing (NGS) is shown in Figure 13.

Figure 13. Current and emerging automated, semi-modular or non-integrated TB NAATs; their intended laboratory location and release date (actual or anticipated)



Overall, more diagnostic companies are now registering their intent to create products for TB diagnosis and genotyping drug susceptibility via a variety of methods. The NAATs on offer are intended for use in a variety of intended settings from the national reference laboratory to the microscopy centre. The test intent, complexity and user requirements of the NAAT dictates where they may be best positioned (Table 1). There is a need for improved diagnostic tests to reduce the approximate one third of active TB cases currently either not diagnosed or notified.¹ The bulk of this testing will be performed in lower-tier laboratories and as such these tests need to be affordable, easy to use, have improved performance to SSM and low to moderate throughput to meet projected demand.^{6,7,37} Drug resistance testing to first-line drugs via an integrated or reflexive test is needed at the microscopy centre level where treatment is administered and genotyping second-line drug resistance, strain genotyping and/or whole genome sequencing for molecular epidemiology used by mid- or upper-tier laboratories.

Table 1. 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	Test complexity	Hardware cost	Cost/test
WGS	Ref.	High	Surveillance/ DST/ treatment	High	High	High
Genotyping	Ref.	High/ moderate	Surveillance	High	High	High/ 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 ^a	High	High	High/ moderate
LPA	Ref./Int.	Moderate	MTB/NTM/ Dx/ DST ^{a,b}	Moderate	Moderate	Moderate
Modular NAATs	Ref./Int.	Moderate	Dx/DST ^b	Low	High	Moderate
SSM replacement	Int./Per.	Moderate/ Low	Dx/DST ^b	Low	Moderate/ low	Low

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

For other abbreviations, please see the list at the start of this report.

^a Some assays are available to rule in other common types of NTM.

^b DST may be included in the Dx test or as a reflexive test after MTB infection is detected.

The performance of NAATs is relative to the amount of target presented for testing. For diagnosis of PTB, SSM-positive samples have high sensitivity as there are large numbers of bacilli that are processed yielding a relatively high amount of DNA or RNA targets for subsequent amplification. However, paucibacillary specimens are common in PTB and, therefore, assays need to have a low limit of detection (LOD) if tests are to supplant SSM.^{8,37} Diagnostic assays should have comparable sensitivity in detecting a high percentage of SSM-/C+ isolates. The performance of genotypic DST, certainly for first-line drugs, is also necessary if accurate and timely diagnosis of MDR TB is to be offered.⁸

NAATs are designed to specifically target MTBC genomic DNA or cellular RNA (e.g. 16S RNA). PCR is a commonly used amplification method for many of the TB diagnostic technologies, but the greater simplicity of incubation offered by isothermal amplification methods has led to many of the NAATs targeting microscopy centres where inadequate power supply and limited infrastructure can affect operation of complex instrumentation.³⁶ The detection methods vary across products with most using fluorescence and the simpler tests using visual detection of luminescence or stripes on amplicon capture strips. The processes of nucleic acid extraction, amplification, detection and scoring of the test result range from separate (open) and manual, to partially or fully integrated and automated.^{36,41}

A challenge to using the open or partially integrated platforms is that the sensitivity of the assays can be affected by amplicon or sample contamination and so dedicated areas must be employed to reduce this risk in addition to having well-trained staff and constant quality control to monitor test performance. Despite the large range of NAAT MTB diagnostic products currently available to users, only three have received WHO endorsement: LPAs (Hain Lifescience); INNO-LipA (Fuji-Rebio Europe); and Xpert[®] MTB/RIF (Cepheid). Some have US FDA approval: Xpert[®] MTB/RIF; Amplicor (now discontinued, Roche); Amplified Mycobacterium Tuberculosis Direct (AMTD, Hologic); and BDProbeTec[™] (Becton Dickinson),⁴² which reflect the small market niche that TB diagnostics have in the USA, a low-burden country. Most products have CE-IVD marking for Europe and several hold further national regulatory approval (e.g. CFDA).

Commercial NAATs for use in reference and intermediate-tier laboratories

Many of the products listed in Table 1 are intended for use in hospital or reference laboratories. This is in part due to their intended use – for example, genotyping for epidemiologic purposes – but most often the complexity and sensitivity of the platform and reagents require appropriate infrastructure for storage and operation. The test complexity also requires skilled and trained staff for test implementation and routine maintenance. The primary markets are developed countries where TB incidence is typically low and, therefore, the range of products is small. There are few tests that have previously received US FDA approval, and the Xpert® MTB/RIF assay is the first to receive US FDA approval since 2009. This highlights that the TB diagnostic tools for high-income countries is not a primary area of focus for test developers interested in this small yet high-value market. The third edition of the landscape report described the pipeline of these technologies in detail.⁴³ This section presents the range of technologies in this space, including automated MTBC diagnosis platforms, non-automated MTB NAAT assays, LPAs and microarrays.

Automated MTBC diagnosis platforms: High-throughput screening can offer benefits in terms of the cost per test, uniformity of processing and increased test capacity once the initial cost of equipment procurement and infrastructure upgrades (if necessary) are met. An alternative to direct purchasing is via equipment lease systems or via reagent purchase schemes. For NAATs to be considered for high-throughput testing, the technology must not just perform multiple tests within a short timeframe, but also at volume. Ideally, this includes automated extraction and liquid handling to expedite sample preparation and minimize user introduced error. In addition to faster turnaround times, this format creates a more carefully controlled environment where sample integrity is less compromised and fewer adequately trained staff are necessary, which provides uniformity to the process. Two leading international diagnostic companies, Roche Diagnostics (Switzerland) and Abbott Molecular (USA), market technologies for TB diagnostic testing in primary facilities. Hain Lifescience (Germany) has developed low to high-throughput systems for MTBC diagnosis and is working on a DST assay. Becton Dickinson is also currently developing an MTB assay and protocol for use with its BD Max™ platform (Figure 17). All of these companies offer other diagnostic tests for use with their platforms, including HIV viral (Abbott Molecular and Roche Diagnostics) or hospital acquired infections, for example, *Clostridium difficile* (Becton Dickinson and Hain Lifescience). Qiagen has also developed an automated tool for the molecular genotyping of MTBC isolates.

Figure 14. Roche Diagnostics products for diagnosis of MTBC: COBAS® TaqMan® MTB Test kit (left) and the COBAS® TaqMan® 48 real time PCR machine (right)



Source: Images reproduced with permission from Roche Diagnostics.

Roche Diagnostics offers the COBAS® TaqMan® MTB Test, in which up to 44 patient test reactions can be performed in its COBAS® TaqMan® 48 real time PCR machine (Figure 14). Recent information supplied by the company notes that it also offers the AMPLICOR® Respiratory Specimen Preparation Kit, a manual extraction kit (100 samples) that complements its other products. It notes the product can be used with liquefied, decontaminated and concentrated human respiratory specimens, including sputum and bronchial alveolar lavages. The subsequent amplification is by real time PCR targeting the gene responsible

for 16S RNA. The scoring of results is automated and test data stored electronically. In terms of assay performance, Roche Diagnostics claims it has 100% analytical specificity for MTBC with an analytical sensitivity of 18 cfu/mL. The clinical sensitivity with SSM + /C + sputum samples is reported as 96.4%, while for SSM- / + ve the sensitivity decreases to 76.8%. The entire process for RNA extraction and testing takes approximately 240 minutes of which only 35 minutes are required for hands-on time. The company estimates that batched testing will permit a throughput of over 100 samples per day.

Currently, Roche Diagnostics does not offer a reflexive assay for DST. Equipment maintenance is minimal with a yearly change of the air filter the only required step for the COBAS® TaqMan® 48 real time PCR machine. Training of an operator takes one day. The cost of this suite of components is not available, but the company is targeting high-income country markets in addition to HBCs. These products are available and have CE-IVD markings and have received regulatory approval from Health Canada and JMHLW. While there are over 150 peer-reviewed articles describing the performance of earlier versions of the AMPLICOR® MTB assay, there is limited evaluation of the latest version. One recent study compared this assay with the Xpert® to detect MTB in pleural fluid.⁴⁴ The study found that the sensitivity of the COBAS® TaqMan® MTB Test was 29%, while only 3% with the Xpert® MTB/RIF. Using sputum as the test samples, several studies noted good performance with SSM + /C + specimens, but poorer performance with scanty or SSM-ve/C + .⁴⁵⁻⁴⁷

The Abbott Molecular RealTime MTB is an assay that can be integrated with a fully automated DNA extraction platform (the *m2000sp*) and performed by the *m2000rt*, a real time PCR detection platform (Figure 15). This assay is for the detection of MTBC from sputum or bronchoalveolar lavage and NALC prepared sediments (from sputum or bronchoalveolar lavage). Sample pretreatment involves inactivation reagent for a minimum of one hour. These DNA can be extracted manually or automatically via the *m2000sp*. Automated extraction of up to 94 samples and takes approximately seven hours to process and analyse 94 specimens. The analytical sensitivity is reported to be 17 cfu/mL. The clinical sensitivity in SSM + /C + samples is 97%, while SSM- /C + is 81% with a specificity 97%. A recent study using 214 clinical specimens gave similar data to the manufacturer's performance claims.⁴⁸ The assay has CE-IVD marking. Abbott Molecular has also developed an MDR TB companion assay that targets resistance to RIF and INH and has also recently received CE-IVD marking.

Figure 15. Abbott Molecular platforms for automated sample preparation (m2000sp, left) and real time PCR analysis (m2000rt, right) for MTBC and first-line DST



Source: Images reproduced with permission from Abbott GmbH & Co.

Training takes from three to four days and both platforms require an annual maintenance inspection. Abbott Molecular lists the price of the *m2000sp* and *m2000rt* technologies at US\$ 162 000 and US\$ 45 000, respectively, while the cost per test or other components is not known. As noted, the products have CE-IVD marks and are available. No further regulatory information is currently listed, while further peer-

reviewed publications demonstrating the performance of this assay are in preparation. The RealTime MTB is included in a Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund) framework agreement as part of an expanded assay menu (together with HIV viral load, HIV early infant diagnosis, hepatitis B virus, hepatitis C virus, human papilloma virus and Chlamydia/*N. gonorrhoea*) at the same low access price. Abbott Molecular offers planning for scale-up as well as assistance with scale-up, including training and performance monitoring based on country needs. Terms vary dependent upon volumes and specific needs.

Figure 16. Automated workflow to integrate the Hain Lifescience FluoroType® MTB assay using the GenoXtract® (left) and FluoroCycler® PCR machines (12- and 96-well, centre) utilizing LATE-PCR amplification, melt curve analysis with automatic scoring of MTBC test



Source: Images reproduced with permission from Hain Lifescience.

In addition to its current product range of LPAs for the detection of MTBC and DST, Hain Lifescience has developed a suite of technologies for moderate to high-throughput testing for the diagnosis of MTBC. Throughput can be in either a 96-well format for extraction and amplification (GenoXtract®96 with the FluoroCycler® 96) or in smaller sets of 12 using scaled instruments (GenoXtract®12 with the FluoroCycler® 12). The company offers two kits for nucleic acid extraction, the GenoLyse® and FluoroLyse®, and the GXT. All kits can extract MTBC nucleic acids from most specimen types, but not whole blood. The GenoLyse® and FluoroLyse® kits are manual kits and are used for preparing nucleic acids for either LPAs or use with its MTB assay with amplification in the FluoroCycler®, a real time PCR platform (Figure 16). The FluoroLyse® GXT is the kit used in conjunction with the automated GenoXtract® platform. This platform uses a silica-coated magnetic bead-based purification strategy.

The FluoroType® MTB assay is used for the FluoroCycler® and amplifies MTBC DNA by PCR and detects amplification with molecular beacons and fluorescence-based end-point detection using melt curve analysis to add specificity. Scoring of the test data is automated via Fluoro-Software® IVD hosted on a computer (included with the product). Using the smaller, 12-specimen system, Hain Lifescience estimates one complete test cycle can be completed in three hours. To improve upon data tracking, a barcode reader is supplied with the FluoroCycler® to reduce data entry errors during the specimen entering the test regime. Depending on the instrumentation used, from one to several hundred specimens per day may be tested. The analytical sensitivity of the MTB assay is 15 cfu/mL (BCG). The clinical performance of the MTB assay includes 99% specificity for MTBC and in SSM + /C+ samples the sensitivity is 100% and 90.2% reported for SSM-/C+ The products are available and annual maintenance is necessary. Some extra equipment is necessary, including a water bath, pipettors, pipettor tips and 1.5 mL screw cap tubes. Training for using the products takes only two days. All products have CE-IVD marking. Cost for each component is not available, but the FluoroCycler® 12, including computer software and a barcode scanner, is offered at €4650. To date, there are two studies that have evaluated the performance of the FluoroType® MTB assay.^{49,50}

Hain Lifescience is also in the late development of the FluoroType® MDRTB, a multiplexed assay to not only detect MTBC, but to also interrogate for RIF and INH resistance. This assay can use either manual or automated extraction and uses the FluoroCycler® 96, which requires a heat sealer for the 96 plate. The

company has an exclusive license with the Wangh Laboratory at Brandeis University (USA) to access its linear-after-the-exponential (LATE) PCR and “lights on/light off” probe technology that permits the accurate identification of mutations.^{51,52} LATE PCR is an asymmetric PCR method that selectively biases amplification of the strand that complements the detection probes. The “lights on/light off” probe technology uses molecular beacon probes with both fluorophore and quencher moieties at either end of the probe and when the probe binds to its target spatially separation of each allow fluorescence when excited by light of the appropriate wavelength. This is “lights on”. The “lights off” describes when a quencher-only labelled probe binds to a sequence adjacent to a “lights on” probe binding site. The positioning of a “lights off” quencher adjacent to the “lights on” fluorophore creates a sequence unique fluorescent fingerprint when a high-resolution melt curve is applied after LATE-PCR. Unique fingerprints are generated for each allelic variant of drug resistance mutations. The RIF assay targets mutant alleles in the *rpoB* gene, while INH resistance focused on *katG* and the promoter region of *inhA*. The throughput of this system is identical to the Fluorotype® MTB assay protocol depending on level of automation used. Performance data are not yet available for this test. The FluoroCycler® 96 is intended for this product and targeted pricing for this is €24 500 and the plate sealer is listed at €3898.

Figure 17. BD MAX™ platform, with a new multiplexed TB/DST assay under development



Source: Image courtesy of Becton Dickinson. Reprinted with permission.

Becton Dickinson is now developing a multiplexed real time PCR assay for the detection of MTBC and a concurrent assay to genotype of RIF and INH resistance using its BD MAX™ platform. Whereas other technologies use either manual or automated sample preparation before transfer for amplification and detection on a separate platform, the BD MAX™ is a fully integrated, single platform. Up to 24 sputum specimens can be tested in a single run and the company estimates that throughput could be as high as 72 in an 8-hour working day. As this product is in development, there are no performance data available yet. Equipment pricing also has to be established and regulatory approvals will be sought once the product is readied for market release. Becton Dickinson anticipates the release date to be in Q3 2017. It anticipates offering the product to both high-income and HBC markets.

Figure 18. The QIAxcel system for automated screening and scoring of MIRU-VNTR analysis of MTBC isolates



Source: Image reproduced with permission from Qiagen.

A new product is being offered by Qiagen for the high-throughput genotyping of MTBC strains via the QIAxcel automated genotyping system (Figure 18). This system fully automates sensitive, high-resolution capillary electrophoresis of up to 96 samples per run. Ready-to-run gel cartridges allow 96 samples to be analysed with a minimum of hands-on interaction, reducing handling errors and eliminating the need for gel preparation that is used with conventional testing. While not a diagnostic test, genotyping MTBC is essential for epidemiological purposes to investigate the prevalence or introduction of specific genotypes and is an approach commonly used in reference laboratories for molecular epidemiology. The tool uses capillary electrophoresis to size PCR amplicons as compared to DNA size standards.

The method targets variably sized regions of the MTBC genome using a methodology known as MIRU-VNTR. MTBC genomic DNA, resulting from culture-derived clonal isolates, is used. The DNA is first extracted and then specific primers are used to PCR amplify genomic targets from the MIRU-VNTR. Up to 24 loci and 4 hyper variable loci are recommended for use by European and American reference laboratories for accurate genotyping of MTBC.⁵³ The QIAxcel ScreenGel software ensures automated and convenient analysis that includes a report with the genotype and data documentation. Throughput depends on the number of loci analysed, for example, 174 samples in eight hours with 7 isolates using all 24 loci or 11 isolates using only 15 loci. A robotic loading system can increase throughput to 20 x 96 well plates in 24 hours. Training takes less than one day and annual maintenance is recommended. Qiagen is marketing this tool to HBCs and cost will vary depending on the country. Studies in two peer-reviewed articles have demonstrated the use of the technology.^{54,55} Qiagen also notes that the same platform is being used in the development of a high-throughput assay to screen for RIF and INH resistance alleles with completion scheduled in Q2 2016.

Tosoh Bioscience (Japan) offers the TRCRapid® M.TB assay that amplifies MTBC RNA via its proprietary amplification method, transcription-reverse transcription concerted (TRC) reaction.⁵⁶ The assay targets MTBC 16S ribosomal RNA that is multiplexed with an internal control reaction to indicate the presence of inhibitors to amplification. The reaction and data analysis are performed in the TRCRapid®-160 analyzer with the time to result from starting sample preparation as low as one hour, depending on the RNA extraction method used. There is some peer-reviewed literature describing the performance of this assay with one reporting clinical sensitivity of 90.7% with SSM+/C+ and 44.8% with SSM-/C+.⁵⁶⁻⁶⁰ The product has been approved by JMHLW. Unfortunately, the authors of this landscape report are unable to currently provide a more informed description of this product, its specifications and approximate cost. Tosoh Bioscience has a global network of offices and so it is assumed that product distribution is intended for many of the global markets.

NanoBioSys Inc. (Republic of Korea) is reported as developing technologies for rapid batched preparation and analysis of specimens using microfluidic chips for sample processing and for DNA amplification.⁴³ The semi-automated sample extraction platform, the UltraFast LabChip Sample Prep G2 test, can process up to 12 samples simultaneously in only 15 minutes. For amplification and detection, a microfluidic chip, the LabChip G2-3, analyses up to 10 samples via real-time PCR in 30 minutes. Reaction mixture preparation and sample addition is manual. The current availability of this technology is unknown, but data from a small clinical evaluation study were recently presented in 2014.⁶¹ Via independent financial reports, it would appear that NanoBioSys Inc. is currently focused on other areas of diagnostics. The authors cannot confirm the company's interest in marketing its MTB assay.

Conclusion: This section shows an increase in interest from established diagnostic developers to create platforms that can meet mid- to high-volume-throughput via semi- or fully automated systems. Many of the developers are also investing in integrated or reflexive assays to first-line drugs and this will add greater utility to testing and potentially improving clinical management and containment of MDR TB. There is currently limited evidence regarding the performance of these platforms in country settings as the assays have only been recently developed. While the hardware for these technologies is typically expensive, in some cases it is already available for other diagnostic tests and, therefore, the production and robustness of the core technologies are established and present limited risk in terms of higher failure rates due to early platform designs. Furthermore, since these are products from large and established diagnostic companies, they have global distribution and maintenance services being offered in many HBCs. One area that needs greater clarity is how these systems may be implemented into a test regime. For example, WHO does not offer guidelines on the application of automated NAAT systems into a TB programme. None has received WHO endorsement, but many of the tests on offer have CE-IVD marking. Procurement may be available for some systems such as those offered by Abbott Molecular via recent agreements with the Global Fund.

Autonomous MTB NAAT assays: The last part of this section focuses on MTB NAAT assays that can be used with a variety of real time PCR machines for the diagnosis of MTB infection and/or for DST. The assays are targeted to upper-tier laboratories as complex equipment is used, the reagents are not cold chain independent, and assay setup is manual and skilled personnel are required to perform testing. Many companies offer such kits, typically for real time PCR and many have CE-IVD marking. However, there is no strong evidence base or peer-reviewed publications describing their performance. The two groups of products listed here have additional regulatory approval other than the CE-IVD mark.

The first set of products is from Seegene (Republic of Korea), which offers highly multiplexed NAAT assays around its key technologies based on unique primer and probe design or function. Its technology expands the colour range of detection to 10 channels using its MuDT software to analyse data and generate results from its assays. Currently, they recommend the Bio-Rad Laboratories (USA) CFX96 real time PCR platform to perform testing. Sample preparation is not supplied by the company and so a different product must be used to first prepare MTB DNA for real time PCR analysis. The Anyplex™ series of assays 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 for INH and RIF resistance alleles in addition to a further 13 alleles associated with XDR (7 alleles for FLQ and 6 for injectable drugs).

The Seegene proprietary Dual Primed Oligonucleotide (DPO™) primer design permits highly multiplexed PCR.⁶²⁻⁶⁴ The primary MTB sample can be used to diagnose MTBC and genotype MDR and then reflex tested 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. Of this suite of assays, the Anyplex™ MTB/NTM has been assessed via a prospective evaluation on existing DNA extracted from clinical specimens. The authors noted that the assay had a very similar performance (96.4%) to the Hain LifeScience MTB-DRplus v1.0 assay with sensitivity/specificity for MTBC and NTM of 100%/96% and 100%/97%, respectively.⁶⁵ A recent study compared the performance of the Anyplex™ MTB/NTM assay for RIF and INH resistance genotyping with the Xpert® MTB/RIF and the MTBDRplus assays using culture isolates.⁶⁶ All assays had similar sensitivity (59/61; 96.7%) and specificity (53/54; 98.1%) for RIF resistance genotyping. With INH resistance, the authors noted that the assay had a poor specificity of 82.4% as compared to DNA

sequencing as the gold standard. Four other recent evaluations have also been performed, including a multicentre evaluation,⁶⁷ investigating MTB detection from paraffin embedded tissue,⁶⁸ DST performance⁶⁵ and for MTBC viability.⁶⁹ The tests are currently CE-IVD marked and have regulatory approval from the Republic of Korea Ministry of Food and Drug Safety. Its pricing is available through the distributors. Seegene currently licenses DPO™ to QuantuMDx (UK), where product development will be discussed in POC MTB assay development.

The second suite of products is offered by Xiamen Zeesan Biotech Co. Ltd (China). It has developed the MeltPro® Drug-Resistant TB Testing Kits, real time PCR assays to detect resistance alleles to RIF and INH and is developing further assays for resistance to ethambutol (EMB), streptomycin (STR) and fluoroquinolones (FLQs). The first product has an analytical sensitivity of 100% at 10⁴ cfu/mL and specificity at 100%. The clinical data include sensitivities of 95.8% for RIF and 90.2% for INH; the specificities of 96.0% for RIF and 96.40% for INH. The time to result is under four hours and manual set means a skilled operator is required. The MeltPro® Drug-Resistant TB RIF and Drug-Resistant INH kits have received CFDA regulatory approval and the second-line drug assay is anticipated for approval in Q4 2015. Two studies have reported on the performance of the INH and STR kits.^{70,71} The first market for these products is China.

Conclusion: The use of autonomous or equipment-agnostic assays permits greater application of existing equipment within a laboratory, permitting cost savings as no purchase of new hardware is required. The assays still require highly skilled staff and dedicated spaces to ensure the correct operation of testing and to limit contamination. The application of these tests as a reflex assay may save time in the identification of drug resistance. There are a limited amount of data on test performance and there are no guidelines as to how to best incorporate this type of test into a diagnostic programme.

LPAs: Two LPAs were the first NAAT products to be endorsed by WHO in May 2008 for the diagnosis of MTBC and also genotyping for MDR.⁷² These were the GenoType® MTBDRplus v1.0 from Hain LifeScience and the INNO-LipA Rif.TB from FujiRebio Europe (formerly Innogenetics; the Netherlands). LPAs or reverse blot hybridization assays are a relatively low-cost tool and have been developed for a variety of areas around MTB diagnostics, including MTBC-specific assays, speciation of NTMs and assays that genotype first- or second-line drug resistance alleles (Table 2). While the technology is relatively simple, the assays involve diligent processing steps to prevent contamination events. For these and other reasons, the test is used only in upper- and middle-tier facilities. It is not typically used at the microscopy centre level and LPAs are often used in conjunction with initial culture isolates to rapidly screen clones for MDR TB and XDR TB.⁷³ An LPA can more rapidly inform on drug-resistant MTB than phenotypic culture-based DST methods that take months from receipt of the initial sputum specimen for diagnostic testing. All LPAs can use DNA from culture isolates, some can use SSM+ sputum and two products are claimed for use with SSM-/C+ samples via improved PCR reagent design.

DNA extraction can be manual or automated and the DNA is amplified by asymmetric PCR to create a population of biotin-labelled single-stranded DNA amplicons. Oligonucleotide capture probes with sequences specific to the target amplicons are printed as an array of stripes on a nitrocellulose strip. The amplification mixture is then exposed to a strip under conditions to optimize the specific hybridization of amplicons to their sequence match probe printed on the nitrocellulose. Detection of hybridization of amplicons to the probe stripe is achieved via a streptavidin-linked enzyme that creates a colorimetric reaction visualized as defined stripes. The developed LPAs are read manually with comparison to a printed score chart or can be scanned and automatically analysed by attendant software. Workflow can be increased by using automated workstations for the hybridization/wash component and analysis of developed strips. The use of LPAs needs strict quality control procedures to ensure controlled manipulation of the test components. The methodology requires open tubes containing amplified DNA, which can contaminate the workspace and then compromise all future test data. The automated workstations alleviate this to some degree by providing controlled conditions that better contain reaction mixtures.

A comprehensive list of LPA products was first included in the 2014 landscape report and no new products have been identified other than Hain LifeScience reporting a new iteration of its second-line drug resistance assay, the GenoType® MTBDRsl v2.0. This test has increased sensitivity via an improved PCR

methodology and may be used on SSM-/C+ sputum. The original version of this assay was presented for STAG-TB evidence-based review in 2013 when it was noted that the test was effective in identifying second-line drug resistance, but it could not definitively rule out drug resistance. Rather, it could be used to rule in drug resistance. A Cochrane review on the pooled performance of MTBDRsl v1.0 had a similar conclusion where a positive MTBDRsl v1.0 result for FLQ resistance, kanamycin (KAN)/STR resistance or XDR TB can be treated with confidence, whereas as with negative tests a more sensitive DST method (e.g. culture) could be used to confirm XDR negatives samples.⁷⁴ The MTBDRsl v2.0 has a total of 27 probes for the detection of resistance to second line drugs and includes two new target genetic regions to improve detection of KAN and FLQ resistance via the *eis* promoter region (in addition to *rrs*) and the *gyrB* quinolone resistance determining region (QRDR) (in addition to *gyrA*) respectively.⁷⁵ The EMB (*embB*) target region has been removed from this assay.

A recent evaluation of the REBA MTB-XDR LPA (YD Diagnostics, Korea) yielded similar findings in that the assay could be used to rapidly rule in cases of XDR but not rule out XDR cases.⁷⁶

Recently, two different groups in the Congo and the Democratic Republic of Congo reported cases of false-positive FLQ resistance identified when using the MTBDRsl v1.0. A silent double mutation in the *gyrA* gene (80A and 90A) does not bind to the wild type probe and, therefore, FLQ resistance assumed as the wild type allele is not detected. This was not due to a poor-quality test as Hain Lifescience is regarded as a supplier of quality products for MTB, but rather these events highlight the complexity faced in accurately discriminating allelic variation using a relatively simple test that can detect, but cannot precisely discriminate exact allelic variance like DNA sequencing methods.

Since the initial endorsement of two LPAs in 2008 there are number of manufacturers making LPAs to detect MTBC and various drug resistances (Table 2). To assess the performance of the newer LPA designs, FIND has been performing non-inferiority studies with two LPAs: the NTM+MDRTB Detection Kit 2 (NIPRO Co.); and the GenoType® MTBDRplus v2.0 assay (Hain Lifescience). Their performance is being assessed against the original GenoType® MTBDRplus v1.0 assay endorsed by WHO. The studies have entered the second phase at three WHO Supranational Reference Laboratories (Germany, South Africa and Uganda) that have access to sputum samples with significant rates of drug resistance. In phase 1, completed in November 2013, testing of 600 strains was completed by all sites and the interim report submitted to WHO. In phase 2, completed in 2014, testing of 900 sputum samples was completed in 2014. A final report on test performance was expected to be submitted to WHO in June 2015 for STAG-TB review. The performance of the MTBDRplus v2.0 assay was recently assessed with other genotyping assays using RIF- and INH-resistant culture isolates.⁶⁶ The assay had similar sensitivity (59/61; 96.7%) and specificity (53/54; 98.1%) for RIF and the authors noted that the MTBDRplus v2.0 assay had the best overall performance for MDR TB. A recent study in Thailand noted good performance of the NTM/MDR TB LPA from Nipro Co. (Japan) for the rapid and accurate detection of MDR TB on SSM-/C+ specimens.⁷⁷

Table 2. 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
FujiRebio Europe	INNO-LiPA MYCOBACTERIA v2.0	Y/Y/Y	No	Y	Auto-LiPA 48	N/A	N/A
FujiRebio 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 v2.0	Y/Y/N	RIF/INH	Y	GT-Blot 48	GenoScan®	2012
Hain Lifescience	GenoType® MTBDRs/ v1.0	Y/Y/N	FLQ/ AMG/ ETB	Y	GT-Blot 48	GenoScan®	2009
Hain Lifescience	GenoType® MTBDRs/ v2.0	Y/Y/N	FLQ/ AMG	Y	GT-Blot 48	GenoScan®	2012
Hain Lifescience	GenoType® Mycobacterium CM	Y/Y/Y	No	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

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

Conclusion: LPAs are rapid and sensitive tests when using either TB culture or SSM positive specimens. Many offer the diagnosis of mycobacteria in addition to MTBC and genotype resistance to first-line drugs in a single test. The assays can interrogate a large number of targets within a single assay and are comparatively low cost as compared to culture-based DST. Newer tests are available that can expand the range of genotyping for other first- and second-line drugs. The tests have relatively low instrumentation costs and can use generic equipment (e.g. thermocyclers) or can be applied to semi-automated systems for higher throughput in addition to automatic scoring and archiving of test results. The tests and their application are relatively well understood and there are WHO guidelines regarding their implementation within country programmes.⁷⁸ 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. In some instances not all resistance genotypes can be accurately identified for many drugs – for example, pyrazinamide (PZA) – and conversely, as shown with *gyrA*, silent mutations in drug-sensitive strains can be misclassified as drug resistant leading to unnecessary treatment options.

Microarray-based platforms: A microarray leverages off the same basic principles as LPAs and can be perhaps considered as a miniaturized LPA, but with some significant advantages. Rather than printing a series of oligonucleotide capture probe stripes onto a nitrocellulose strip, the probes used in microarray are printed as clearly defined spots on a surface and so, by virtue of their smaller size (typically > 200 µm), many more spots can be printed in a very small area to interrogate amplified target DNA. For example, several spots (typically three) of the same probe are printed per array to add confidence to scoring test data and more probes can be added to an array; either to add greater stringency to allelic identification of drug resistance or add more genotypic drug susceptibility tests onto a single array. Therefore, microarrays potentially offer greater discriminatory power to a wider variety of drug resistance markers in a single-test format.

Typically, culture isolates or SSM+ specimens can be used to provide sufficient DNA for amplification via asymmetric PCR to create labelled complementary strands of amplified and labelled target DNA that can bind to the arrayed probes. Due to the small size of each capture spot, microarrays are normally read with an instrument, and detection is often by measuring the fluorescence intensity of each spot versus controls and the data then scored *in silico*. The tests when used in an open platform are complicated to perform and require sensitive and complex equipment. Some developers are creating partially or fully integrated microarray products that have reduced user input to allow their use outside of national reference laboratories.

AutoGenomics (USA), CapitalBio (China), Akonni Biosystems (USA) and Veredus Laboratories (Singapore) offer marketed products in this space. iCubate (USA) no longer provides MTB assays for use with its platform, but the platform is available as an open system where groups may create their own assay designs.

The AutoGenomics INFINITI® PLUS Analyzer platform is US FDA approved. A higher throughput system is also available, the INFINITI® High Throughput System (HTS). AutoGenomics offers two MTB microarrays, the MDR-TB BioFilmChip® for MTBC and first-line DST (RIF, INH and PZA) and a second array for MTBC diagnosis, the OCTA, in which eight samples per array are processed to increase throughput. Manual extraction and PCR is required for this instrument, while hybridization, washing, reading and data analysis are all automated. This method can process 48 samples in 3.5 hours using the INFINITI® and MDR-TB BioFilmChip®. AutoGenomics also offers an amplification and labelling kit, the MDR-TB Amp Mix and the Intellipac® reagents for the array preparation that are loaded into the INFINITI®. There are no evaluation data available for these products other than from the manufacturer and no other national regulatory approvals other than for the instrument. Available costs associated with this technology remain at US\$ 95 for the MTBC and US\$ 320 for the OCTA BioFilmChip® and the analyzer at US\$ 227 445.

CapitalBio offers two microarray products that have been approved for use by the CFDA. One, the Mycobacterium Identification Array Kit, is for MTBC diagnostic and NTM speciation. The *M. Tuberculosis* Drug Resistance Detection Array Kit is for MTBC diagnosis and genotyping RIF and INH resistance. The kits include reagents for PCR labelling of extracted DNA. The use of these tests requires manual processing and

key equipment such as a PCR machine, hybridization and wash chambers, and a microarray reader. The processing time to result is six hours and test throughput is unknown, but likely to be very limited due to manual operation. The specimen types noted for use are SSM + samples or culture isolates.⁷⁹ Evaluation data are limited to three peer-reviewed reports.⁷⁹⁻⁸¹ One study compared performance of the *M. Tuberculosis* Drug Resistance Detection Array Kit to culture methods and noted high, but variable performance using culture isolates as compared to sputum samples for RIF resistance (91.8% for isolates and 94.6% for sputum) and INH resistance (70.2% for isolates and 78.1% for sputum).⁸⁰ These products have CE-IVD marking and are available with CapitalBio looking at global market supply. The cost of the first assay is US\$ 25 per array with the MDR TB assay listed at US\$ 21.

The first two microarrays listed above are expensive and/or complex to perform, requiring highly skilled users familiar with molecular techniques, which limits their immediate application to MTB diagnosis and DST in terms of proving timely information for patient treatment. Other companies are developing microarray systems that may be applied in intermediate facilities based upon their cost and more integrated systems. Their utility is not only in diagnosing or confirming MTB infection, but also in accurately identifying MDR strains. Akonni Biosystems offers a range of products, including microarray assays and instrumentation for processing, reading and analysis and aims to simplify microarray workflow to permit the routine use of MDR TB microarray-based diagnostics in intermediate facilities.⁸²

The TruArray[®] MDR-TB microarray can identify MTB and *M. avium* and detect resistance alleles 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 hybridization reaction mixture and has relatively short hybridization and wash times to ease use and enable higher throughput. The arrays are read on the TruDiagnosis[®] System. Akonni Biosystems is currently developing simpler array protocols and has developed a field portable device to analyse the labelled arrays (the Dx2100 portable imager). A recent study noted a workflow of eight hours to process 24 samples with a single laboratorian. Akonni Biosystems is a sub-grantee of a current project funded by the United States National Institutes of Health (NIH) (US\$ 29 million) and, therefore, has an opportunity to complete development. Currently, the company aims to reduce the hybridization time from three hours to only 15 minutes and to have cold chain independent reagents. Results will be scored automatically. A new array is undergoing development where screening for alleles conferring EMB and STR is included with an array that uses 96 unique probes for 39 drug-resistant mutations across 5 genes.^{82,83} Akonni Biosystems offers a range of reagents and equipment for all of the steps for use with its microarrays, including DNA extraction, PCR, hybridization, array reading and software for analysing test data. These are not CE-IVD marked and are for research use only. Further information on the company's product development is not currently available.

Figure 19. VerePLEX™ Biosystem and VereMTB™ Detection Kit from Veredus Laboratories: 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. For analysis each chip is manually inserted into the reader that interrogates the array (right).

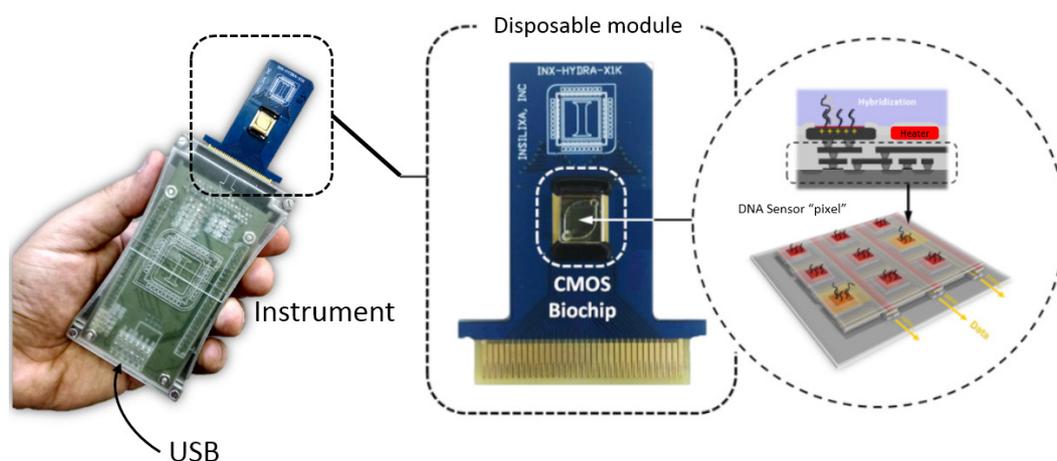
Source: Image reproduced with permission from Veredus Laboratories.

Veredus Laboratories offers the VereMTB™ Detection Kit, which is currently listed for research only, but the developments have created the most integrated array test system that is close to market (Figure 19). The company has partnered with ST Microelectronics (Switzerland), one of the world's largest semiconductor and microelectronics manufacturers for the development of the array chip and associated technology. What makes this array unique from the others listed is that the extracted DNA is added to the chip and all other processes are performed on the chip with no user input other than moving the chip from the PCR/hybridization module to the array reader to generate test data. The PCR chamber is split into two reactors to increase the ability to multiplex assays. The assay is intended for use with either culture or SSM+ samples.

The array addresses MTBC detection via *IS6110*, and, by interrogating the 16S RNA gene, the array can confirm MTBC and speciate a further eight NTMs (*M. avium*, *M. simiae*, *M. intracellulare*, *M. Kansasii*, *M. abscessus*, *M. scrofulaceum*, *M. chelonae*, *M. xenopi*).⁸⁴ In addition to MTBC/NTM speciation, the same array has probes that genotype alleles associated with either RIF or INH resistance. In total, the array has 500 probes. An additional spoligotyping array has also been noted.⁸⁴ The VereMTB™ chips are processed on the VerePLEX™ Biosystem and analysed with VereID software. After the manual addition of 23 µL of extracted sample, the test result is produced after only two hours. The system is semi-automated and modular with up to five samples simultaneously processed before individually analyzing on the reader. An analytical limit of detection of 100 genome equivalents was recently reported.⁸⁴ The platform and assay were released in 2012 as research use only and the company identified China, Australia, South-East Asia and the Russian Federation as its primary potential markets. Currently, Veredus Laboratories are looking to build a new array that can genotype resistance to EMB and also for second-line drugs, including FLQ, and injectables. One group has recently published performance data comparing the performance of the VerePLEX system with phenotypic DST, the MTBDR_{plus} (Hain) and DNA sequencing to assess the detection of MTBC and associated drug resistance alleles in 80 smear +ve clinical isolates, 91 clinical isolates and 116 MTBC culture -ve specimens.⁸⁵ The data was promising with the array having similar performance to the MTBDR_{plus} assay when compared to the gold standard phenotypic DDST. Currently CE-IVD marking is targeted for Q2 2016 and the Veredus recently noted that they are currently not considering US FDA approval. Current pricing for LMICs is unknown but pricing in the range of US\$ 100 has been proposed for high-income countries.

The final product to be noted in this section is the HYDRA 1K development from Stanford University (USA) and Insilixa Inc. (USA) (Figure 20). This product utilizes advances in microelectronics and employs complementary metal-oxide semiconductor (CMOS) technology for digital imaging and uses heating at each individual spot on the array. The goal of this technology is to create a fully integrated microarray technology that can be used in microscopy centres or scaled for use in upper-tier facilities. The sample preparation module is in development and details have not been shared. The developers note that the array can host up to 1024 probes. One aspect that makes this technology very novel is that PCR is performed in the array area so binding of the labelled amplicon to the fixed probes can be measured in real time with amplification, hybridization and detection occurring in parallel rather than as distinct processes, saving significant time and requiring no user input or handling.

Figure 20. Hydra 1K hand-held platform (left) and chip (centre)



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

Source: Images reproduced with permission from Insilixa Inc.

A further highlight of this technology is the application of CMOS technology that allows melt curve analysis of amplicon binding at each probe spot on the array in parallel. This is a highly significant component as it notes the binding of every amplicon to its probe during PCR and also the thermal characteristics of each amplicon across a temperature range during the melt curve analysis step. In principle, this can provide additional information about the bound amplicons and create fingerprints that may help further discriminate silent from active mutations, something current microarrays cannot effectively do. The larger size of the array may enable new probes to be added and it is not constrained to a particular target region as with PCR genotyping methods, for example, the inability of the Xpert® MTB/RIF assay to detect *rpoB* I491F due to this allele being outside of the PCR amplicon generated by this assay.⁸⁶

The current goal is to develop this technology to diagnose active PTB for the purpose of treatment initiation; diagnosis of MDR TB and XDR TB by genotyping of mutations that confer resistance to RIF, INH, FLQ, PZA, aminoglycoside (AMG) and injectable drugs. All reagents will be housed on the cartridge and sputum liquefaction/inactivation and loading steps performed by the user. The test will be used in a hand-held device or in a modular platform that will perform all other tasks. Further test attributes are being designed to meet the TTP for a low-cost tool for use in microscopy centres.⁷ The developers recently received an NIH grant of US\$ 1.5 million to fund the development work. The group anticipates that product development will be complete by Q3 2017.

Conclusion: Microarrays are powerful tools for MTB diagnosis, speciation and with which to interrogate allelic variation for genotyping of drug resistance at a scale typically greater than LPAs. They offer a higher-quality test to LPA in terms of greater stringency in addressing probe detection and, due to the

potential number of probes that may be printed, offer the potential to screen greater numbers of targets, including NTMs or a broader range of drug resistance targets on a single array. The arrays are read by a scanner that makes result interpretation less compromised or subjective. The arrays in development aim to further simplify the complex methodology by integrating processing steps to give a faster time to result with reduced user time or input. The relatively high cost of microarrays and their platforms, however, is a barrier to uptake. Certain arrays are typically intended for reference facilities, and with the introduction of whole genome sequencing and/or pyrosequencing target genes in reference laboratories, their value may decrease. For the intermediate and lower-tier facilities, there is a clear need for rapid DST and diagnosis, and integrated arrays could be useful for rapid diagnosis and DST. By their nature of probe-based detection, the array formats can be quickly updated to accommodate new alleles for DST. As with the other NAAT-based tests, there has not been adequate evaluation of these technologies in their intended use settings and until this is done there cannot be effective guidelines on how they may best be implemented in TB control programmes.

Modular, cartridge-based, fully automated NAATs: An increasing number of developers are now designing modular or single cartridge-based NAATs platforms with which to diagnose infectious diseases. In terms of diagnostics for MTB, there are very few integrated and/or modular technologies with assays that target lower-income countries. Of those that do, only Cepheid Inc. and its Xpert® MTB/RIF assay (Figure 21) has made any significant advances as a more rapid and accurate test to replace SSM. A significant amount of data is available on the performance of this tool in settings all over the world.⁸⁷ In the most recent Cochrane review from 2014,⁸⁸ the authors identified several positive key findings, including that for suspected cases of active PTB (with or without HIV infection) the Xpert® MTB/RIF is sensitive and specific, substantially increases TB detection among culture-confirmed cases and, while less sensitive in SSM-/C+, still has added value in retesting SSM specimens. In terms of indicating RIF resistance, the Xpert® MTB/RIF provides accurate results and can allow rapid initiation of MDR TB treatment, pending results from conventional culture and DST.⁸⁸

Figure 21. GeneXpert® 4 platform with four independent modules for processing test cartridges (left) and the MTB/RIF cartridge (right)



Source: Images reproduced with permission from Cepheid Inc.

As the technological aspects of Xpert® MTB/RIF have been described in detail in many reviews and publications, it is assumed that the readership is generally familiar with this system. Briefly, the Xpert® MTB/RIF is a modular platform that drives a cartridge-based assay that includes sample preparation, semi-nested real time PCR amplification of *rpoB* with automatic scoring of data for MTB infection and genotypes common RIF resistance alleles. A sputum sample is diluted 1 : 2 with sample reagent buffer that inactivates and liquefies the sample. Then 2 mL of the sample is pipetted into a barcoded cartridge that is sealed, barcode scanned and subsequently placed into a module of an Xpert® machine. Thereafter, all processes are automated. The entire process takes approximately two hours and a typical 4-module Xpert®

platform can process 16 specimens in an 8-hour working day. A smaller version and several larger Xpert® platforms exist. The fully automated INFINITI® Xpert® platform has 80 modules and can process over 1000 samples in a 24-hour period. All test materials are stored in the MTB/RIF cartridge and the PCR reagents stabilized for storage at 2–28 °C for up to 18 months.

The most recent policy guidelines for the implementation of the Xpert® MTB/Rif for diagnosis of PTB and EPTB in adults and children were released by WHO in 2013.⁸⁹ Five key findings were summarized by the review group when using the Xpert® MTB/RIF to diagnose PTB and RIF resistance in adults and children:

1. Xpert® MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in adults suspected of having MDR TB or HIV-associated TB (strong recommendation, high-quality evidence).
2. Xpert® MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in children suspected of having MDR TB or HIV-associated TB (strong recommendation, very low-quality evidence).
3. Xpert® MTB/RIF may be used 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).
4. 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).
5. Xpert® MTB/RIF may be used as a follow-on test to microscopy in adults suspected of having TB, but not at risk of MDR TB or HIV-associated TB, especially when further testing of smear-negative specimens is necessary (conditional recommendation acknowledging resource implications, high-quality evidence).

Regarding the use of Xpert® MTB/RIF to diagnose EPTB and RIF resistance in adults and children, a further two recommendations were made:

1. Xpert® MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test for cerebral spinal fluid (CSF) specimens from patients suspected of having TB meningitis (strong recommendation given the urgency for rapid diagnosis, very low-quality evidence).
2. Xpert® MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture or histopathology) for testing specific nonrespiratory specimens (lymph nodes and other tissues) from patients suspected of having EPTB (conditional recommendation, very low-quality evidence).

WHO released guidelines in 2014 advising on implementation of this technology within TB programmes.⁹⁰ A more detailed description of market analysis and implementation to date is provided in section 5: Market landscape.

Use of Xpert® in microscopy centres: While Xpert® can be hosted in intermediate facilities, there have been concerns regarding the suitability of lower-tier laboratories to effectively host TB testing using Xpert® MTB/RIF-based screening due to infrastructure requirements and logistical constraints in some peripheral facilities.⁵ A recent multicentre demonstration study from India investigated the effects on the operational feasibility of introducing Xpert® MTB/RIF within existing microscopy centres functioning under the Revised National TB Control Programme (RNTCP) of India. The study was aimed at measuring the performance of Xpert® MTB/RIF under routine conditions in existing microscopy centres, to assess test failure rates and the impact of key implementation factors on the assay in decentralized settings.⁹¹ All settings were adapted as necessary to include a 2-hour universal power supply, solar power to augment electricity

supply and an air-conditioner in the room hosting the Xpert® platform. The failure modes were assessed in the screening of 40 035 suspected cases with 39 680 (92.8%) receiving a valid result upon the first test. The greatest areas of failure were with inadequate sample treatment or the equipment at 3.9%. The authors noted concerns where 32% of Xpert® instruments required modular replacement and that inter-lot cartridge performance variation was significant. Dust blocking the cooling fan on Xpert® was noted as a common issue, but with the comment that preventive maintenance via more regular replacement of filters helped reduce failure rates. Key findings were concluded with the authors noting that minimal infrastructure modifications were required for Xpert® installation and that concerns about adequacy of human resources under public sector facilities and temperature extremes were unfounded.

A more recent study investigated the effects on MTB diagnosis and treatment of active TB cases when diagnosis was offered by Xpert® MTB/RIF in a rural area of South Africa as opposed to being shipped to a central facility.⁹² Patients in rural areas have very limited access to health care and laboratory testing and, therefore, can experience longer delays before diagnosis and treatment of TB.⁹³ The study indicated that the use of single module Xpert® instruments in rural settings resulted in more rapid diagnosis and reduced the time to anti-TB treatment after producing a sputum sample. Many patients in the rural arm of the study received a same-day result and were placed on treatment, but overall the numbers of new MTB cases diagnosed did not significantly vary at each laboratory tier.⁹² The Omni is a new platform in development by Cepheid is being designed to specifically address many of the logistical constraints faced in microscopy centres, whilst using cartridges already in use for the current GeneXpert® platforms (see later text for a complete description).

Cepheid Inc. is currently developing two new MTB assays to be hosted on the Xpert® platform: the Xpert® MTB/RIF Ultra and XDR assays. Both are being developed in the laboratory of Professor David Alland from Rutgers New Jersey Medical School (USA) who led the development of the MTB/RIF assay. The Ultra is designed to have an LOD similar to that of culture to more accurately diagnose PTB from paucibacillary specimens. In order to increase sensitivity, a series of designs were re-optimized for the PCR assay, cartridge and software engineering. For assay development, a new assay targeting MTBC specific regions, the insertion sequences IS6110 and IS1081, was developed.^{94,95} In many MTBC strains, these are typically multicopy and so present more target for PCR amplification.⁹⁶ The new assay also undergoes an initial round of nested PCR, rather than the hemi-nested method in the MTB/RIF. This further increases the amount of template DNA prior to the multiplexed diagnostic assay. Sloppy molecular beacons are used to detect MTBC via the two targets.⁹⁷ A process control using *B. globii* is still included to ensure appropriate test performance. An assay to target *rpoB* is also incorporated, but with four molecular beacons targeting the key RIF resistance alleles in the RIF resistance-determining region (RDRR) rather than the five used in the current MTB/RIF assay.

In the new test protocol, Xpert® employs a high-resolution temperature melt curve analysis on the PCR amplicons after completion of PCR amplification. Rather than comparing cycle threshold values from the five molecular probes in real time (as is performed with the current MTB/RIF assay), the high-resolution melt curve generates specific fluorometric fingerprints for each probe binding to its amplified complement and these may better discriminate allelic variance within each probe region. A further significant design change is that the Ultra cartridge uses double the volume of input sample material as compared to the MTB/RIF cartridge, with 50 µL rather than 25 µL. The LOD for MTBC using this format is claimed to be only 5 cfu when testing analytical sensitivity. The current Xpert® MTB/RIF assay has a sensitivity of ~ 150 cfu/mL sample. While more data are not available at this time, Cepheid Inc. notes that the targeted clinical sensitivity for SSM-/C+ specimens is > 90% with 100% specificity. The release date for the Ultra is anticipated in Q1 2016 and the cost is not yet known.

The XDR assay is designed to be a reflexive test when an Xpert® (or Ultra) MTB/RIF positive test indicates an infection with RIF-resistant MTBC. The XDR assay will further genotype common resistance alleles to INH, FLQ and AMG. Genotyping for INH resistance will add confidence to an RIF-based MDR TB test result and FLQ and AMG are important second-line drugs. The technical challenges of creating a multiplexed NAAT assay to precisely interrogate multiple and variant targets are being met by using novel large Stokes shift dyes that can permit more channels than currently applied for fluorescence detection without physi-

cally altering the excitation or detection channels already used in Xpert®.⁹⁸ In addition to these new dyes, sloppy molecular beacon probe designs are being developed to better discriminate the key mutations associated with INH, FLQ and AMG resistance. This assay will also use a high-resolution melt curve after the completion of PCR amplification to fingerprint the fluorescence profiles generated from each probe bound to its target DNA amplicon. In principle, each amplified resistance allele may create a unique melt curve profile that can be used to note drug resistance with a higher degree of confidence than measuring the delay (in real time) of a specific probe hybridizing to its target region. Cepheid Inc. anticipates the release of this product in 2017.

Modifications to Xpert® instrument to operate these new assay cartridges will need only software upgrades and the recalibration of the optics system. New machines are not required and thus these new assays can be introduced for use on the Xpert® platforms already implemented. There are no performance data available for the XDR assay. An evaluation of the XDR design is being led by FIND and the United States Critical Path to Tuberculosis Drug Regimens (CPTR). The study will assess the sensitivity and specificity of the investigational Xpert® XDR test for the detection of drug resistance, using phenotypic drug susceptibility testing, mycobacterial DNA sequencing and the Xpert® MTB/RIF test as the reference comparators.

Two other modular and integrated NAATs were identified in the 2014 landscape report: the Stat-Diagnostica (Spain) DiagCORE and the Enigma Diagnostics (UK) mini laboratory (ML) platforms. Stat-Diagnostica has noted that it currently is not developing its MDR TB assay. The Enigma Diagnostics ML platform has CE-IVD marking and the company is developing a real time PCR assay for MDR TB for integration into this platform. In Q4 2014, the company signed a joint venture agreement with Beijing Leadman Biochemistry with a US\$ 50 million investment. Presumably, this initiative is intended to introduce the ML technology into the Chinese diagnostics market. Currently, there is no further information around the progress of this effort and when the product may be available.

Semi-automated NAATs for use in peripheral microscopy centres: Moving NAAT testing from the higher-tier laboratory to the peripheral laboratory represents a variety of challenges in terms of sufficient infrastructure, robustness of equipment, reagent stability, limited user skills and a requirement for improved performance to that offered by SSM.^{37,99,100} Given the volume of tests currently performed via SSM, costs also must be similar to SSM and, therefore, developers are faced with challenges spanning from developing appropriate technology, understanding supply logistics and having sufficient access to a relatively undefined yet potentially large global market. There has been a focused effort to define many of the infrastructural and operational challenges presented in microscopy centres in low-income HBCs and, in addition, attempts to better estimate the value of replacing SSM with NAATs or other tests.^{5,6,9,10,37,100}

There is increasing interest from developers to create products that compete in this area and more products than ever are now in development in this space (see the Figure 13 NAAT product pipeline). The first wave of NAATs to compete with Xpert® MTB/RIF, the so-called “fast followers”, are now being validated, receiving national regulatory approval and entering their intended initial markets. These technologies typically use open or partially integrated systems in order to reduce equipment complexity, cost and/or increase the tolerance of technology to the adverse environmental extremes encountered in some facilities. The next generation of NAATs is now in development and anticipated to enter the market in one to four years, and these products typically offer a greater degree of integration than the first generation and offer fully automated processing from sample collection to test result. These technologies diagnose MTBC and in some instances drug resistance testing is offered in the same test or via a second reflexive test upon initial diagnosis of MTBC infection.

Commercially available products: Previous landscape reports have focused on four products: the Eiken (Japan) Loopamp™ MTBC assay; the Epistem (UK) Genedrive® MTB assay; the Molbio Diagnostics (India) Truelab™ RealTime micro PCR System; and the Ustar Biotechnologies (China) EasyNAT™ TB assay. Each of these products is addressed by alphabetical order (based on the company name).

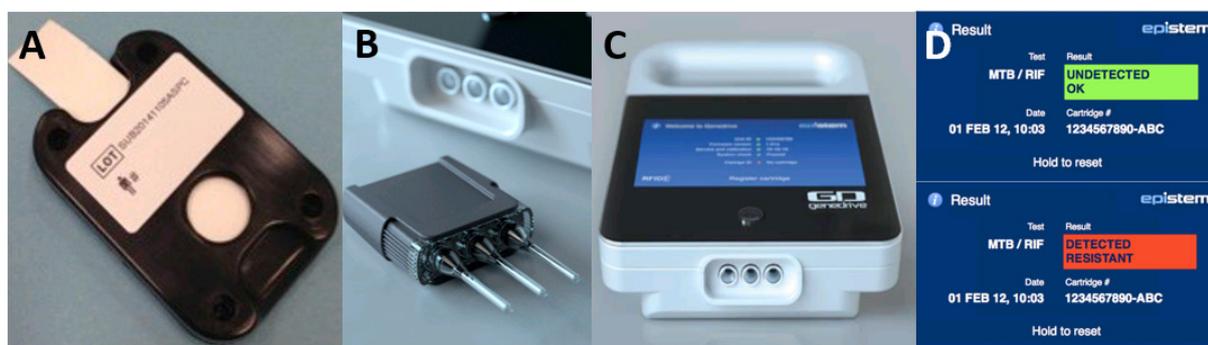
Eiken’s Loopamp™ MTB kit was commercially released in 2011 and uses loop-mediated amplification (LAMP) as the amplification technology. Eiken, with the assistance of FIND, has developed the assay

for use in low-resource settings. The system involves significant user input via sample processing, reaction setup and data interpretation. The assay uses 60 µL of raw sputum that is added to a lysis tube and subsequently liquefied and MTBC cells inactivated and lysed under a combination of heat (95 °C for five minutes) and basic conditions. The Loopamp LF-160, a mains electricity-powered instrument provides receptacles for the simultaneous heat treatment of eight samples. Neutralization reagents are then added to the treated sputum via an interlocking neutralization tube and the reaction contents mixed. The user completes the sample preparation components by locking an applicator nozzle to each purification tube. In this format, the liquefied contents can then be manually applied to the test reaction by squeezing the sides of the purification tube.

The LAMP assays are supplied as glassified reagents in each cap of a strip of eight 200 µL micro tubes. The reaction volume is not wholly critical and can operate with 25–35 µL of sample, with 30 µL being ideal. One strip of 6 test reactions or two strips with 14 reactions can be prepared in a batched test setup. The negative control and then positive control are added and the tubes inverted and gently mixed to re-suspend the LAMP reagents and mix the reaction contents. The LF-160 has a heat bar that incubates the samples at 65 °C before inactivating them at a higher temperature to stop the reaction. The heating processes are timed and an audible alarm notifies the user when heating steps are completed. The user then interprets each test reaction by exposing the completed reaction tubes to an LED. The use of calcein, a fluorescent green dye, permits the visible scoring of tubes where DNA has been amplified. By comparing each test reaction to the controls, the user addresses quality control of the assay and, in addition, can discriminate positive for negative reactions. With 14 samples being tested, it is estimated that the system with one user can process 56 samples in an 8-hour working day. The reagents are stabilized for storage at up to 30 °C for one year and the extraction reagents are stable for two years under the same conditions. User training takes two days.

While a variety of “homebrew” LAMP assays are described in peer-reviewed literature, there are only three studies that assess the clinical performance of this product for PTB.¹⁰¹⁻¹⁰³ Two involved small cohorts in Japan and a larger cohort was used in China with testing at two county-level facilities. Each study indicated better sensitivity with the Loopamp™ MTBC assay compared to SSM. Pooled data from a three-country demonstration of the assay were presented to the STAG-TB committee in 2013.¹⁰⁴ While not endorsed, it was noted that broader study populations were necessary, including greater geographic diversity and in areas of high- and low-HIV prevalence. To address this, Eiken and FIND worked with 14 countries representing global diversity in terms of ethnicity, geography, MTB and high or low HIV prevalence. The performance of the Loopamp™ MTBC Detection Kit was compared to the Xpert® and LED smear microscopy with liquid and solid culture as reference standards. This project is completed and the data have been collated. A WHO Expert Group reviewed the performance of LAMP in June 2015, and recommendations are pending. The current assay is CE-IVD marked and also has been approved by JMHLW. Eiken lists the cost of the LF-160 at US\$ 1000 and US\$ 6 per test.

Figure 22. Epistem Genedrive® Mycobacterium iD® assay



Notes: Paper lysis device v2.0 for rapid sample preparation (A); test cartridge with three reaction tubes for MTBC, RIF and control (B); Genedrive® instrument (C); test data output with a negative result (upper) and positive result (lower) (D).

Source: Images reproduced with permission from Epistem.

Epistem offers the Mycobacterium iD® Test-kit for use with its Genedrive® instrument (Figure 22). Currently, the test assays only for diagnosis of MTBC with a control reaction, but a third test to genotype MDR TB via RIF resistance is in the final phases of internal validation by the company. This assay can test one sputum sample at a time and requires some manual preparation of test material. Once the reagent cartridge is prepared and inserted into the machine, real time PCR analysis is performed and the results automatically scored after melt curve analysis in an approximately one-hour process. The cartridge assays screen for three individual components: an MTBC assay, an inhibitor control and a genotyping assay targeting *rpoB* via the RRDR.

Sample preparation is minimal where raw sputum is applied to a sandwiched paper pad and allowed to dry for 5–20 minutes. The wicking paper is removed from the device and a disposable punch removes a 1 mm disc of the capture/lysis paper and one disc is added per reaction tube within the disposable reaction cartridge. A further 20 µL of nuclease-free water is added to each reaction tube to rehydrate the dried PCR reactions and move the disc into the liquid volume; the lid is snapped on and a flick of the wrist pulls the liquid and disc to the bottom of each tube. The cartridge is placed in the Genedrive® instrument and the user starts the device via a touchscreen interface. Amplification and analysis is via real time PCR and after end-point analysis via melt curves, the results are scored without user input. One device could process eight specimens in a working day. Data capacity onboard the instrument allows for up to 1000 test runs to be stored. The test reagents are stable to 28 °C for one year. The analytical performance of the assay is claimed to be > 95% for both sensitivity and specificity and clinical performance of > 95% for SSM + / C + sputa. Clinical performance data are currently limited to a single study.¹⁰⁵ The assay showed 90.8% sensitivity and 100% specificity using contrived samples of sputum spiked with cultured MTB cells with sensitivity 100% if spiking with cfu/mL was > 1000 cells.

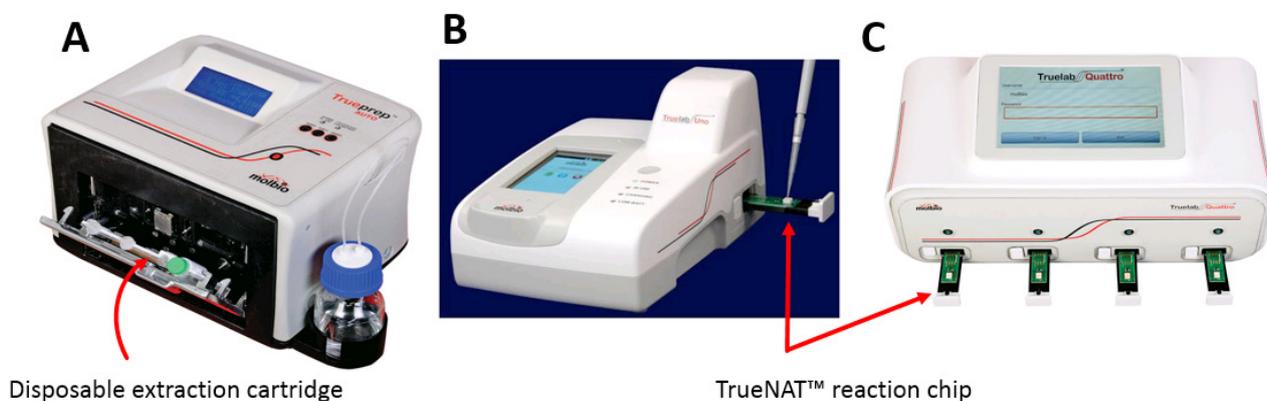
Epistem is currently revising its lysis device design based upon the lysis paper concept. The company noted that if a pelleted sputum sample is used with the lysis device, then sensitivity is further improved to detect SSM-/C + specimens. Epistem is working with FIND and other partners to validate this test. A non-inferiority study with this technology is being performed by FIND and performed by CPTR with comparison to SSM, Xpert® and culture.¹⁰⁶ The device is currently being evaluated on 500 participants and, in addition, the Liverpool School of Tropical Hygiene and Medicine is performing a further larger study (1400 participants) of this technology in Nigeria. Epistem had targeted India as the first major market to introduce the product and has been working with Xcelris Labs (India) as a partner in this process. The technology has the CE-IVD mark and in April 2015, the Drug Controller General of India (DCGI) issued a three-year import license to Xcelris Labs. This will enable Epistem and Xcelris Labs to start preparing for the launch of Genedrive® and the Mycobacterium iD® Test-kit into the Indian and Indian subcontinent markets.

In 2014, Epistem signed a collaborative funding agreement with the United Kingdom Global Health Investment Fund LLC and received a five-year convertible bond totalling US\$ 8 million (£4.7 million) with which to fund ongoing final development, external validation, manufacturing and marketing of this technology. As part of the collaborative funding agreement, Epistem has agreed to commit to global access with its technology and target other HBCs, including China and Africa. In terms of manufacturing, Epistem now has partners for both the cartridge and the Genedrive® instrument. The cost of the equipment and assay is still not known, but the company notes that it will be competitive to similar MTB diagnostic NAATs. The assay is not available for sale on the general market, but will soon be in use in India.

Molbio Diagnostics represents a joint venture between a large diagnostics manufacturer (Tulip Group; India) and a technology developer (Bigtec Labs; India). Molbio Diagnostics offers the Truelab™ RealTime micro PCR System consisting of a semi-automated DNA extraction device and a second instrument for DNA amplification and detection of MTBC (Figure 23). These products were first released in 2013. All equipment is powered via onboard rechargeable batteries for uninterrupted testing from six to eight hours. Sample preparation uses 0.5 mL of sputum that is first treated with 2.5 mL lysis reagent and applied to a tube in the Trueprep™ MAG Sample Prep device. This approach uses silica-coated magnetic beads to allow the capture, washing and elution of MTB DNA from the beads. This step is manual as the user must use pipettes to add and remove fluids during DNA purification. The instrument heats and mixes reagents per a programmed run protocol stored onboard the device and an alarm sounds when the user input is needed.

The company supplies a kit for performing the sample preparation and DNA extraction in addition to a set of precision pipettes for liquid transfer.

Figure 23. Molbio Diagnostics technology: current products and those in developments for NAAT-based detection of MTBC



Notes: Trueprep™ AUTO, an automated and cartridge-based sample preparation extraction tool (A); Truelab™ Uno and Truelab™ Quattro, single and 4x scaled test reactors for amplification and analysis of test data from the TrueNAT™ reaction chip (B, C).
Source: Images reproduced with permission from Molbio Diagnostics.

Amplification of MTBC DNA is performed via the TrueNAT™ reaction chip that houses lyophilized real time PCR reagents and is automatically processed via the Truelab™ UNO RealTime micro PCR System that measures fluorescence in two channels for MTBC detection and an internal control, introduced in the extraction step. An Android phone embedded in the Truelab™ UNO is the controller and processes the real time data to automatically generate a test result. Data entry and user inputs are via the touchscreen interface. The reaction chip requires 6 µL of extracted sample and a heat labile wax seals the PCR reaction upon initial heating to contain reagent evaporation and amplicon contamination of equipment. Time to result in is under one hour. The LOD per assay is reported by the company to be 10 genome equivalents per test. In terms of clinical performance, Molbio Diagnostics claims a sensitivity of 99.6% with SSM + / C + samples and 75.6% with SSM-/C + . A recent study compared the performance of the TrueNAT™ MTB to the Xpert® MTB/RIF on 274 patient specimens using culture as the reference standard.¹⁰⁷ Both assays had similar sensitivity on SSM + ve samples, while with SSM-/C + samples the sensitivity of the TrueNAT™ was reported to be 86.2% as compared to 90.1% for the Xpert® MTB/RIF.

The implementation of an Android phone as a controller also allows test data export for automated disease surveillance and device/test/user monitoring. In addition, other functions offered include data on GPS/GPRS, GSM, Wi-Fi and Bluetooth enabled. A Bluetooth, battery-powered printer can also provide paper copies of test results if required. Up to 5000 test results can be stored on the device and data entry and operation is via the touchscreen interface of the phone. Reagents are stable for 12 months at 30 °C and Molbio Diagnostics notes stability for three months at up to 45 °C. User training takes an estimated one to days. The device is CE-IVD marked and the company is licensed to manufacture the TrueNat™ MTB by the Directorate of Food and Drugs Administration, Goa State. For companies located in India, licenses to manufacture are approved by state regulatory bodies. Currently, it lists the price of the Trueprep™ and Uno Systems at US\$ 7000 and US\$ 14 per assay. The public sector will receive a further discount. Annual maintenance is not required.

Molbio Diagnostics has also developed several new products for its TB technology suite with the intent to increase automation and test throughput and to include a reflexive DST assay for MDR TB. The first, the Trueprep™ AUTO is an automated extraction platform that employs a single-use disposable cartridge and a proprietary extraction process (Figure 23A). The user steps are limited to preparing 0.5 mL of sputum in 2.5 mL of liquefaction/kill buffer and waiting from three to five minutes before loading this into the

cartridge using a plastic pipette (provided in the kit). This houses other critical reagents onboard. After insertion into the machine all further extraction and purification steps are automated and performed in the cartridge in a process taking 12 minutes. Liquid reagents are introduced via an onboard reagent reservoir. Liquid waste that is generated (e.g. some sputum; dilution buffer; wash buffers) is held in an onboard reservoir containing absorbent material to ensure unidirectional flow of spent fluids and prevention of backflow. The 100 µL of elute produced at the end of processing is manually collected by the user and transferred for storage and/or using 6 µL to add to a reaction chip for PCR analysis. The extraction cartridge is discarded into a laboratory waste container. The company estimates that up to 48 samples can be processed by a Trueprep™ AUTO instrument in an 8-hour working day and anticipates its release in Q3 2015.

To match the faster extraction methodology and enable optimal test throughput, Molbio Diagnostics is developing a new instrument that can independently run up to up to four reaction chips, the Truelab™ QUATTRO (Figure 23C). This is a modular device and the prepared reaction chips can be inserted into the device independently of other chips already undergoing processing. Data entry for the QUATTRO is via the touchscreen interface and the onboard application operates the test reactor and scores test data. Comparable to the UNO, this device is enabled for GPS/GPRS, GSM, Wi-Fi and Bluetooth. The memory can save data from up to 30 000 test results. The company anticipates its release in Q3 2015. A fully integrated device incorporating automated sample preparation, DNA amplification and test data interpretation is in early development by Molbio Diagnostics, but further information is not yet available. The company is also validating an RIF resistance genotyping assay as a reflexive test after the MTBC test identifies MTBC infection. There is no further information on the assay at this point.

Peer-reviewed evidence of the performance of the Truelab™ RealTime micro PCR System in clinical settings is still limited, but one study noted that the assay had high sensitivity and specificity with SSM + /C + of 99.2%/100%, respectively, and with SSM-/C + of 75.96% and 100%. A more recent study compared the technology to SSM, culture, a PCR assay and the Xpert®. The sensitivity of the TrueNAT™ was 99% as opposed to 100% for the Xpert® on SSM + samples. When compared to culture on all test data, the sensitivity of the TrueNAT™ was 94.6% as compared to 96% for the Xpert® MTB/RIF.¹⁰⁷

A non-inferiority study clinical evaluation of this technology with comparison to SSM, the Xpert® and culture is being led by FIND and performed by CPTR.¹⁰⁶ It is significant to note that Molbio Diagnostics is the first developer to be chosen for the Support for Success (S4S) initiative being initiated by FIND.¹⁰⁸ This new programme is envisaged to drive the development and uptake of promising fit-for-purpose diagnostics in low-resource countries. The goal of S4S is to assist developers via a comprehensive network of experts from the diagnostics industry and other critical fields in the product development pipeline such as regulatory pathways. The entire package of support will include market needs assessment, product requirements, feasibility and streamlining of the development process, and look to industrial upscaling, manufacturing, product evaluation, regulatory clearance, market access and logistics. The programme will also provide access to FIND's sample and strain banks and its worldwide network of clinical study sites. In India, Molbio Diagnostics has also been chosen to participate in a diagnostics initiative led by a joint venture of the Department of Bio-technology, the Indian Council of Medical Research and the Ministry of Health & Family Welfare. The Indian Council of Medical Research is leading an effort for promoting "Indigenous diagnostic technologies for diagnosis of TB and MDR/XDR-TB" developed by Indian scientists/companies.^{106,109} The company has started to supply private health-care groups in India with its technology.

Ustar Biotechnologies is producing the EasyNAT™ TB assay intended for the diagnosis of MTB infection in lower-tier laboratories. This a manual assay using sputum specimens and requires only quite basic laboratory equipment with which to perform the assay: a centrifuge, block heater or water bath and pipettors. The assay utilizes the company's isothermal amplification strategy, cross-priming amplification (CPA) technology, and reagents are supplied as a glassified pellet.¹¹⁰ Reactions are incubated in a water bath, heat block or PCR machine. This assay is intended for batch processing in microcopy centres. The reagents are stable outside of cold chain for two weeks, but Ustar Technologies recommends from four to eight weeks, or -20 °C storage for up to 12 months. For low-throughput testing, the company recommends using its syringe-driven DNA purification method that uses a heat block to lyse MTB cells. For higher-throughput

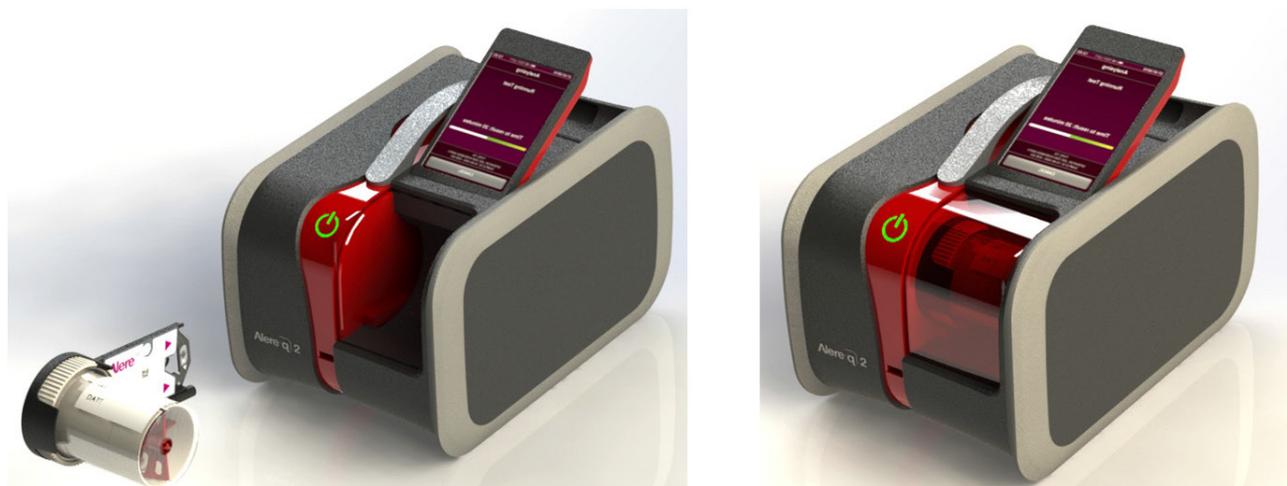
testing, it recommends centrifuge-based DNA microcolumns. The assay also includes a process control and target IS6110. CPA amplification of TB DNA is indicated using a sealed immunochromatographic strip that captures and detects hapten-labelled CPA amplicons in a process that takes five minutes. The system is sealed to prevent amplicon contamination of the test site.

Ustar Biotechnologies estimates that up to 40 samples can be processed in an 8-hour working day. The company claims an analytical sensitivity of 10 cfu/mL and 100% specificity and reports clinical sensitivity for SSM+/C+ of 98.1% and SSM-/C+ of 77.8% with a specificity of 89.2% versus culture. A multicentre evaluation of the EasyNAT™ TB assay in China with a pooled cohort of 2200 TB-suspected patients had sensitivity and specificity with SSM+/C+ samples of 84.1% and 97.8%, respectively. For SSM-/C+ samples, the sensitivity was 59.8%.¹¹¹ A study in Tanzania noted good specificity of the EasyNAT assay but unlike earlier studies that had shown high sensitivity, this was only 10% with SSM-/C+ samples.¹¹² The CFDA approved the EasyNAT™ TB assay in 2014. Ustar Biotechnologies is targeting markets in China and South-East Asia as its entry points with a price point of US\$ 6–8 per test. The company is currently developing a fully integrated technology that will process the sample and perform all test steps in a single disposable cartridge onboard with no extra reagents required. The platform housing test cartridges will have battery power, an onboard microprocessor for operation and data scoring/storage.

Conclusion: The first wave of NAAT-based assays to challenge the current market dominance of the Cepheid Inc. Xpert® is finally starting to enter its intended markets. The primary challenge to these groups has been to create a sufficient independent evidence base to achieve regulatory approval for use. All of the developers listed above have now achieved regulatory or licensing approval to allow their products to be used in intended markets. The Eiken Loopamp™ MTBC assay is of particular importance as the data from the multicounty validation studies were reviewed by the WHO STAG-TB committee in June 2015.

Further validation efforts are being led by FIND and its partners to assess the Epistem and Molbio Diagnostics products and the release of data is anticipated from the ongoing Nigerian study investigating the Epistem Genedrive® system. While all of these technologies show potential, their utility in diagnosing PTB in low-resource settings cannot be understood without a larger user-driven evidence base to indicate which technologies are best in class. In particular, informed evaluations of assay performance of populations with significant HIV comorbidity are critical to understand if the products have the necessary sensitivity to diagnose PTB from paucibacillary specimens. It is anticipated that more peer-reviewed data will be available in 2016. However, the paucity of validation data highlights that a more rapid and comprehensive approach to informing donors and the TB community as to the performance of these tools is insufficient. In the following section, more emerging technologies are described and while this is generally encouraging news for the fight against TB, all of these products need to be more effectively assessed so that the best in class can be more quickly offered to the global TB community.

NAATs in development: In this section, the focus is on NAATs that are being designed as competitors in the market of tests aimed at replacing SSM. New developers have been added to the pipeline since the 2014 landscape report and each technology is specifically aimed at automated tests with limited or no user input required to prepare sputum specimens for testing (Figure 13). These technologies are intended to have performance and limited user input similar to the Xpert®, but with inherent robustness and reduced cost that permit their rollout in austere settings without the need for upgrading infrastructure. Clinical performance data are typically not described for these technologies as in many cases they are aspirational due to their early development phase.

Figure 24. Alere™ q instrument for TB testing, currently in development

Source: Images reproduced with permission from Alere Inc.

The q platform was released by Alere Inc. as a single fully automated NAAT and the original design can host test cartridges to diagnose HIV1/2 or assess viral load of HIV 1/2.¹¹³ A grant and low-interest loan totalling US\$ 42.6 million was provided by the Bill & Melinda Gates Foundation to support Alere Inc. in modifying the q technology designs (instrument and cartridge/assays) to also incorporate high-volume sputum samples into the test cartridge and instrument (Figure 24) and to develop TB diagnostic and drug resistance genotypic assays for the platform. The intent is that a patient expectorates sputum into the cartridge specific cup, which is then immediately screwed onto the test cartridge and inserted into the q instrument for subsequent processing and testing. No other user input is needed to prepare or operate the test. The cartridge houses reagents to liquefy and inactivate the sample prior to processing the MTBC cells to purify DNA, amplify and detect MTBC, and then qualitatively score the result for the user. The MTB diagnostic assay uses nicking enzyme amplification reaction (NEAR), a specific and highly rapid isothermal method to amplify TB DNA. The q instrument provides fully automated results analysis and reporting with display of the simple final result onscreen. Alere Inc. estimates the time from collection to result at 20 minutes.

Alere Inc. is also developing a reflexive test for MDR TB in patients who test MTB positive. The cartridge design is the same as are the processes to prepare the sample and purify MTBC DNA, but the MDR TB technology used thereafter is different. DNA is amplified by PCR, not NEAR, and detection of the drug resistance alleles is afforded by a microarray of probe specific targets that compete with the amplified DNA for the sequence specific fluorescent probes in the reaction mixture.¹¹⁴ An internal control and other process control methods are integrated into the device and test cartridge. Data are processed and the results automatically scored by the q instrument and shown on a visual display on the LCD touchscreen. Data can be stored and downloaded via a USB port with multiple connectivity options for reporting. This test takes 45 minutes.

In terms of flow through, this is dependent on incidence and assumes 100% DST testing of TB patients. An example would be that the user could perform 18 TB tests and two MDR tests in an 8-hour working day. The instrument is battery-powered to tolerate intermittent mains electrical power and to protect against power surges. Reagents are intended to be stable at 5–40 °C for two years, and the q instrument will tolerate similar conditions. User training will take one day at most. Alere Inc. notes that routine maintenance and calibration are unnecessary; every cartridge contains controls for auto-focus and auto-calibration for each and every test cartridge. Alere Inc. is confident that the q platform and TB assay will receive CE-IVD marking in 2016 and in this same period will begin independent performance assessment. The price of the q platform and the cartridges is yet to be established. The test is aimed at global markets including LMICs where MTB prevalence is high.

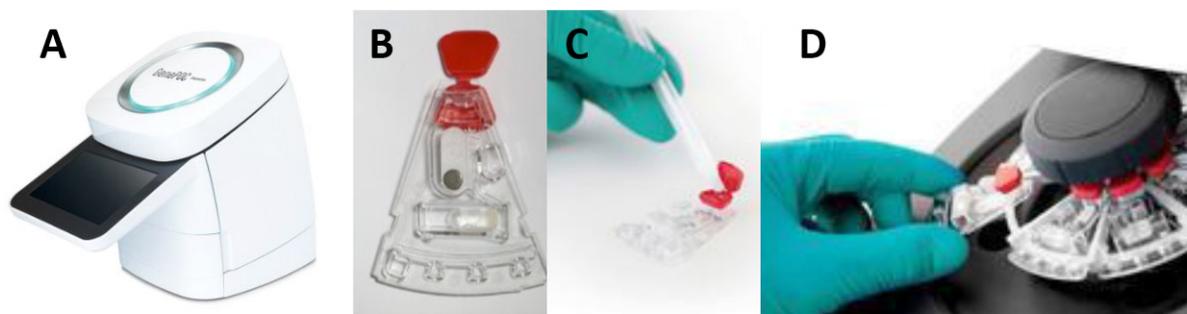
Figure 25. Cepheid Inc. GeneXpert® Omni: a standalone tool, currently in development, for the independent processing of the Xpert® test cartridges at POC



Source: Image reproduced with permission from Cepheid Inc.

In July 2015, Cepheid Inc. released news that they are developing a new tool, the GeneXpert® Omni (Figure 25), capable of processing Xpert® cartridges in more austere settings than the current Xpert® instruments. The Omni is small (9 x 3 x 4.2 inches) and portable (2.2 pounds). The Omni uses the same test cartridges already in use with the Xpert® platforms (e.g. MTB/RIF). Cepheid Inc. notes that the Omni has undergone significant engineering changes to provide better durability, portability and connectivity, while lowering power consumption. The reduction of component parts is also envisaged to improve reliability and serviceability. An internal battery allows up to four hours of use and an additional external battery provides sufficient electrical power for two days operation without mains electricity. Other details of the technology are unknown. Unlike the current Cepheid Inc. Xpert® platforms, the Omni is operated via a dedicated mobile device, no PC interface is required for operation. A mobile device controls a single module and the Omni can store up to 20 000 test results. The mobile device is designed to permit secure cloud-based connectivity that integrates real-time data streams for improved monitoring of productivity and performance for external quality assurance (EQA) purposes. Other details of this technology are currently not unknown. Cepheid Inc. plans the commercial release of the Omni into emerging and POC markets in the first half of 2016 at an expected cost of US\$ 2895.

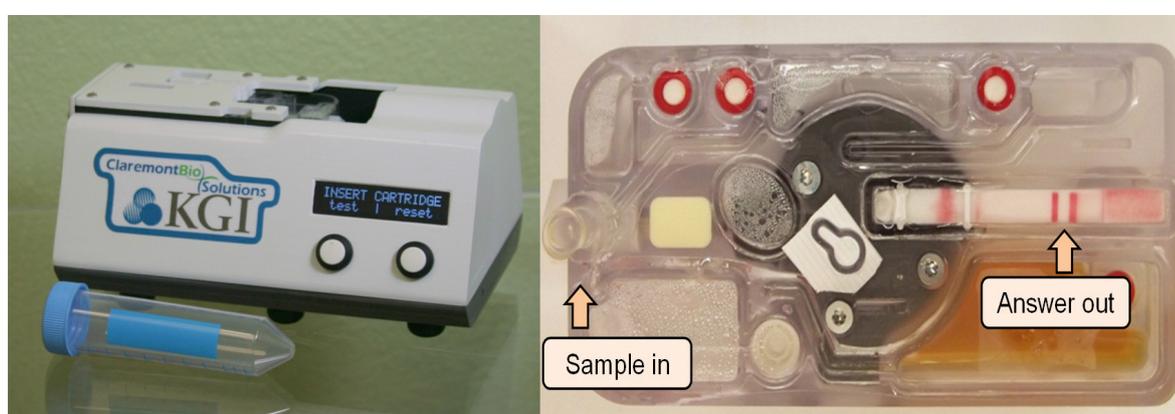
Figure 26. GenePOC automated test device



Notes: GenePOC Diagnostics automated test device (A); reaction cartridge and sample loading (B, C); loading of the test cartridge (D).
Source: Images reproduced with permission of GenePOC Diagnostics.

GenePOC Diagnostics (Canada) has a standalone system with an automated POC molecular diagnostic tool (Figure 26). The system is composed of an instrument and a disposable test cartridge to receive and process a sample to a result. This technology uses real time PCR and fluorescence detection to identify positive results during amplification. All reagents are housed in the cartridge (Figure 26B). The sample is added to the cartridge and up to eight cartridges can be prepared at one time and processed in parallel in the instrument (Figure 26C and D). The company estimates the time to result will be one hour from starting the machine. Data are automatically scored and there is a visual display and an LCD touchscreen for data entry. Using the maximum of eight cartridges per test run, it estimates that 64 samples per machine can be processed in an 8-hour working day. Reagent and platform stability are under assessment. The device runs off mains power and data are downloaded via USB port. GenePOC Diagnostics aims to have a TB assay and the platform marketed by 2018. Current costs are estimated to be US\$ 7000 for the platform and US\$ 20 for the cartridge. The test is primarily aimed at European markets.

Figure 27. KGI TBDx system: instrument (left) and test cartridge (right)



Source: Images reproduced with permission from KGI.

The TBDx system (Figure 27) is being developed by the Keck Graduate Institute (KGI; USA) in collaboration with Claremont BioSolutions, the University of Washington, the Seattle King County TB clinic, Learndon Solutions (all USA) and Ustar Biotechnologies. The system is designed to be compact, simplistic for very low-cost instrumentation, robust and able to perform all processing steps in the analysis of liquefied and disinfected sputum onboard a single disposable cartridge. MTBC cells are captured, washed, lysed and then the DNA eluted using a derivative of Claremont BioSolutions's PureLyse® technology. This novel solid-phase extraction method does not require chaotropic salts or organic solvents and, therefore, significantly simplifies nucleic acid preparation. The process is performed in a microbead beater and eluate DNA used to solubilize an MTBC assay that uses CPA as its core reagents. After amplification, the test reaction is interrogated for MTBC amplicons via an immunochromatographic strip and the user scores this visually. All onboard liquid handling processes within the cartridge are operated through inexpensive electrolytic pumps (epumps). KGI and its partners intend to complete development of this technology by Q2 2018. The technology will not require onsite calibration and a swap-out system is envisaged to replace faulty instrumentation. Current cost estimates are US\$ 150 for the instrument and under US\$ 8 per test cartridge. The test is targeted at HBC markets.

The Northwestern Global Health Foundation (USA) is developing an integrated MTBC assay for the Savanna Molecular Platform in partnership with Quidel Inc. (USA). This system consists of a heat block to first lyse MTBC cells and purify the DNA using a system with immiscible oil interfaces that greatly reduces volumes for washing and does not need fluidic control.¹¹⁵ Other information is limited, but a reflex assay for MDR TB is also in development. The instrument will have battery power and the system requires no user involvement after the lysed sample is added to the cartridge and inserted in the platform. Throughput is thought to be 13 tests per 8-hour working day. The cost per device is currently estimated at below US\$ 12 000 with cartridges at under US\$ 10. The targeted date for release is in 2016. The test is targeted at LMIC markets.

Figure 28. Point of Need (PON) technology in development by Qiagen: a schematic rendering of test cartridge design (left) and an image of a prototype instrument (right)



Source: Images reproduced with permission from Qiagen.

Qiagen is developing a fully integrated test platform, the Point of Need (PON) (Figure 28), intended for use in low-resource settings with a minimally skilled user. The PON is for the diagnosis of active PTB in adults and children and diagnosis of MDR TB by isothermal nucleic acid amplification-based detection of drug resistance to RIF, INH and FLQ. All reagents will be housed onboard the cartridge. The user, a health-care worker, adds the specimen to the test cartridge and all steps thereafter are automated. Training is estimated to be less than one day. Qiagen anticipates a 1-hour time to result. The number of test cartridges to be housed by the instrument is still undergoing review. The company projects that 8–24 samples per day can be processed pending the final design of the instrument. The instrument and test reagents have a targeted stability of up to 40 °C for one year. The instrument will be battery-powered and use a touchscreen interface for data entry. Data export options will include USB, Wi-Fi, GSM and GPRS. The technology will not require onsite calibration and a swap-out system is envisaged to replace faulty instrumentation. The cost per instrument is targeted at under US\$ 10 000 and at under US\$ 8 per test. Qiagen aims to complete development in 2018-2019.

Figure 29. Rendering of the Q-POC™ platform under development from QuantuMDx



Source: Image reproduced with permission from QuantuMDx.

The Q-POC™ platform is under development by QuantuMDx (UK) (Figure 29). It is intended as a hand-held, fully integrated device to diagnose PTB with application for the microscopy centre and higher-tier levels. The patient expectorates sputum into a cup and subsequently liquefied and decontaminated via reagents housed in the test cartridge. The MTBC cells are concentrated via a paramagnetic bead concentration step. The lysis of cells is via micro bead beater and the MTBC DNA is amplified via an isothermal, asymmetric PCR method that takes only four minutes to amplify. QuantuMDx has licensed the DPO™ technology from Seegene, which enables highly multiplexed PCR while limiting the risk of primer-dimer formation and so enhancing efficiency of complex multiplexed PCR assays.^{63,116} The Q-POC™ platform uses an amplicon detection method that does not require complex optical detection tools that typically increase costs and can compromise equipment performance in austere settings. It detects specific amplification of MTBC targets via the amplicons binding to complementary oligonucleotides arrayed on silicon nanowires. The binding of amplicon DNA to their complement fixed on the nanowire alters the resistance of the nanowire and this can be measured. The nanoscale of these wires creates the potential for many nanowires to be individually arrayed and monitored. QuantuMDx notes the potential for tens or even thousands of detector nanowires that could be placed into the single fluidic channel of their cartridge.

An assay is estimated to take only 20 minutes after insertion of the test cartridge. All reagents will be housed onboard the test cartridge and current stability targets are up to 40 °C for up to 18 months. The Q-POC™ platform will be operated via a touchscreen interface and from a battery capable of running 20–25 tests per charge. Data interpretation will be automatically performed and the device could store up to 250 test results. As the instrument is fully automated, user training will be under one day. Software updates will be updated via USB and units replaced if a fault occurs. The estimated release date for the Q-POC™ assay is 2017. The cost of instrumentation is estimated to be under US\$ 2000. Assay cost has yet to be released.

Figure 30. Rendering of the collection unit of the Wave 80 Biosciences EOSCAPE-TB System



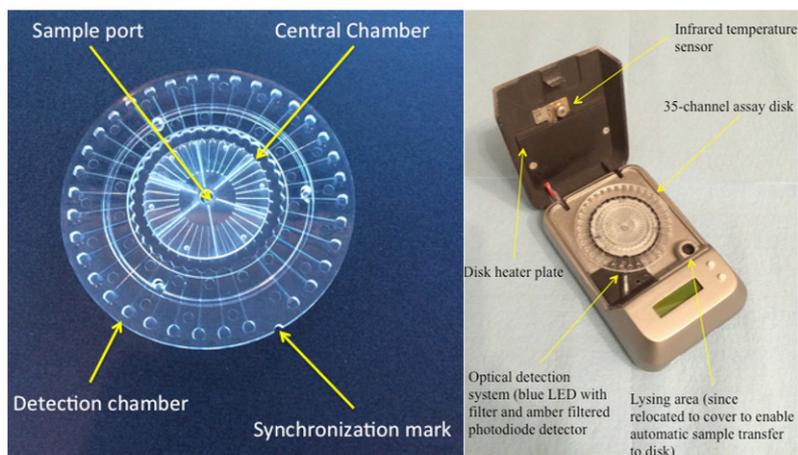
Source: Image reproduced with permission from Wave 80 Biosciences.

Wave 80 Biosciences (USA) continues to develop its EOSCAPE-TB System (Figure 30) to detect MTBC from sputum. It is also developing the EOSCAPE-RIF-FQ test, a reflexive test to genotype RIF and FLQ resistance alleles. The assays are integrated and require that the patient expectorates into a custom collection cup that can liquefy the sample. The user inserts the single-use disposable test cartridge into the collection container via the snap feature and inserts this into the processor unit. All other reagents are housed in the test cartridge. The EOSCAPE-TB system is unique to the other platforms in that the cartridge-based extraction and amplification components are performed in small battery-powered processing units, by which multiple tests can be run in parallel. Upon completion of a test, each processing unit is docked into the EOSCAPE-1 Analyzer that generates the test result. One analyzer can be used with multiple processors. Wave 80 Biosciences notes the development of a new analyzer that can interpret multiplexed reactions, the EOSCAPE-Mplex Analyzer. The time to result is one hour and the company estimates that up to 50 specimens can be processed in one day when using multiple processing units. Targeted reagent stability is at 30 °C for 12 months with two days exposure at 50 °C. The processor and analyzer are all battery powered. User training will take under one day. Wave 80 Biosciences intends to release these products in 2016. The

cost of the TB and RIF-FQ assay are estimated at US\$ 12 and US\$ 20, respectively. For the analyzers, the EOSCAPE-1 is projected at US\$ 5000 and US\$ 10 000 for the Mplex device.

Tosoh Bioscience has developed a new standalone device for integrated molecular diagnostics, the TRC-Ready® 80 platform that leverages off its TRC amplification technology.¹¹⁷ It is currently unknown if the company intends to develop a TB-specific assay for this platform. Due to the fact that it is already marketing the TRCRapid® M.TB as a batched assay on a different platform (TRCRapid 160), the authors of this report assume that the company intends the TRC-Ready® 80 platform to host the same assay.

Figure 31. Tangen Biosciences TB diagnostic test disc (left) and platform (right)



Source: Images reproduced with permission from Tangen Biosciences Inc.

Tangen Biosciences Inc. (USA; hereinafter Tangen) is a start-up company developing a fully integrated TB platform (Figure 31). The technology is being developed to include specimen collection as part of the specimen collection and processing device. The sputum is liquefied and disinfected within the collection cup once it is screwed shut. The sample is then filtered to capture the MTB cells on a filter that is then transferred to the 35-channel test disc where lysis and subsequent isothermal amplification via LAMP and detection take place. The instrument rotates the test disc to facilitate liquid transfer to test reaction chambers, and then provides heating and optical detection of reactivity in each test chamber. The time to result is 30 minutes and the test data are automatically scored and pooled for a test result. All core reagents are housed on disc or in the sample collection device. Tangen estimates 8–16 specimens can be processed in an 8-hour working day. Training of a health-care worker will take less than one day. The instrument is battery operated and uses touchscreen interface. Up to 10 000 test results can be stored. The device is Bluetooth enabled for a wireless printer. The company currently estimates the cost per cartridge at US\$ 10 and the device at US\$ 1400. The projected time to market is in Q4 2016.

Several groups are pursuing PCR based detection of MTBC DNA based upon proprietary tools that claim to have optimized primer design for the more efficient amplification of MTBC target DNA. Fluorocentric Inc. (USA) offers a real time PCR assay based on its extreme PCR (XCR™) assay designs developed via proprietary primer design algorithms. Any real time PCR machine can be used with this assay, but the limitations of heat transfer during thermal cycling can significantly extend the time to result. In light of this, Fluorocentric Inc. is developing a hand-held, battery-powered instrument that is optimized for the rapid amplification and real detection of individual XCR™ assays. Fluorocentric Inc. claims that their MTBC XCR™ assay can complete amplification in under 10 minutes using their prototype reactor. Currently, there is no evaluation data to back this claim.

Similarly, Co-diagnostics and Thisis (both USA) also use novel primer design algorithms with which to develop better assays for the detection of MTBC using PCR. In June 2015, Co-diagnostics announced an agreement with Mapmygenmome (India) to release SMART TB™, a real time PCR assay to detect MTBC

via the 16S RNA gene. The assay also uses an internal control and is in an open format that can be used on a variety of real time PCR platforms; the user is required to first extract DNA from samples to be tested. The LOD is estimated to be ~ 200 cfu/mL with detection within 30–60 minutes depending on the real time platform used. There are no performance data, information on regulatory qualifiers or pricing at this stage. This is a spinoff company from the Boston University School of Medicine, which has developed assays using totally optimized PCR (TOP); one is designed to detect MTBC at a claimed LOD of 4 cfu; and another assay to genotype MTBC into five genogroups. Currently, the TOP assays use an open platform with confirmation of positivity via a colorimetric assay after amplification. A validation of these assays has recently been completed in the Brazil, Uganda and the USA using discarded specimens and a manuscript has been prepared for peer review. Data suggest that the assays are sensitive to detecting MTBC and the genotyping assay identified a genogroup only associated with HIV coinfection. This is currently looking to place their TOP assays onto an existing diagnostic platform.

Scanogen is the final developer to be included in this section. In terms of their underlying technology, it is not strictly considered a NAAT because while it uses nucleic acids (DNA) as MTBC-specific targets, it does not amplify from them. The core technology is based on single molecule scanning (SM-Scanning), a novel non-enzymatic technique capable of detecting a target nucleic acid in body fluids samples without PCR amplification. The key innovation of SM-Scanning is the detection of target molecules based on the displacement under force of DNA tethered micrometer beads. Scanogen claims that this approach enables single molecule detection with extremely low background noise and high sequence specificity. In the context of limited-resource setting TB diagnosis, SM-Scanning may offer important advantages over the available amplification-based molecular platforms. The reagents for SM-Scanning are DNA molecules and beads, which are inexpensive and stable. In SM-Scanning, beads are imaged and their displacement measured using inexpensive and low-power equipment comprised of LED ring illumination, a lens and a digital CMOS camera. The assay does not require a specific incubation temperature and, therefore, instrumentation complexity and attendant power to incubate and perform test reactions are greatly reduced. As this technology is in early development, a more complete description is not available. The device will be hand held and the intended use is by a health-care worker in a microscopy centre setting or similar. The test is aimed to take 30 minutes to perform and preliminary claims of performance by Scanogen are an LOD of 25 cfu. The intended cost per test is US\$ 6 and the instrument US\$ 2000. The time to product release has not yet been established.

Conclusion: From the expansion of NAATs in development, it is clear that diagnostics developers see a sufficient market to develop new products for the diagnosis of PTB. These new products are typically fully integrated and targeted at diagnosing MTB at the peripheral level, for example, the microscopy centre and upwards. All incorporate cartridge-based platforms that house all reagents and user steps are typically minimized to preparation of the specimen into the cartridge before testing. Some systems also incorporate the sputum transfer step to further reduce user input as evidenced by the Alere™ q, the Tangen and Wave 80 Biosciences EOSCAPE platforms. The recent announcement of the Cepheid GeneXpert® Omni represents a significant challenge to these nascent technologies, especially given that the Xpert® MTB/RIF assay has been already been introduced and adopted by many countries. The Omni is designed to target the current gaps seen with NAAT-based testing in lower tier facilities such as microscopy centres. Overall, new tests and technologies are being developed to cope with intermittent power and provide greater resilience to high heat, humidity and other environmental confounders such as dust. Most developers are also developing reflexive tests for genotypic DST including MDR TB and, in some cases, both MDR and XDR TB. Processors to host data entry, test algorithms, data processing, result storage and connectivity to centralized data systems are now integral components of the new platforms in development. The following section highlights a new initiative to accurately collate all the MTB genotypes associated with drug resistance with commonly used drugs. This resource is intended to provide developers with the current and fully factual data so that efforts can be focused on technology development and assay design rather than parsing literature to identify the key alleles.

As noted in the 2014 WHO *Global Tuberculosis Report*, in 2013, approximately three million people are left undiagnosed or their test data are not acted upon.¹ To address this gap, nearly all of the new technology

platforms host automated scoring of test data and its storage for upload via USB or via cellular networks and/or Wi-Fi. Availability of information on the location of testing is also enhanced by many technologies incorporating GPS into their platforms. Therefore, in principle, these new technologies should improve the diagnosis and recording of patient data that can be tracked. Other features that are often difficult to quantify, such as case detection rates, rate of testing, cartridge failure and/or user errors, will also be highlighted by improving remote communication with the potential for real time monitoring by country TB control programmes. If these can be combined as optimistically envisaged, then improved diagnosis and a clearer understanding and tracking of TB testing is possible. Each of the technologies claim high performance of diagnosing SSM + /C + , but a key challenge is for these technologies to detect SSM-/C + with a high degree of sensitivity (e.g. > 90%) thus there remains a challenge to effectively diagnose PTB in PLHIV. If the turnaround time and cost per test are low, repeat testing may be an option. Only independent validation of these technologies with this population will provide this answer.

Hardware cost varies significantly and is typically reflective of the complexity of the instrumentation that is used. Some of the smaller technologies do not need routine maintenance and a swap-out model is proposed for failed devices. Given that many of these groups are relatively small in size, a more realistic final cost of the tests or equipment will be achieved with production at scale.

4.2.10. EQA systems for NAATs

While the NAAT products incorporate controls to address the performance of specimen processing, test reagents and the technology bed during processing, there is a further need where TB programmes are assured of equipment and test performance after installation or repair. EQA is an important component of the quality system required for diagnostic testing programmes, but it must be complemented by routine monitoring of performance indicators and instrument verification. With the initial scaled implementation of the Xpert® across South Africa, the NHLS encountered this problem and has assessed a variety of products that can be used for this purpose. In developing an EQA programme for the Xpert®, the NHLS noted five key qualifiers:¹¹⁸

1. The testing material must contain whole *M. tuberculosis*.
2. Transportation of EQA material needs to be safe.
3. The testing procedure needs to be safe and compatible with the Xpert® MTB/RIF current testing protocol.
4. Health-care workers who do not have laboratory skills must be able to perform the testing in non-laboratory settings.
5. The programmes need to be cost effective and sustainable.

Currently, five commercial sources can provide MTBC materials for Xpert® verification and EQA, including a lyophilized sample (Vircell; Spain), a dried tube specimen (United States Centers for Disease Control and Prevention [CDC]), liquid (Maine Molecular Quality Control Inc.; USA), artificial sputum (GLI) and a dried culture spot (DCS) developed by the NHLS. All of these were subjected to a multicentre evaluation to assess their performance at 11 independent sites.¹¹⁹ The study noted little overall differences in scores between the Vircell, CDC, GLI or NHLS panels, indicating that all are likely to be suitable for use in an EQA programme, while the Maine Molecular Quality Control Inc. panel was less favourable as it required cold chain and the material was difficult to transfer to the Xpert® cartridge. The NHLS has continued to develop its DCS product for use within the South African TB programme and 21 other country programmes have used this product. As its name implies, the DCS is a spot of quantified MTB cells that was first qualified as inactivated before spotting and drying on Munktel filter cards (Figure 32).

Figure 32. Dried culture spot card used for Xpert® verification and EQA programme management; each circle contains a sample spot and barcoding simplifies data management



Source: Image reproduced with permission of the University of Witwatersrand

The DCS is offered as two separate products. The verification panel has unblinded results and reporting is managed manually by receiving laboratories. Instrument verification involves testing standardized material on a diagnostic instrument to ensure that it is “fit-for-purpose” before clinical specimens can be tested. Verification should be performed on each instrument (module) when an instrument is installed, repaired, moved and post calibration. DCS for verification is available in a 5-spot and 1-spot card format. GLI has endorsed the use of DCS technology in Xpert® instrument verification and Cepheid Inc. uses this post installation of new Xpert® platforms.

The second product is the EQA panel that is used to ensure ongoing quality monitoring of diagnostics, from pre- to post-analytical processing. The EQA DCS cards are offered three times per year. Management of EQA result for the Xpert® platform is automated through a web reporting system (www.tbgxmonitor.com). Each EQA panel comprises four DCS (combinations of MTB RIF susceptible; MTB RIF resistant; NTM; and negative). Further application of DCS on other NAATs has been demonstrated with its use in conjunction with the Hain GenoType MTBDRplus v1.0 and GenoType MTBDRplus v2.0.¹²⁰ DCS is stable after storage at 37 °C for one year. Current production of the DCS cards is performed at the University of Witwatersrand (South Africa) and a commercial spinoff company, SmartSpot, is anticipated. DCS is available as a 5-spot or a 1-spot paper card and currently cost US\$ 38 per card and includes access to the TBGX monitor. In May, 2015, the African Innovation Foundation awarded the inventors of the Smartspot TB Check (the DCS) with an Innovation Prize for Africa, the Special Prize for Social Impact.

4.2.11. Whole genome sequencing and its application for TB diagnosis and treatment

The advent of whole genome sequencing (WGS) via a growing number of test platforms and technologies is starting to impact TB diagnosis in a number of ways and is now yielding detailed information that was unthinkable even five years ago. The third edition of the landscape report in describing the pipeline first highlighted the application of this incredibly powerful technology and how it is already being applied in the TB landscape. In terms of complexity, this is a tool for the supranational or national reference laboratory, but with the complexity of hardware, assays and data analysis being rapidly simplified and streamlined, NGS may soon be more broadly applied. In 2015, there is a large number of peer-reviewed articles describing a broad variety of applications for NGS in the context of MTB. An obvious use for NGS is in a more accurate classification and characterization of phylogenetic lineages and specifically in tracking the prevalence of circulating strains or sudden emergence of new ones from disparate geographies. Recent examples of the application of WGS data for genotyping include the molecular evolution of *M. tuberculosis* Beijing strain,¹²¹ the appearance of an Iberico-American strain in Tibet¹²² and a study that noted that WGS provides greater resolution than MIRU-VNTR, the current tool for molecular genotyping of circulating strains.¹²³

Unlike many other bacterial pathogens that can become drug resistant by acquiring plasmid, transposon or phage-mediated elements, *M. tuberculosis* develops drug resistance exclusively through chromosomal mutations, in particular, single-nucleotide polymorphisms.¹²⁴ Historically, this has made identifying drug resistance elements in MTB very challenging, especially for alleles that lie outside of drug resistance hotspots such as the RIF resistance-determining region for RIF, and even with RIF there are alleles outside of *rpoB* that are associated with resistance.¹²³ Recent examples of using NGS to elucidate new drug resistance mechanisms include PZA resistance via deletions,¹²⁵ STR resistance,¹²⁶ novel INH resistance genotypes,¹²⁷ and, most importantly, screening for allelic variance associated with resistance to new drugs and compounds.^{124,128,129} This last point is key to developing proactive diagnostic tests where it is possible to identify associated genotypes and resistance mechanisms therein *before* clinical application rather than waiting for drug resistance to emerge after drugs are approved and in widespread clinical use. It is now possible to identify putative associated genotypes and resistance mechanisms therein before clinical application, although it is noted that observed rates of drug resistance with MTB are reduced under contrived conditions of clonal culture in the laboratory.

However, the precision and comprehensive analysis offered by NGS methods presents an opportunity for new diagnostics tests to be released in conjunction with new drug regimens so that evolution of resistance within populations is more carefully measured and identified much more quickly than with previous tools that cannot do this effectively. WGS has already been applied to patients with MDR TB in order to inform on the course of treatment.^{130,131} NGS has the potential to identify if a relapsed patient has been infected with a new strain or whether the original infection was not fully curtailed by the initial treatment.^{132,133}

There is an ever growing variety of NGS technologies that is available to the user and specific applications can vary. Due to the application of WGS for a broad variety of inheritable diseases or their use as cancer diagnostics, many of the platforms are US FDA approved or undergoing this process to gain approval. In the 2014 landscape report, the authors noted small laboratory footprints for equipment such as the MiSeq (Illumina; USA), the Ion Personal Genome Machine® (PGM™) System (Life Technologies Incorporated; USA) or the 454 FLX Junior (Roche Diagnostics; Switzerland). Qiagen is preparing to release its platform, the GeneReader, in 2015 and the GnuBIO is another NGS system from Bio-Rad Laboratories, soon to enter the market. Other tools such as the Pac Bio (USA) are also being described for WGS of MTB.¹³⁴ Other, potentially more simplistic technologies are being presented, including the GridION™ and the MinION™ from Oxford Nanopore Technologies (UK), in addition to QuantumDx, the developer of the Q-POC™ described earlier, which is also developing an NGS platform.

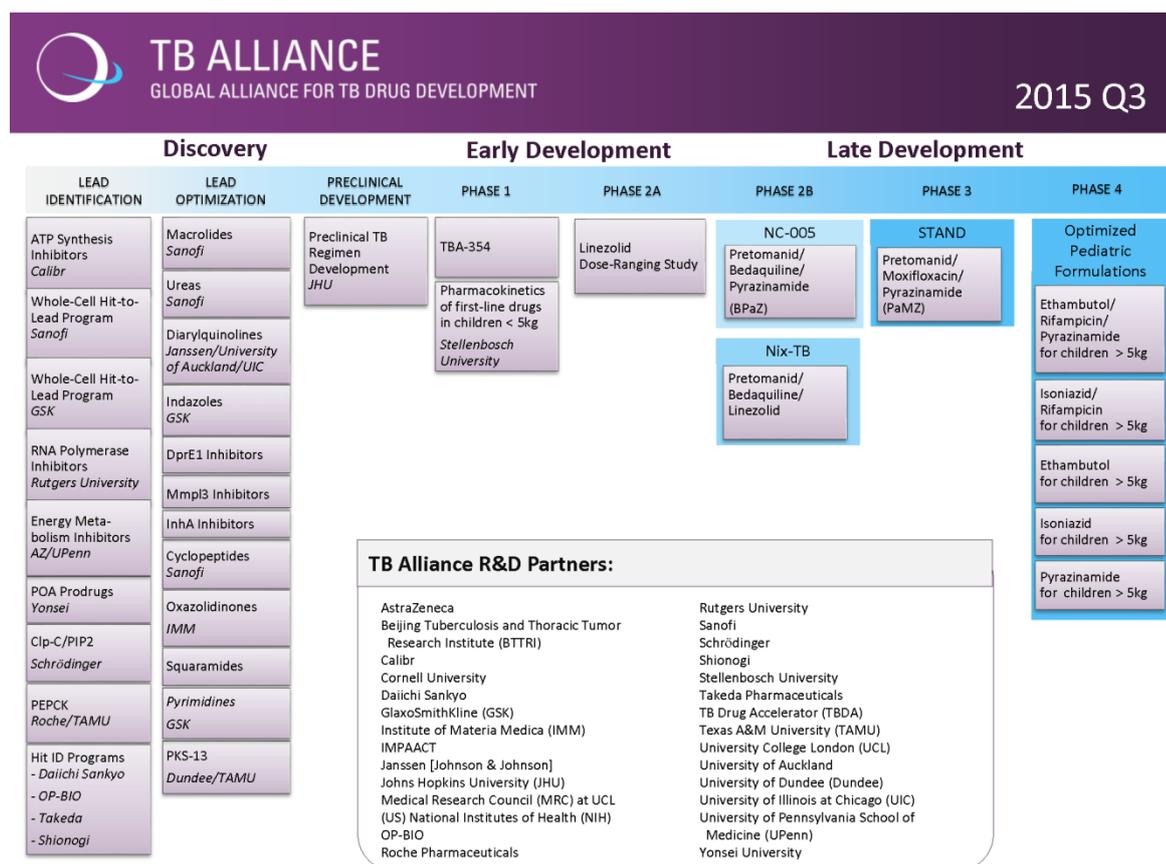
A challenge with so many systems producing massive data sets is how to parse and store these datasets and to create host systems where data are open access and in fully annotated formats that are uniform. The huge data sets are best hosted by cloud-based servers, but as yet there is no uniform approach to data management and annotation. Several groups have created software applications with which to provide systems to accommodate WGS variance, display variance via phylogenetic trees and effectively incorporate drug resistance alleles.¹³⁵⁻¹³⁹ The closing section of this landscape report describes a focused effort to list fully characterized resistance alleles to existing and new drugs in clinical trials with an aim to providing technology developers and researchers with fully accurate and up-to-date resources to inform on genotypic DST assay development. Currently, there are no guidelines for TB programmes to use in terms of how to best apply NGS regarding molecular epidemiology or for improved and rapid DST. Data uniformity will be critical for global surveillance of MTB. The laboratory techniques using NGS have typically followed traditional molecular epidemiologic techniques where a subset of culture-derived clinical isolates is used for testing. Risks involved with this are contamination, further variance through culture methods and when a heterogenous population is present, thus the clonal nature of culture may remove the variance in the patient. One group has demonstrated a procedure where early MGIT™ positive culture can provide sufficient material for NGS.¹⁴⁰ Most pertinently, a recent peer-reviewed publication describes a method to directly perform NGS from sputum samples that allows for a more comprehensive assessment of the direct genetic variance seen within a population of infectious cells without bias from culture or preparation methods used therein.¹⁴¹ Currently, the only product being offered for sequencing of MTB isolates in relation to drug resistance screening is from Longhorn Vaccines & Diagnostics that offers primer sets from

which 10 full-length genes associated with drug resistance to first- and second-line drugs may be amplified via PCR and then sequenced on the Ion Torrent platform from Life Technologies Incorporated.^{21,22}

Conclusion: The application of NGS is already occurring in many reference and research laboratories in LMICs. The discriminatory power of this evolving technology is unrivalled and will play an ever increasing role in understanding the pathology and epidemiology of MTB. This information is already translating into reflexive technologies such as using target sequences to create lower cost and more rapid PCR assays to genotype common MTB strains known to be in circulation.¹⁴² NGS can provide rapid and detailed information as to the prevalence of strains and drug resistance alleles in addition to providing raw data that will be of key use to national and regional prevalence data. However, to be most impactful, guidelines for the most appropriate use of NGS are now required so that uniformity of methods can be applied with careful parsing to avoid deposition of poor data. It is possible that a global repository of whole genome data could be available in the next three or four years if methodology can be streamlined and all HBCs are willing to fully contribute the test data.

An accessible repository of assessed and fully annotated drug resistance alleles: The current pipeline of new TB drugs is shown in Figure 33. The range of new products and regimens in development provides an indication of the evolving challenges developers face in creating required diagnostics when they are needed.

Figure 33. Pipeline of new TB drugs and regimens



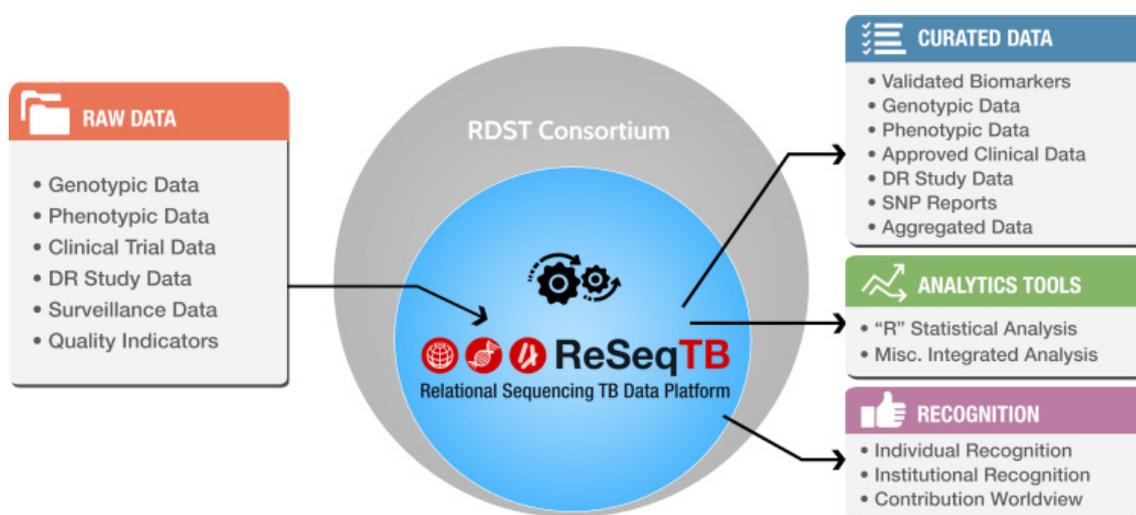
Source: Reproduced with permission from the Global Alliance for TB Drug Development (<http://www.tballiance.org/pipeline/pipeline.php>).

The CPTR programme of C-Path has recognized the need for an international data-sharing platform (Figure 34) to enable the acceleration in development of safer, faster-acting and more efficacious drug regimens for TB treatment. The development and implementation of the Relational Sequencing TB Data Platform,

or ReSeqTB, will be undertaken by the CPTR Rapid Drug Susceptibility Test (RDST) consortium as part of its overall goal to help accelerate development of rapid drug susceptibility tests – crucial to creating and evaluating potential new therapies (<http://www.cptrinitiative.org/working-group/>). This resource will be designed to provide researchers and clinicians with valuable and standardized international TB patient data that are currently contained within an array of private and public databases.

To aid in this complex, international undertaking, RDST has formed strategic partnerships with the Critical Path Institute, FIND, WHO, NDWG, CDC and the National Institute of Allergy and Infectious Diseases. After being populated with relevant patient and laboratory data from research, pharmaceutical, government and academic sources, the ReSeqTB will identify correlations between MTB genetic mutations and clinically relevant phenotypic resistance patterns, pinpoint and complete knowledge gaps, aid in the development of new rapid drug susceptibility tests, facilitate international research and collaboration and, eventually, directly enable sequencing data interpretation for patient care, giving rise to personalized TB treatments encompassing geographically based resistance trends.

Figure 34. Anticipated role of the ReSeqTB initiative and RDST consortium in developing a comprehensive, evidence-based understanding of drug resistance



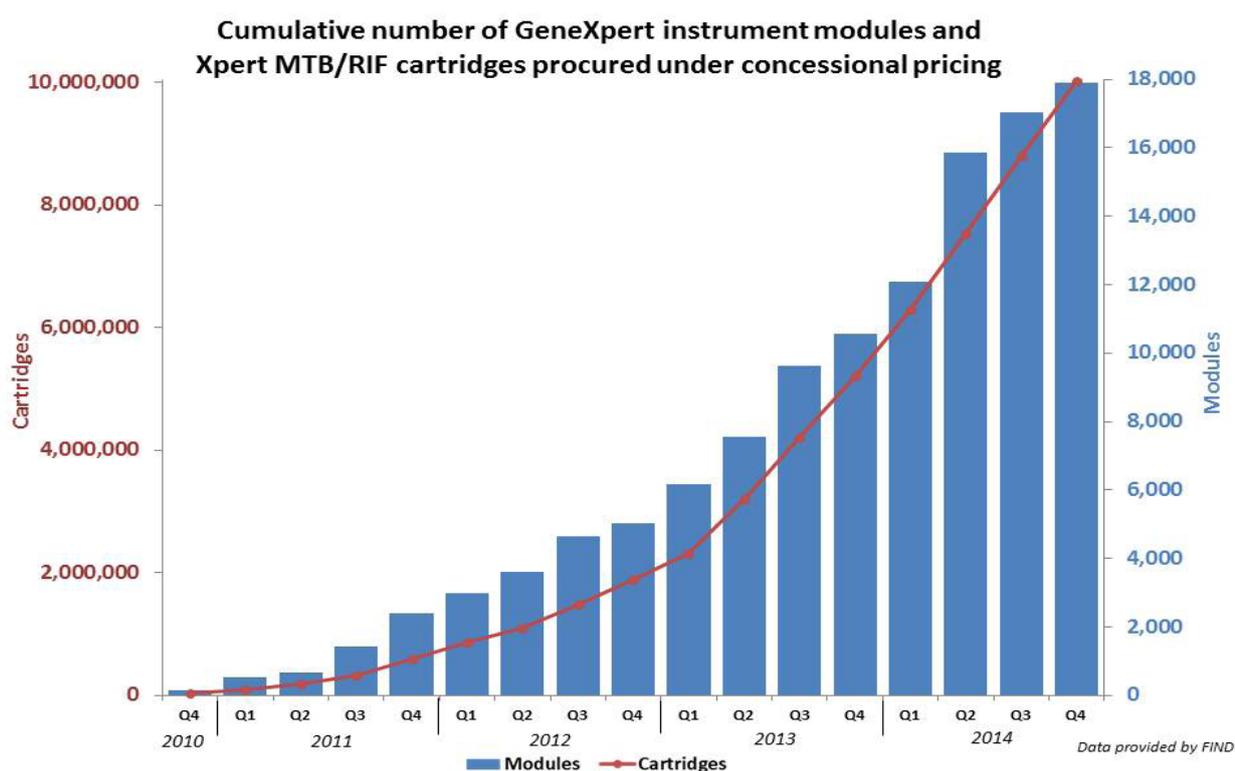
Source: Image reproduced with permission from C-PATH.

5. Market landscape

5.1. 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 31 December 2014, a cumulative number of 3763 Xpert® instruments and 10 million Xpert® MTB/RIF cartridges had been procured in the public sector in 116 of the 145 countries eligible for concessional pricing (Figure 35), with South Africa accounting for over half of all the cartridges procured to date.

Figure 35. WHO monitoring of Xpert® MTB/RIF scale-up



As of 31 December 2014, a total of 3,763 GeneXpert instruments (comprising 17,883 modules) and 10,013,600 Xpert MTB/RIF cartridges had been procured in the public sector in 116 of the 145 countries eligible for concessional pricing.

Notes: As of 31 December 2014, a total of 3763 Xpert® instruments (comprising 17 883 modules) and 10 013 600 Xpert® MTB/RIF cartridges had been procured in the public sector in 116 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 continuing to support Xpert® scale-up, including UNITAID, the United States President's Emergency Plan for AIDS Relief, 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 GLI meeting, and in peer-reviewed publications. The largest study of Xpert® was published in May 2015.¹⁴³ This demonstration study was implemented in 18 subdistrict-level TB programme units (TUs) in India in diverse geographic and demographic settings covering a population of 8.8 million. A baseline phase in 14 TUs captured programmatic baseline data, and an intervention phase in 18 TUs had Xpert® MTB/RIF offered to all presumptive TB patients. In the 14 study TUs, which participated in both phases, 10 675 and 70 556 presumptive TB patients were enrolled in the baseline and intervention phase, respectively, and 1532 (14.4%) and 14 299 (20.3%) bacteriologically confirmed PTB cases were detected. The implementation of Xpert®

MTB/RIF was associated with increases in both notification rates of bacteriologically confirmed TB cases (adjusted incidence rate ratio [aIRR] 1.39; CI 1.18–1.64), and the proportion of bacteriological confirmed TB cases among presumptive TB cases (adjusted risk ratio [aRR] 1.33; CI 1.6–1.52). More importantly, compared with the baseline strategy of selective DST only for patients at high risk of drug-resistant TB, Xpert® MTB/RIF implementation increased RIF-resistant TB case detection by over fivefold. This highlights the critical importance of upfront DST, right at the time of diagnosis.

While a large number of studies have confirmed the high accuracy of Xpert® MTB/RIF, new studies are starting to address the issue of how the test impacts patient outcomes.⁸⁸ The first randomized controlled trial of Xpert® MTB/RIF was published in October 2013.¹⁴⁴ 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.¹⁴⁵ Xpert®, compared to microscopy, increased the proportion of 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 were published in December 2014.¹⁴⁶ In this trial of 14 primary care laboratories in two Brazilian cities, replacing smear microscopy with Xpert® MTB/RIF increased confirmation of PTB. An additional benefit was the accurate detection of RIF resistance. However, no increase in overall notification rates was observed, possibly because of high rates of empirical TB treatment. A follow-up study of this trial was published in April 2015.¹⁴⁷ The proportion of patients successfully treated did not increase with Xpert® MTB/RIF implementation, with high loss to follow-up rates in both arms. The researchers did observe a 35% reduction in TB-related mortality, which they hypothesized might be explained by less advanced disease among the smear-negative patients diagnosed by Xpert®. Thus, Xpert® MTB/RIF introduction did not substantially improve TB treatment outcomes in Brazil.

A common theme in these pragmatic 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 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.¹⁴⁸ 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 recent survey of 22 HBCs suggested that while a majority has 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.⁴ The survey used the ratio of smear volumes for initial diagnosis to the number of Xpert® cartridges procured during a roughly similar time period as an approximate index of Xpert® market penetration in the public sector. The ratio in South Africa was 1.6, significantly lower than most other HBCs where approximately 40–70 smears were performed for each Xpert®. 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.

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 its 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 CAD 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 over 105 member laboratories across India. These member laboratories collectively account for over 3500 collection centres, covering approximately 80% of the districts in India. Since inception, IPAQT labs have collectively tested over 200 000 presumptive TB cases with average quarter-on-quarter growth being ~ 25%.

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. 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.

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

While the Xpert® MTB/RIF assay is a much-needed breakthrough, it was not originally designed to reach lower tiers of the health-care system, and not intended to meet all needs – for example, 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 and colleagues conducted a priority-setting exercise to identify the highest priority tests for TPP development and investment in research and development.¹⁰ 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. Based on this analysis, the following were identified as the highest priorities:

- a POC sputum-based test as a replacement for smear-microscopy (“smear-replacement test”);
- a POC non-sputum-based test capable of detecting all forms of TB via the identification of characteristics biomarkers or biosignatures (“non-sputum-based biomarker test”);
- a POC triage test, which should be a simple, low-cost test for use by first-contact health-care providers as a rule-out test (“triage test”);
- rapid DST at the microscopy centre level (“rapid DST”).

Given the variety of felt needs, and diversity of sites where testing can occur, 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 have been addressed by several partners.

In April 2014, the WHO Global Tuberculosis Programme convened a meeting on behalf of GLI and NDWG to develop consensus on the minimal and optimal specifications of four different types of TB diagnostic tests that were identified by multiple stakeholders to be high priority. Extensive work by McGill University, FIND, Médecins sans Frontières, NDWG of the Stop TB Partnership and GLI informed this process. The consensus meeting report, published in October 2014, includes four TPPs. A special supplement on TB diagnostics, published in the *Journal of Infectious Diseases* in March 2015, covered all TPPs in detail (included in the Appendix of this landscape report), and also provided additional information on the work being done to identify mutations associated with drug resistance, market projections for priority tests, and cost versus affordability analyses for HBCs.

In addition to the above, FIND is currently working to develop other TPPs, in collaboration with several stakeholders:

- latent to active disease progression: a test that is better able to predict disease in people who are actively infected;
- treatment monitoring: a test that is better able to predict failure when performed in early phases of therapy;
- test of cure: a test that can define cure and thus end of therapy;
- connected diagnostics: the requirements needed for a test to provide data to a data repository in order to deliver the data for use applications (e.g. text messaging of provider; stock management; surveillance).

5.3. Efforts to estimate market for new TB diagnostics

Efforts are also ongoing to quantify the potential market value around the various priority TPPs. A recent series of studies have attempted to quantify the current served available market value of TB diagnostics. A recent survey showed that 22 HBCs performed a total of 77.6 million sputum smears annually at a value of US\$ 137 million in over 42 000 microscopy centres.⁹ Of these, 61 % was done in Brazil, China, India, the Russian Federation and South Africa.

A detailed analysis of the amount Brazil spent on TB diagnosis showed that in 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.¹⁴⁹ 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.

Another analysis estimated the expenditure on TB diagnosis in South Africa in 2012–2013.¹⁵⁰ This study showed that South Africa has a sizeable TB diagnostic market both in terms of volume and value. In 2012, during Xpert® scale-up, the public and private sectors performed a total of 9.2 million TB diagnostic tests at an estimated total value of US\$ 98 million. The public sector accounted for 93 % of the overall

test volume and value, with microscopy and culture accounting for the majority of tests performed. In 2013, the public sector market value increased to US\$ 101 million (10% increase over 2012), while Xpert® volumes increased by 166% and the total TB test volumes decreased by 12%, compared to 2012.

Similar analyses are being completed for China and India. Data from China suggest that, in 2012, the China Centre for Disease Control and Prevention and hospital sectors performed a total of 44 million TB diagnostic tests at an overall value of US\$ 294 million.¹⁵² Tests used by the China Centre for Disease Control and Prevention sector were smear microscopy, solid and liquid culture and DST, while the hospital sector additionally used IGRAs, NAATs, adenosine deaminase and serology. The hospital sector accounted for 76% of the overall test volume and 94% of the market value.

Preliminary data from India showed that, in 2013, India's public sector performed 19.2 million tests, with a market value of US\$ 22.9 million.¹⁵³ The private sector performed 13.6 million tests, with a market value of US\$ 60.4 million when prices charged to the patient were applied. The overall market was US\$ 70.8 million when unit costs from the ingredient approach were used for the 32.8 million TB tests performed in the entire country. SSM was the most common test performed (21.1 million, 64% of all tests) and accounted for 25% of the overall market value (US\$ 17.8 million).

Based on these analyses, Kik and colleagues have made projections on the potential available market for the four priority TPPs that have been developed.⁹ Their results indicated that, out of the four TPPs, the greatest potential available market in terms of value would be for a sputum-based TB detection and DST upfront test. A test that can be deployed at lower levels of the health-care system and could be used for the detection (or rule out) of all forms of TB, such as a biomarker test or a triage test would have the largest potential market volume.

Overall, the publication of landscape reports, TPPs and market size estimates are all intended to stimulate increased investments in the area of TB diagnostics. While the overall trend is positive (as seen in the number of products and companies), TB research and development as a whole continues to be severely underfunded. A 2014 annual research and development funding report by the Treatment Action Group showed that the world invested only one third of the required US\$ 2 billion needed every year for new drugs, diagnostics and vaccines to fight the global TB epidemic effectively (<http://www.treatmentactiongroup.org/tbrd2014>).

5.4. Market shortcomings

The unprecedented scale-up of Xpert® MTB/RIF has, in many ways, reinvigorated the market for TB diagnostics. New investments, product developers and a growing pipeline of promising technologies include smaller, simpler and more robust options that may be more amenable to use at the POC, or address unmet and evolving diagnostic needs. The Xpert® MTB/RIF 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.

Despite progress, however, challenges persist. The 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 the original format of this test in lower tiers of the health-care delivery system (i.e. primary care centres and peripheral microscopy labs) are important concerns. Also, pragmatic trial data suggest that the impact of Xpert® on TB transmission and mortality may be limited due to widespread empiric therapy, weak health systems and lack of adequate linkages between diagnosis and treatment/follow-up.^{144,148} 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 the following.

Innovation: 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 (e.g. 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.

Availability: There is no true, simple, inexpensive POC TB diagnostic test: Xpert® still requires basic laboratory infrastructure (though this may change with the recently announced Omni and other platforms in the development pipeline). 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 deter wider application of newer NAATs. Unclear potential market and lack of clarity on available market share after Xpert® 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, the numbers of tests performed remain low in most HBCs, with the exception of South Africa. Lack of resources for second-line TB drugs (which are expensive) and effective programmatic management of MDR TB can deter commitment to universal DST (i.e. NTPs can be reluctant to move towards universal DST without first ensuring capacity to manage MDR TB).

Demand and adoption: Barriers to adoption of novel innovative technologies hinder uptake.

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

Demand and adoption: Currently, most NTPs do not offer universal DST, resulting in less than one in two 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.

Demand and adoption: 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 NTPs in many countries.

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 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).

Affordability: New technologies are expensive: the Xpert® 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 is 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.

Delivery: Supply constraints affecting delivery of Xpert® 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.

5.5. Potential opportunities for market intervention

As noted in section 4, the 2015 TB diagnostics technology pipeline may soon offer 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 bedaquiline, the European Medicines Agency 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 test-and-treat strategies.

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 that 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. 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 links between diagnostics manufacturers and drug development teams, to ensure better alignment of new DST products with emerging TB drug regimens.*
- *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 previous editions of this landscape report, 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.*

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Appendix. TPPs for priority diagnostics

Appendix 1. TPPs for a smear-replacement test for TB detection

Characteristic	Optimal requirements	Minimal requirements
Scope		
Goal	To develop a sputum-based test for detecting PTB at the microscopy centre level of the health-care system to support the initiation of TB therapy during the same clinical encounter or the same day	
Target population	Target groups are all patients suspected of having PTB who are able to produce sputum, in countries with a medium prevalence to a high prevalence of TB as defined by WHO ^a	
Target user of the test^b	Health-care workers with a minimum amount of training (i.e. with skills that are similar to or less demanding than those needed for performing smear microscopy)	
Setting (level of the health-care system)	Microscopy centre level (primary health-care centres with attached peripheral laboratories) or higher levels of the health-care system	
Performance characteristics		
Diagnostic sensitivity^b	Sensitivity should be >95% for a single test when compared with culture (for smear-negative cases it should be >68%; for smear-positive it should be 99%)	Sensitivity should be >80% for a single test when compared with culture (for smear-negative cases it should be >60%; for smear-positive it should be 99%)
Diagnostic specificity^b	>98% specificity when compared with culture	
Possibility of using test for treatment monitoring	Yes: a test that is able to replace smear microscopy and also be used to monitor treatment is more likely to be adopted and more likely to completely replace smear microscopy	No
Operational characteristics		
Manual preparation of samples (steps needed after obtaining sample)	No steps or 1 step; precise volume control and precise timing should not be required	A maximum of 2 steps; precise volume control and precise timing should not be required
Reagent integration	All reagents should be contained in a single device	A maximum of two external reagents should be required; these should be part of test kit
Data export (connectivity and interoperability)	Integrated ability for all data to be exported (including data on use of the device, error rates and rates of invalid tests, and personalized, protected results) over a USB port and network	Integrated ability for all data to be exported (including data on use of the device, error rates and rates of invalid tests, and non-personalized results) over a USB port
Time to result^b	<20 minutes	<2 hours
Power requirements	Battery operated with recharging capability and a circuit protector	

Maintenance and calibration^b	Preventive maintenance and calibration should not be needed until after 2 years or 5000 samples; only simple tools and minimal expertise should be required; an alert to indicate when maintenance is needed should be included; the device should be able to be calibrated remotely or no calibration should be required	Preventive maintenance should not be needed until after 1 year or 1000 samples; only simple tools and minimal expertise should be required; an alert to indicate when maintenance is needed should be included; the device should be able to be calibrated remotely, should calibrate itself or no calibration should be required
Operating temperature and humidity level	Between +5 °C and +50 °C with 90% humidity	Between + 5 °C and +40 °C with 70% humidity
Reagent kit – storage, stability, and stability during transport	2 years between 0 °C and +50 °C with 90% humidity; should be able to tolerate stress during transport (72 hours at +50 °C); no cold chain should be required	12 months between 0 °C and +40 °C with 70% humidity; should be able to tolerate stress during transport (72 hours at +50 °C); no cold chain should be required
Internal quality control	Full internal process controls are necessary, including controls for sample processing and amplification (for NAAT)	
Pricing		
Price of individual test^b (costs of reagent only; after scale-up; ex-works [manufacturing costs only, excluding shipping])	<US\$ 4 for detecting TB	<US\$ 6 for detecting TB
Capital costs for instrument^b	<US\$ 500 per module	<US\$ 1400 per module

NAAT, nucleic acid amplification test

^a High-prevalence countries are those with >40 cases per 100 000 population; medium-prevalence countries are those with 20–40 cases per 100 000 population; and low-prevalence countries are those with <20 cases per 100 000 population.

^b These characteristics were considered to be the most important, and specific consensus was asked for and reached through a Delphi survey. Sources: Adapted with permission from the WHO consensus meeting report on TPPs151 and Denkinger et al.⁷

Appendix 2. TPPs for a rapid non-sputum-based biomarker test for TB detection

Characteristic	Optimal requirements	Minimal requirements
Scope		
Goal	To develop a rapid biomarker-based test that can diagnose PTB and optimally also EPTB using non-sputum samples (e.g. urine; blood; oral mucosal transudates; saliva; exhaled air) for the purpose of initiating TB treatment during the same clinical encounter or on the same day	
Target population	Target groups are adults and children, including those who are HIV-positive and suspected of having active PTB or EPTB in countries with a medium prevalence to a high prevalence of TB as defined by WHO ^a	
Target user of the test^b	Health-care workers with a minimum of training	Trained microscopy technicians
Setting (level of the health-care system)	Health posts without attached laboratories (i.e. levels below microscopy centres) or higher levels of the health-care system	Primary health-care clinics with attached laboratories; peripheral microscopy centres or higher levels of the health-care system
Performance characteristics		
Diagnostic sensitivity for PTB in adults^b	Sensitivity should be $\geq 98\%$ for smear-positive culture-positive PTB, and $\geq 68\%$ for smear-negative culture-positive PTB in adults (i.e. sensitivity should be similar to that of the Xpert [®] MTB/RIF assay) Overall pooled sensitivity should be $\geq 80\%$ in adults with HIV infection	Overall sensitivity should be $\geq 65\%$, but should be $>98\%$ among patients with smear-positive culture-positive PTB (i.e. sensitivity should be similar to that of smear microscopy) Overall pooled sensitivity should be better than the sensitivity of smear microscopy in adults with HIV infection
Diagnostic sensitivity for EPTB in adults	Ideally, sensitivity should be $\geq 80\%$ for all forms of microbiologically confirmed EPTB ^{c,d}	Diagnosis of EPTB is an important need, and a test that can diagnose EPTB in addition to PTB will have significant benefits for individual patients; additionally, it is likely to be better accepted in the community of care providers No lower range of sensitivity was defined
Diagnostic sensitivity in children^e	Sensitivity for childhood intrathoracic TB should be $\geq 66\%$ for microbiologically confirmed TB (i.e. similar to the sensitivity of the Xpert [®] MTB/RIF assay)	Diagnosis of childhood TB is an important need, and a test that improves the diagnosis of TB in children will have significant benefits for individual patients; additionally, it is likely to be better accepted in the community of care providers No lower range of sensitivity was defined
Diagnostic specificity^b	At least as specific as the Xpert [®] MTB/RIF assay for detecting PTB, EPTB and childhood TB (i.e. the test should have 98% specificity when compared against a microbiological reference standard); the test should distinguish between active TB and latent or past infection	

Operational characteristics		
Sample type	Not invasive or minimally invasive, non-sputum samples (e.g. urine; blood; oral transudates; saliva; exhaled air)	
Manual preparation of samples (steps needed after obtaining sample)	Sample preparation should be integrated or manual preparation should not be required	A limited number of steps only; precise measuring should not be needed for any step (such as precise measuring of volumes or time)
Time to result^b	<20 minutes, including time spent preparing the sample	<1 hour, including time spent preparing the sample
Instrument and power requirement	No instrument needed	Small, portable or hand-held instrument (weighing <1 kilogram) that can operate on battery or solar power in places where power supplies may be interrupted
Maintenance and calibration^b	Disposable, no maintenance required	Preventive maintenance should not be needed until after 1 year or >1000 samples; only simple tools and minimal expertise should be required; an alert to indicate when maintenance is needed should be included; the instrument should be able to be calibrated remotely or no calibration should be needed
Operating temperature and humidity level	Between +5 °C and +50 °C with 90% humidity	Between +5 °C and +40 °C with 70% humidity
Result capturing, documentation, data display	An instrument-free test with the ability to save results using a separate, attachable reader	The test menu must be simple to navigate; the instrument should have an integrated LCD screen, simple keypad or touchscreen, and the ability to save results using either the instrument or a separate reader
Internal quality control	Internal controls should be included for processing the sample and detecting TB	Internal control included only for processing the sample
Pricing		
Price of individual test^b (costs of reagents and consumables only; after scale-up; ex-works [manufacturing costs only, excluding shipping])	<US\$ 4	<US\$ 6

HIV, human immunodeficiency virus; LCD, liquid-crystal display

^a High-prevalence countries are those with >40 cases per 100 000 population; medium-prevalence countries are those with 20–40 cases per 100 000 population; and low-prevalence countries are those with <20 cases per 100 000 population.

^b These characteristics were considered to be the most important, and specific consensus was asked for and reached through a Delphi survey.

^c The sensitivity for detecting EPTB should also be tested against a composite reference standard that includes culture with or without a nucleic acid amplification test, histology, smear microscopy, biochemical testing, presenting signs and response to treatment with anti-TB therapy, depending onsite of infection. Xpert[®] MTB/RIF testing has an estimated sensitivity for diagnosing TB of 84% for lymph node aspirates or other tissue samples, and 55% sensitivity for samples of cerebrospinal fluid, when compared with a composite reference standard, but Xpert[®] MTB/RIF testing requires invasive samples.

^d The Xpert[®] MTB/RIF has an estimated sensitivity for microbiologically confirmed TB of 85% for detecting TB in lymph node aspirates or other tissue samples, 80% for cerebrospinal fluid and 44% for pleural fluid, but testing requires invasive samples (from aspiration, biopsy, lumbar puncture or thoracentesis).

^e The test's sensitivity in children should be evaluated against a composite reference standard as defined by an international panel of experts.

Sources: Adapted with permission from the WHO consensus meeting report on TPPs151 and Denkinger et al.⁷

Appendix 3. TPPs for a community-based triage/referral test for identification of TB suspects

Characteristic	Optimal requirements	Minimal requirements
Scope		
Goal	To develop a test that can be used during a patient's first encounter with the health-care system to identify patients with any symptoms of or risk factors for active TB , including patients coinfecting with HIV, those who do not have TB and those who need referral for further confirmatory testing	To develop a test that can be used during a patient's first encounter with the health-care system to identify patients with any symptoms of or risk factors for active PTB , including patients coinfecting with HIV, those who do not have TB and those who need referral for further confirmatory testing
Target population	Adults and children with signs and symptoms of active TB at any site in countries with a medium prevalence to a high prevalence of TB as defined by WHO ^a	Adults and children with signs and symptoms of active PTB in countries with a medium prevalence to a high prevalence of TB as defined by WHO ^a
Target user of the test^b	Community health workers and informal providers who have had a minimum of training	Health workers trained to the level of auxiliary nurses
Setting (level of the health-care system)	Community level or village level or higher levels of the health-care system	Health posts and primary care clinics or higher levels of the health-care system
Performance characteristics		
Diagnostic sensitivity^b	Overall sensitivity should be >95% when compared with the confirmatory test for PTB; ^c no lower range of sensitivity was defined for EPTB ^d	Overall sensitivity should be >90% compared with the confirmatory test for PTB ^c
Diagnostic specificity^b	Specificity should be >80% compared with the confirmatory test	Specificity should be >70% compared with the confirmatory test

Operational characteristics		
Sample type	Non-sputum samples (such as urine, oral mucosal transudates, saliva, exhaled air or blood from a fingerstick)	Sputum; non-sputum samples are preferred (such as urine, oral mucosal transudates, saliva, exhaled air or blood from a fingerstick); imaging technology
Manual preparation of samples (steps needed after obtaining sample)	Sample preparation should be integrated or manual preparation should not be required (excluding waste disposal); precise timing and measuring should not be required	2 steps (excluding waste disposal); precise timing and measuring should not be required
Time to result^b	<5 minutes	<30 minutes
Instrument and power requirement	None	Small, portable or hand-held device (weighing <1 kilogram); should have an option for battery power or solar power
Maintenance and calibration^b	Disposable, no maintenance required	Preventive maintenance should not be needed until after 1 year or 1000 samples; only simple tools and minimal expertise should be required; an alert to indicate when maintenance is needed should be included; the device should be able to be calibrated remotely, should calibrate itself, or no calibration should be required
Operating temperature and humidity level	Between +5 °C and +50 °C with 90% humidity	Between + 5 °C and +40 °C with 70% humidity
Result capturing, documentation and data display	An instrument-free test with visual readout and with the ability to save results using a separate, attachable reader	The test menu must be simple to navigate; the instrument should have an integrated LCD screen, a simple keypad or touchscreen, and the ability to save results using either the instrument or a separate reader
Internal quality control	Internal controls should be included for processing the sample and detecting TB	Internal control included only for processing the sample
Pricing		
Price of individual test^b (costs of reagents and consumables only; after scale-up; ex-works [manufacturing costs only, excluding shipping])	<US\$ 1.00	<US\$ 2.00

HIV, human immunodeficiency virus; LCD, liquid-crystal display

^a High-prevalence countries are those with >40 cases per 100 000 population; medium-prevalence countries are those with 20–40 cases per 100 000 population; and low-prevalence countries are those with <20 cases per 100 000 population.

^b These characteristics were considered to be the most important, and specific consensus was asked for and reached through a Delphi survey.

^c The performance characteristics of the triage test need to match those of the confirmatory test that will be used.

^d The sensitivity of the triage test should be compared with the sensitivity of a composite reference standard (that includes culture with or without a nucleic acid amplification test, histology, smear microscopy, biochemical testing, presenting signs and response to treatment with anti-TB therapy, depending on site of infection) to account for the fact that the test may detect cases of early TB or EPTB in cases in which a standard microbiological reference standard might not perform well.

Sources: Adapted with permission from the WHO consensus meeting report on TPPs151 and Denking et al.⁷

Appendix 4. TPPs for a molecular DST for TB used at the microscopy centre level

Part A. Scope of drug susceptibility testing at the microscopy centre level

Characteristic	Optimal	Minimal
Goal of test	Diagnosis of TB disease and detection of drug resistance to inform decision-making about the optimal first-line regimen (HRZE, PaMZ or other FLQ-based regimens) for treatment, and possibly to detect the presence of additional resistance to second-line anti-TB agents and the need for further testing	
Target population	Target groups are all patients suspected of having TB, with a special focus on those at high risk of morbidity and mortality from drug-resistant TB, such as PLHIV and those at high risk of having MDR TB (e.g. household contacts of patients diagnosed with MDR TB and people with a history of TB, especially those for whom first-line therapy has failed) in countries with a medium prevalence to a high prevalence of TB as defined by WHO ^a	
Target user of test	Health-care workers with training necessary for performing smear microscopy	
Lowest setting of implementation (health system level)	Microscopy centre level or higher levels of the health-care system	

HRZE, isoniazid, rifampicin, pyrazinamide, ethambutol; PaMZ, Pa824, moxifloxacin, pyrazinamide; MDR TB, multidrug-resistant TB

^a WHO categories: High-prevalence countries are those with >40 cases per 100 000 population; medium-prevalence countries are those with 20–40 cases per 100 000 population; and low-prevalence countries are those with <20 cases per 100 000 population.

Part B. Performance characteristics

Characteristic	Optimal	Minimal
Diagnostic sensitivity – TB detection	Sensitivity for detecting TB should be >95% for a single test when compared with two liquid cultures; for smear-negative TB it should be >68%; for smear-positive TB it should be 99%	Sensitivity should be >80% for a single test when compared with culture (for smear-negative cases it should be >60%; for smear-positive it should be 99%)
Diagnostic specificity – TB detection	Specificity should be >98% for a single test when compared with culture	Specificity should be >98% for a single test when compared with culture
Priority of drugs tested	In order of decreasing importance: <ol style="list-style-type: none"> 1. RIF 2. FLQ; including moxifloxacin (MOX) 3. INH and PZA; equally important 4. AMG/capreomycin (AMG/CAP) Optimally, all drugs would be included, but as a minimum at least RIF should be included	
Diagnostic sensitivity for DST compared against genetic sequencing as the reference standard	Sensitivity should be >98% for detecting targeted SNPs for resistance to RIF, FLQ, PZA, INH and AMG/CAP when compared with genetic sequencing	Sensitivity should be >98% for detecting targeted SNPs for resistance to RIF, and 95% sensitivity for detecting targeted SNPs for resistance to FLQ, PZA, INH and AMG/CAP when compared with genetic sequencing
Diagnostic sensitivity for DST compared against phenotypic DST as a reference standard	>95% sensitivity for detecting RIF, FLQ, PZA, INH and AMG/CAP resistance in comparison to recommended phenotypic culture reference DST for specific anti-TB agent	>95% sensitivity for detecting RIF resistance; >90% for detection of FQ, PZA, INH and AMG resistance in comparison to recommended phenotypic culture reference DST for specific anti-TB agent

Diagnostic specificity for DST compared against genetic sequencing as the reference standard	Specificity should be $\geq 98\%$ for any anti-TB agent for which the test is able to identify resistance when compared against genetic sequencing as the reference standard	
Diagnostic specificity for DST compared against genetic sequencing as the reference standard	Specificity should be $\geq 98\%$ for any anti-TB agent for which the test is able to identify resistance when compared against genetic sequencing as the reference standard	
LOD – TB detection (1st reaction)	Should be better than the Xpert® MTB/RIF for TB case-detection, i.e. < 4.5 genome equivalents/reaction and $< 10e2$ cfu/assay using one sample	Should be between smear microscopy and the Xpert® MTB/RIF for TB case-detection, i.e. between $10e2$ cfu/assay and $10e5$ cfu/assay using one sample
LOD – TB detection (in the context of 2nd reaction for resistance testing)	Should be no worse than the Xpert® MTB/RIF for TB case-detection, i.e. ≥ 4.5 genome equivalents/reaction and 131 cfu/mL of sputum	Should be between smear microscopy and the Xpert® MTB/RIF for TB case-detection, i.e. between $10e2$ cfu/assay and $10e5$ cfu/assay using one sample
Analytical specificity – TB detection	No cross-reactivity with other organisms, including NTM	No cross-reactivity with other organisms, including NTM
Indeterminate results detection	$< 2\%$	$< 5\%$
Reproducibility	Interassay coefficients of variance should be $\leq 10.0\%$ at the high and low extremes of the assay	
Interfering substances	No interference should be caused by those substances known to occur in the human respiratory and pulmonary tracts, including blood that could potentially inhibit a PCR reaction, and substances used to treat or alleviate respiratory disease or symptoms	
Assay design	The assay should be designed in such a manner that the addition of or removal of analytes does not require extensive analytical and clinical re-verification and revalidation of the assay	
Treatment monitoring capability	Yes	No

DST, drug-susceptibility testing; PZA, pyrazinamide; RIF, rifampicin; FLQ, fluoroquinolone; MOX, moxifloxacin; INH, isoniazid; AMG, aminoglycoside; CAP, capreomycin; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction

Part C: Operational characteristics

Characteristic	Optimal	Minimal
Sample type	Sputum raw	Sputum raw
Acceptable range for sample volume	Any sample from 0.1–10 mL is acceptable	Any sample from <0.5–2 mL is acceptable
Manual sample prep (total hands-on steps after obtaining sample)	No steps or 1 step; precise volume control and precise timing should not be required	Maximum of 2 steps; precise volume control and precise timing should not be required
Reagent integration	All reagents should be contained in a single device	A maximum of 2 external reagents should be needed and if required, should be included in the test kit
Time-to-result	<30 minutes (for detection and resistance testing)	<2 hours (for resistance testing alone)
Daily throughput per module	>25 tests	>5 tests
Sample capacity and throughput	Multiple samples should be able to be tested at the same time; random access should be possible	Batching should be possible
Walk-away operation	These features are required; there should not be a need for operator intervention once the sample has been placed into or on the instrument	No more than 1 step of operator intervention should be needed once the sample has been placed into or on the system
Biosafety	Should have the same requirements as Xpert® MTB/RIF assay	Should have the same requirements as Xpert® MTB/RIF assay
Waste disposal solid	Should require no more than smear microscopy; should have the possibility of recycling some waste	Should require no more than Xpert® MTB/RIF
Waste disposal infectious material	Should require no more than Xpert® MTB/RIF	Should require no more than Xpert® MTB/RIF
Multi-use platform	Yes	None required
Instrumentation	Ideally, would be a single integrated system that is modular to allow throughput to be increased if needed	Up to two instruments within the system that are independent of each other
Power requirements	Battery operated with the ability to run for 1 day on the battery, and with recharging capability (which could be solar powered) and a circuit protector	Capable of running on standard electricity plus an uninterrupted power supply unit to enable a cycle to be completed in case of a power outage; a circuit protector should be included; the uninterrupted power supply and circuit protector must be integrated within the system
Maintenance/calibration	Preventive maintenance should not be needed until after 2 years or >5000 samples; an alert should be included to indicate when maintenance is needed; should be able to be calibrated remotely, or no calibration should be needed	Preventive maintenance should not be needed until after 1 year or 1000 samples; an alert should be included to indicate when maintenance is needed; should be able to be calibrated remotely, or no calibration should be needed
Data analysis	Data analysis should be integrated into the device; a personal computer should not be required; exported data should be capable of being analysed on a separate or networked personal computer	

Result documentation, data display	An integrated results screen and the ability to save and print results should be included; the device should have a USB port	An integrated results screen and the ability to save results should be included; the device should have a USB port
Regulatory requirements	Manufacturing of the assay and system should comply with ISO EN 13485 or higher standards or regulations, and comply with ISO IEC 62304 Medical Device Data Systems; the manufacturing facility should be certified and authorized for use by a regulatory authority that is a member of the International Medical Device Regulators Forum, formerly known as the Global Harmonization Task Force; the assay must be registered for in vitro diagnostic use	
Data export (connectivity and interoperability)	All data should be able to be exported (including data on use of the device, error rates and rates of invalid tests, and personalized, protected results) over a USB port and network; network connectivity should be available through an Ethernet, Wi-Fi or GSM/UMTS mobile broadband modem, or a combination of these; results should be encoded using a documented standard (such as HL7) and be formatted as JSON text; JSON data should be transmitted through HTTP(S) to a local or remote server as results are generated; results should be stored locally and queued during network interruptions use of the device, error rates or rates of invalid tests, and non-personalized results) over a USB port to be sent as a batch when connectivity is restored	Integrated ability for all data to be exported from the device in a user-friendly format (including data on use of the device, error rates or rates of invalid tests, and non- personalized results) over a USB port
Electronics and software	Should be integrated into the instrument	Should be integrated into the instrument
Operating temperature/humidity	Between +5 °C and +50 °C with 90% humidity	Between +5 °C and +40 °C with 70% humidity
Reagent Kit transport	No cold chain should be required; should be able to tolerate stress during transport for a minimum of 72 hours between -15 °C and +50 °C	No cold chain required; should be able to tolerate stress during transport for a minimum of 72 hours between -15 °C and +40 °C
Reagent Kit storage and stability	2 years between +5 °C and +40 °C with 90% humidity; should be able to tolerate stress during transport for a minimum of 72 hours at +50 °C; no cold chain should be required	12 months between +5 °C and +35 °C with 70% humidity; should be able to tolerate stress during transport for a minimum of 72 hours at +50 °C; no cold chain should be required
Additional supplies (not included in kit)	None	None
Internal quality control	Full controls for sample processing, amplification and detection of TB should be included	
Training and education needs	6 work hours for staff at the level of a microscopy technician	3 days (or 24 work hours) for staff at the level of a laboratory technician

GSM, Global System for Mobile Communications; ISO, International Organization for Standardization; UMTS, Universal Mobile Telecommunications System

Sources: Adapted with permission from the WHO consensus meeting report on TPPs,151 and Denkinger CM et al.⁷