

TUBERCULOSIS

Diagnostics Technology Landscape
5th Edition, May 2017

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Diagnostics Technology Landscape



CONTENTS

6	Abbreviations and acronyms
8	Foreword
9	Executive summary
9	<ul style="list-style-type: none">• Tuberculosis (TB) is an urgent public health problem, but many people do not have access to critical diagnostic tools
10	<ul style="list-style-type: none">• Significant events since the last TB diagnostics technology landscape
12	Introduction
13	Methodology
13	<ul style="list-style-type: none">• Acknowledgements and declarations of interest
14	Overview of the TB diagnostics technology landscape
17	Recent policy and recommendation updates for TB diagnostics
23	Screening and triage tools
23	<ul style="list-style-type: none">• Digital chest X-rays
24	<ul style="list-style-type: none">• Volatile organic compounds (VOCs)
26	<ul style="list-style-type: none">• Immune response-based screening tests for MTB exposure
29	Solutions for diagnostic sample transport
31	Automated microscopy
34	Culture-based tools for the diagnosis of TB and DST
35	Biomarkers to detect MTB exposure and TB disease
36	<ul style="list-style-type: none">• Immune response-based tests
37	<ul style="list-style-type: none">• Serologic and antigenic biomarkers of TB
39	<ul style="list-style-type: none">• Other biomarker development news
40	NAATs and sequencing methods for TB diagnosis and DST
40	<ul style="list-style-type: none">• Update to the NAAT pipeline
42	<i>Stratification of diagnostic NAATs in the test continuum</i>
43	<i>Application of NGS to TB diagnosis and control</i>
47	<i>Automated batched PCR</i>
53	<i>Autonomous NAAT reagents for use in open systems</i>
55	<i>LPAs</i>
58	<i>Microarrays</i>
64	<i>Modular, cartridge-based, fully automated NAATs</i>
68	<i>NAATs for use at peripheral centres</i>
79	Appendix
79	<ul style="list-style-type: none">• Status update on NAAT-based technologies for reference and intermediate laboratories
81	<ul style="list-style-type: none">• Update on technology status of NAAT-based technologies intended for use in microscopy centres
82	References

FIGURES

- 15 **Figure 1.** Current FIND TB diagnostics pipeline listing the development phases and types of technologies in development or evaluation
- 24 **Figure 2.** Prototype Nanosynth breath test and detector
- 25 **Figure 3.** Hand-held Aeonose™ device
- 27 **Figure 4.** QuantiFERON-TB Gold Plus kit from Qiagen: reagents and ELISA plates (left) and collection materials (right)
- 31 **Figure 5.** TBDx system
- 38 **Figure 6.** Determine™ TB LAM Ag rapid assay, with strip ready for use shown on the right
- 41 **Figure 7.** Current and emerging automated, semi-modular or non-integrated TB NAATs; their intended laboratory location and release date (actual or anticipated)
- 46 **Figure 8.** Workflow of ReSeqTB bridging targeted NGS platforms to provide rapid patient management decisions
- 48 **Figure 9.** Abbott Molecular platforms for automated sample preparation (*m2000sp*, left) and real-time PCR analysis (*m2000rt*, right) for MTBC and first-line DST
- 49 **Figure 10.** Akonni TruTip® Automated Sample Prep Workstation
- 51 **Figure 11.** Hain Lifescience FluoroType® MTB [A] and FluoroType® MTBDR [B] processes
- 60 **Figure 12.** Akonni TruDx®2000 platform with TruTip® extraction pipette, TruArray® processor and TruArray® scanner (left); TruArray® test, microfluidic valve-less design for simultaneous on-slide PCR and microarray hybridization in a closed format (right)
- 62 **Figure 13.** Veredus Laboratories VerePLEX™ Biosystem and VereMTB™ Detection Kit
- 63 **Figure 14.** Hydra 1K hand-held platform (left) and chip (centre)
- 65 **Figure 15.** Cepheid Inc. GeneXpert® IV System (GX-4) with four independent modules for processing test cartridges (left) and the Xpert® MTB/RIF cartridge (right)
- 66 **Figure 16.** Cepheid Inc. Xpert® MTB/RIF Ultra cartridge
- 69 **Figure 17.** HumaLoopT instrument (from HUMAN Diagnostics Worldwide, top left) and the Pure DNA Extraction kit
- 71 **Figure 18.** Molbio Diagnostics technologies: current and pending products for NAAT-based detection of MTBC and drug resistance
- 73 **Figure 19.** Ustar Biotechnologies EasyNAT™ TB assay
- 75 **Figure 20.** GeneXpert® Omni
- 76 **Figure 21.** KGI TBDx system
- 77 **Figure 22.** Scanogen reader and test cartridge for use in microscopy centres

TABLES

- 42 **Table 1.** Summary of NAATs relating their role in TB diagnosis in terms of intended location of use, throughput and other key factors
- 56 **Table 2.** Current LPA products and associated equipment marketed for MTBC diagnosis, mycobacterial speciation and genotypic DST

ABBREVIATIONS AND ACRONYMS

AMG	aminoglycoside
AMK	amikacin
BCG	Bacillus Calmette-Guérin
BD	Becton Dickinson
BRICS	Brazil, Russian Federation, India, China and South Africa
CAD4TB	Computer Aided Detection for Tuberculosis
CAP	capreomycin
CE	European Conformity (Conformité Européenne) certification
CE-IVD	European Conformity (Conformité Européenne)-in vitro diagnostic
CFDA	China Food and Drug Administration
CFP10	culture filtrate protein 10
cfu	colony forming units
CXR	chest X-ray
DCXR	digital chest X-ray
DST	drug susceptibility testing
ELISA	enzyme linked immunosorbent assay
EMB	ethambutol
ESAT-6	early secreted antigenic target
FDA	Federal Drug Administration
FIND	Foundation for Innovative New Diagnostics
FLQ	fluoroquinolone
HBC	high-burden country
HIV	human immunodeficiency virus
IFN-γ	interferon-gamma
IGRA	interferon-gamma release assay
INH	isoniazid
IRISA™	InterGam Rapid Immuno Suspension Assay
ISO	International Organization for Standardization
KAN	kanamycin
KGI	Keck Graduate Institute
LAM	lipoarabinomannan
LAMP	loop-mediated amplification
LF	lateral flow
LF-LAM	lateral flow urine lipoarabinomannan
LMIC	low- and middle-income country
LPA	line probe assay
LTBI	latent TB infection
MDR	multidrug resistant
MGIT™	mycobacterial growth indicator tube
miRNA	micro ribonucleic acid

mL	millilitre
mm	millimetre
MODS	microscopically observed drug susceptibility
MOX	moxifloxacin
MTB	<i>Mycobacterium tuberculosis</i>
MTBC	<i>Mycobacterium tuberculosis</i> complex
MTB/RIF	<i>Mycobacterium tuberculosis</i> /rifampicin resistance
MTBDRs/	<i>Mycobacterium tuberculosis</i> drug resistance second line
NAAT	nucleic acid amplification test
NGS	next-generation sequencing
NTM	non-tuberculous mycobacteria
NTP	national tuberculosis programme
NWGHF	North Western Global Health Foundation
OFX	ofloxacin
PCR	polymerase chain reaction
PEPFAR	President's Emergency Plan for AIDS Relief
POC	point of care
PLHIV	people living with HIV
PTB	pulmonary TB
PZA	pyrazinamide
Q1, 2, 3, 4	Quarter 1, 2, 3, 4
RDT	rapid diagnostic test
RIF	rifampicin
RR	rifampicin resistant
RR TB	rifampicin-resistant TB
SL-LPA	second-line probe assay
SLID	second-line injectable drug
SSM	sputum smear microscopy
STR	streptomycin
TB	tuberculosis
TOP TB	totally optimized PCR for TB
TPP	target product profile
TRCR	transcription reverse-transcription concerted reaction
TST	tuberculin skin test
UK	United Kingdom
µL	microlitre
US	United States
USA	United States of America
USAID	United States Agency for International Development
USB	universal serial bus
VOC	volatile organic compound
WGS	whole genome sequencing
WHO	World Health Organization
XDR	extensively drug resistant

FOREWORD

Tuberculosis (TB) continues to be a major public health threat despite being a curable disease. Latest figures from 2015¹ indicate an estimated 10.4 million people had TB, and 1.8 million people died (1.4 million HIV negative and 400 000 HIV positive). Of further concern is that 480 000 cases of multidrug-resistant (MDR) TB^a and a further 100 000 that were estimated to be rifampicin-resistant (RR) TB have occurred in the same period.¹

The rapid and accurate diagnosis of TB infection and disease is critical for timely initiation of treatment and, ultimately, control of the disease. Of the 10.4 million people who developed TB in 2015, 4.3 million cases were not diagnosed or notified and only one quarter of RR/MDR TB cases (132 000) were detected and reported. The underdiagnosis and underreporting of TB may be due to limited or delayed access to appropriate diagnosis and care, large private sectors not reporting cases, and the lack of access to appropriate diagnostic tools due to geographic and/or financial barriers.²⁻⁴ Most of the currently available TB diagnostics are ill-adapted to resource-limited settings or specific patient needs; or may be priced out of reach.

Many countries still rely on tools such as sputum smear microscopy but new diagnostics are slowly changing the TB diagnostics landscape. In 2016, the World Health Organization made policy guidance statements for five new or improved TB diagnostic products.⁵⁻⁹ Further changes are expected, with unmet needs identified and articulated in target product profiles,¹⁰ and a technology pipeline promising new products to address these needs. Several of these are currently undergoing evaluation in field studies. This updated report reviews the status of current, emerging and potential technologies.

^a TB strains that are resistant to RIF and INH.

EXECUTIVE SUMMARY

Tuberculosis (TB) is an urgent public health problem, but many people do not have access to critical diagnostic tools.

In 2016, the World Health Organization (WHO) noted that the TB epidemic was larger than previously estimated, with 10.4 million new cases of TB in 2015, the increase driven primarily by improved case notification from the private sector in India. However the TB incidence rate and the number of deaths from TB continue to fall globally.^{1,11-13} In 2015, an estimated 1.4 million people died from this largely curable disease with a further 400 000 deaths from TB reported among people living with HIV.¹ The *Global tuberculosis report* (2015) noted that TB now ranks above HIV as a leading cause of death worldwide.¹¹

Access to accurate TB diagnostics and drug susceptibility tests enable TB programmes to identify TB cases and select appropriate treatment. WHO estimates that almost 40% of all TB cases in 2015 were either not diagnosed or cases not reported to national tuberculosis programmes. The *Global tuberculosis report* (2016) noted: “If everyone with TB had a timely diagnosis and high-quality treatment, the case fatality rate would be low in all countries”.¹ Recognizing the critical importance of drug susceptibility testing, the WHO End TB Strategy includes universal drug susceptibility testing as a key component of its first pillar: integrated, patient-centred TB care and prevention. Several of the molecular tests currently in development also offer genotypic drug susceptibility testing, either integrated into the diagnostic test or as a reflex assay for TB-positive samples.

Improvements in the diagnosis of multidrug-resistant tuberculosis (MDR TB) are associated with greater implementation of molecular tests, in particular, line probe assays and the Cepheid Inc. GeneXpert® MTB/RIF assay (hereinafter Xpert® MTB/RIF). However, an estimated 75% of all MDR TB cases are still not identified and reported.¹

Robust and accurate molecular technologies would have the greatest impact on improving diagnosis if they were made available at microscopy centres, where most people with signs and symptoms of TB seek a diagnosis.¹⁴ Although candidate products are described in this landscape report, to date, no molecular test has been sufficiently assessed to demonstrate the potential to do this.

The WHO treatment guidelines for drug-resistant TB were updated in 2016.¹⁵ They note that rifampicin-resistant TB cases, with or without resistance to isoniazid should be treated with MDR TB regimens, with the recommendation that a shorter regimen (9–12 months) be used for cases that are not resistant to second-line drugs. Furthermore, WHO issued interim guidance on the use of bedaquiline in 2013 and the use of delamanid in 2014;^{16,17} and policy guidance on the use of delamanid for the treatment of MDR TB in children (aged 6–17 years).¹⁸

Children^b accounted for 6.3% of the new and relapse TB disease cases notified globally in 2015.¹ An estimated 1 million children became ill with TB in 2015, while 210 000 children died from TB in 2015. Accurately diagnosing paediatric TB continues to be a challenge. Conventional TB tests rely on identifying bacteria in a sputum sample. However, children can find it hard to produce sputum, and when they do, it is often low in bacterial load.

Current molecular tests can determine whether a patient has active TB disease or has been exposed to TB. Combinations of tests can identify whether a patient has latent TB infection but so far cannot determine the risk of progression to active TB disease. Nor can tests determine if an active TB case has been cured. Though developers have made progress with tests to detect bacterial DNA or antigens they continue to face significant challenges. Target product profiles for tests that predict progression from latent TB infection to active disease, including evaluation protocols to assess these tests, are expected in Q2 2017.

Next-generation sequencing technologies combine diagnosis of the bacteria, drug-resistance genotyping and molecular epidemiology from a single test or sample. To date, next-generation sequencing for clinical purposes has mainly been used in the diagnosis and treatment of noncommunicable diseases such as cancers. But the value of this technique has also been shown for challenging infectious diseases such as TB.

While this technology is not available in resource-limited settings and is aimed at tertiary reference-level facilities, smaller platforms are available. Yet, a number of challenges remain with the use of next-generation sequencing, including acquiring and extracting of enough bacterial DNA, especially from compromised samples, or those with low bacterial load. A number of groups are developing software to help people without bioinformatic skills to process and analyse large sets of raw data. The Foundation for Innovative New Diagnostics (FIND) is reviewing current technologies and methods to identify where an “end-to-end” next-generation sequencing system (a device that processes samples from insertion to results) could be implemented in laboratories in low- and middle-income countries.

Significant events since the last TB diagnostics technology landscape

Since the publication of the fourth edition of the *Unitaid Tuberculosis diagnostics technology and market landscape* report in October 2015, there have been a number of significant events.¹⁹

WHO has released policies on the lateral-flow urine lipoarabinomannan assay (highly restricted use recommendation),⁵ as well as an updated policy on use of molecular line-probe assays for the detection of resistance to isoniazid (INH) and rifampicin (RIF).⁷ WHO has also recently released policy statements on the use of molecular line-probe assays for the detection of resistance to second-line anti-TB drugs,⁸ and a robust molecular assay to detect pulmonary TB in sputum (the TB-LAMP assay⁶ from

^b Aged <15 years.

the Eiken Chemical Corp., Japan). Most recently, WHO recommended the Cepheid GeneXpert® MTB/RIF Ultra assay (hereinafter Ultra MTB/RIF),⁹ a more sensitive cartridge-based assay that is intended to ultimately replace the Xpert® MTB/RIF assay currently used by many national tuberculosis programmes. In addition to these key events, there have been two large company acquisitions involving key manufacturers in the TB diagnostic space. Alere Inc. (USA) is currently in the process of being acquired by Abbott Laboratories (USA). In September 2016, Cepheid Inc. (USA) was purchased by the Danaher Corporation (USA) for US\$ 4 billion.

The molecular test pipeline appears to be contracting. Some products have underperformed in trials, whilst others do not yet have independent field evaluations or have reported major delays. As a result, molecular testing at microscopy centres in the near future may involve the roll out of the Loopamp™ MTBC assay (Eiken Chemical Corp, Japan) in addition to the GeneXpert® platform. FIND have negotiated pricing for this new assay and its associated equipment.²⁰ Previous editions of this landscape report stated that Alere™ q and the Cepheid Inc. Omni systems would be expected in the near future. However, both of these products have experienced challenges to their development. Alere Inc. has stopped development work for its q instrument to host assays for *Mycobacterium tuberculosis* (MTB) and drug resistance. Meanwhile, Cepheid Inc. has announced that production of the GeneXpert® Omni (hereinafter Omni) is delayed due to technical redevelopment challenges associated with manufacturing to ensure compatibility of test cartridges with the Omni platform. Omni will be assessed for equivalent performance with the current GeneXpert® platform and WHO will issue implementation guidance. Field testing in a variety of settings, led by FIND, for the new, high-sensitivity cartridge, the Ultra MTB/RIF, started in Q1 2016. In March 2017, WHO released a statement extending the recommendations for the use of Xpert® MTB/RIF to Ultra MTB/RIF, after the WHO Technical Expert Group concluded that the Ultra MTB/RIF is non-inferior to Xpert® MTB/RIF.⁹ A policy update is expected in 2018 and the timeline for this product's release is not known.

Other developers have noted European Conformity-in vitro diagnostic (CE-IVD) registration of their products in 2016, including Autoimmun Diagnostika (Germany) with three line-probe assays for drug resistance genotyping and Hain Lifescience (Germany) with its FluoroType® real-time polymerase chain reaction assays for detection of MTB and genotyping of drug resistance.

Molbio (India) has increased its portfolio of technologies for genotyping of drug resistance and made improvements to its sample processing or detection technologies. It has received or is about to receive CE-IVD markings for them. However, many of the developers with products in phases 1 and 2 have noted delays in the release dates scheduled for their products. The reasons are varied but typically reflect a lack of funding and/or unanticipated technical challenges during development work.

This diagnostics technology landscape outlines the progress with the development of tools for screening and triage, sample transport, automated microscopy, culture-based tools for diagnosis of TB and drug susceptibility testing, molecular tests to detect MTB exposure and TB disease, including sequencing methods.

INTRODUCTION

Recent World Health Organization (WHO) estimates indicate, in 2015 alone, over 10.4 million people fell ill with active tuberculosis (TB), including 580 000 people with a rifampicin-resistant (RR) form of TB, and over 1.4 million people died.¹ Access to rapid, simple, affordable and reliable TB diagnostics at the point of care (POC) is crucial to accelerating a reduction in TB incidence and achieving the global goals of ending TB. As new regimens for drug-resistant TB develop, screening and diagnostic cascades and technologies need to evolve to keep up with needs of patients to receive a timely and accurate diagnosis of TB and initiation of treatment. In 2015, only 6.1 million TB cases were notified to national tuberculosis programmes (NTPs), 57% of which were bacteriologically confirmed via a WHO-recommended test.¹ The remaining 4.3 million cases were either not diagnosed, or not notified to TB programmes.

Timely access to accurate and reliable diagnostics is necessary for the rational and responsible use of antimicrobials. Universal access to drug susceptibility testing (DST), as called for in the WHO End TB Strategy, can include both phenotypic and genotypic testing methods. The need for access to diagnostics for children with TB and DST to identify cases of drug-resistant TB is crucial in ensuring patients receive timely and appropriate treatment. In 2015, only 30% of the 3.4 million of the biologically confirmed TB cases notified globally were reported to have had DST for at least rifampicin (RIF).¹ Overall, there has been a small increase in DST coverage (22% in 2014) but this varies substantially between countries. Only 23% of the estimated 580 000 cases with RR TB, were detected and notified, with 36% of these notified cases also receiving DST for fluoroquinolones (FLQ) and second-line injectable (SLID) agents.¹

Diagnostics for the detection of latent TB infection (LTBI) are of growing interest. Studies have shown that treating patients with LTBI reduces the risk of active TB in people living with HIV (PLHIV) especially when a diagnostic is used to identify those infected with TB.²⁷ However, those at greatest risk of developing active TB after an infection, e.g. PLHIV and those who are immunosuppressed, often are not detected by using the current immunobased assays.

Therefore, a dynamic understanding of existing and forthcoming technologies is key for stakeholders (including ministries of health, Unitaaid and other funders) in facilitating access to appropriate TB diagnostic tools and improving TB care in high-burden TB and/or HIV settings. This edition of the *Unitaid Tuberculosis Diagnostics Technology Landscape* report is intended to complement earlier reports, and presents a comprehensive overview of TB diagnostic technologies that are commercially available or close to market. Previous editions of this landscape report are available at <http://unitaid.org/landscapes>.

METHODOLOGY

The *Unitaid Tuberculosis Diagnostics Technology Landscape (2017)* was developed by David Boyle (PATH, Seattle). The material in this landscape report was gathered by the author 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 derived by continued outreach to known diagnostic manufacturers and leading technology developers working within the TB diagnostic market, ranging from established multinational diagnostic companies to startups and academic groups. Information was provided through questionnaires addressing their technology, target population(s), intended market, pricing and national or regional regulatory approvals and manufacturing standards. Only photographs received from manufacturers and developers were included in this report. Further information on product development and diagnostic validation studies was acquired via press releases, online technology updates, the peer-reviewed literature and accessing clinical study websites. The author continually assesses peer-reviewed literature to identify new technologies, assays or validation studies on existing tools to update the landscape reports.

With the dissemination of Unitaid landscape reports since 2012, diagnostics developers also now approach the author with unsolicited 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 landscape report are derived only from independent studies that have been published in peer-reviewed literature in an attempt to validate the veracity of claims from developers regarding test accuracy. All images have been reproduced with permission of the respective companies or agencies.

ACKNOWLEDGEMENTS AND DECLARATIONS OF INTEREST

The author and Unitaid are grateful to all the industry representatives who shared information (and images) on their products, and also acknowledge technical input from Madhukar Pai (McGill University, Montreal). Industry and FIND contributions were technical in nature. Unitaid acknowledge contributions and review of the document by Laboratories, Diagnostics and Drug-Resistance unit at WHO. The diagnostic technology pipeline was developed independently by David Boyle with support from Unitaid. David Boyle serves as a consultant to the Keck Graduate Institute (KGI) TBDx System project (R01AI111477) and receives funding from Ustar Biotechnologies (China) on a TB-related project. He has no other commercial/financial interests to declare pertaining to information described in this landscape report.

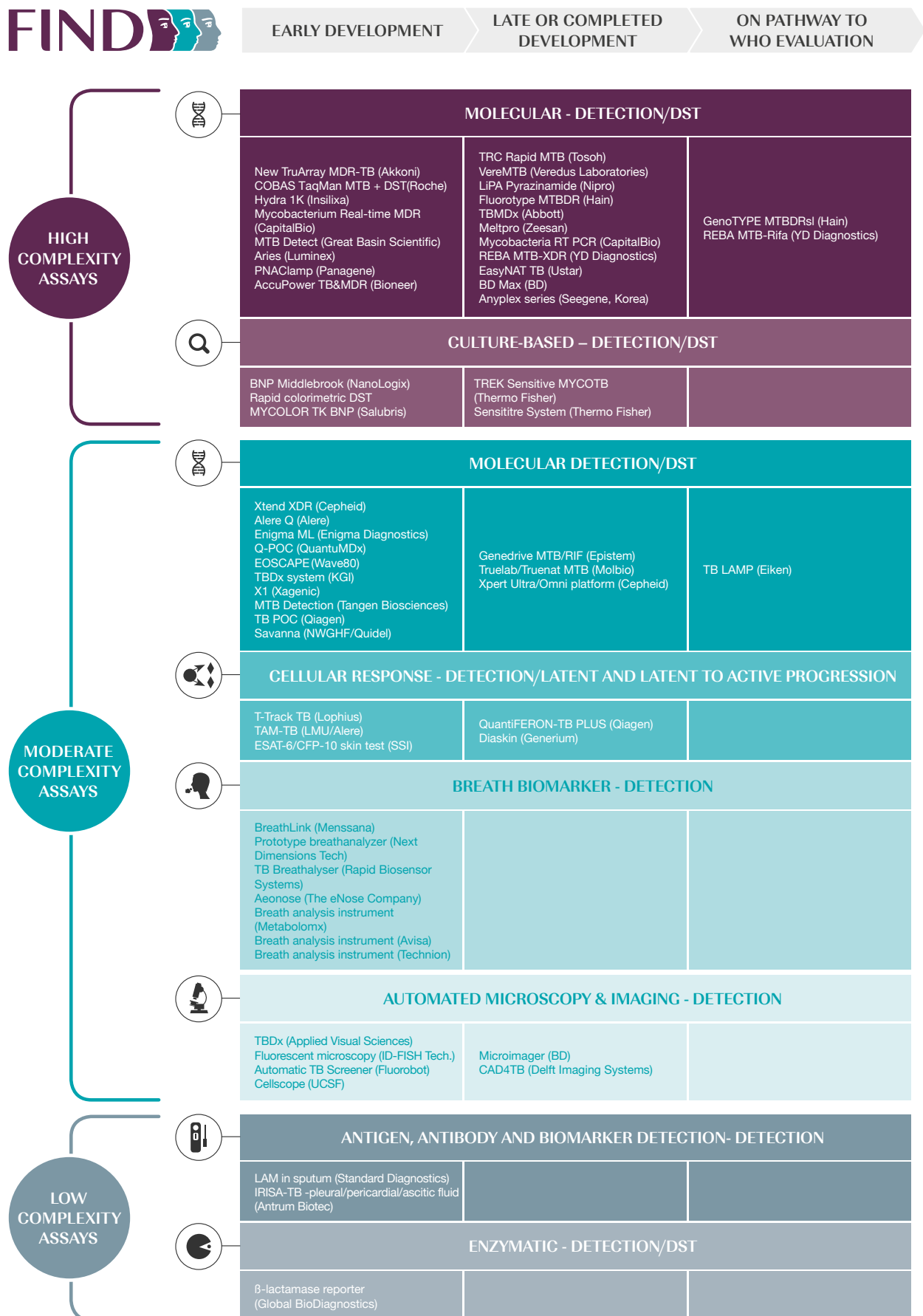
OVERVIEW OF THE TB DIAGNOSTICS TECHNOLOGY LANDSCAPE

This landscape report is an update to the fourth edition,¹⁹ which detailed the primary methods for the diagnosis of pulmonary TB (PTB) and many of the technologies and products associated with these methods. Since the publication of the fourth edition in October 2015, there have been a number of significant events. This landscape report highlights these events, provides the latest updates from developers and diagnostic manufacturers, 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. The product categories discussed in this landscape report include screening and triage tools, solutions for diagnostic transport, automated microscopy, culture-based tools for the diagnosis of TB and DST, biomarkers to detect exposure to *Mycobacterium tuberculosis* (MTB) and TB disease. In particular, this landscape report features information on the advances in “-omics” research and next-generation sequencing (NGS) tools for their potential to identify biomarkers with strong predictive value for TB infection and provide a highly accurate diagnostic method for TB infection, genotypic DST and molecular epidemiologic information. As with earlier editions, this landscape report places a strong emphasis on the development of nucleic acid amplification tests (NAATs) because of their potential to replace sputum smear microscopy (SSM) and/or offer faster, more effective diagnosis of PTB and multidrug-resistant (MDR) TB. Other areas of TB diagnostics are noted, but unless there are significant changes from earlier landscape reports, these areas are only discussed briefly.

This diagnostics technology landscape outlines the progress with the development of tools for screening and triage, sample transport, automated microscopy, culture-based tools for diagnosis of TB and DST, biomarkers to detect MTB exposure and TB disease; and NAATs and sequencing methods for TB diagnosis and DST. Figure 1 shows the updated overall diagnostic pipeline, including the technologies used in the patient screening and diagnosis cascade.

Since the fourth edition of this landscape report, the molecular diagnostic pipeline appears to be contracting, with some products underperforming in trials (or with insufficient evidence) and others reporting major delays in timelines (see Figure 7 in the section “[Update to the NAAT pipeline](#)”). Recently, WHO conditionally recommended the use of loop-mediated amplification (LAMP) as a low complexity NAAT suitable for use at microscopy and higher-tier levels.⁶ The broader use of NAATs has improved the diagnosis of MDR TB.¹¹ In March 2017, WHO recommended Cepheid GeneXpert® MTB/RIF Ultra cartridge (hereinafter Ultra MTB/RIF) that can both diagnose MTB and indicate MDR TB.⁹ This landscape report highlights the development of the extant NAATs assays in terms of product release, regulatory approvals and technologies that are no longer on the market. The route to market of NAATs is generally impeded by a lack of or delay in independent validation studies to inform on the performance of the products in their intended use-case settings. This landscape report notes

Figure 1. Current FIND TB diagnostics pipeline listing the development phases and types of technologies in development or evaluation



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several key policy decisions and peer-reviewed articles describing the performance of certain NAATs, including two of the next-generation tools (previously called the “fast followers”). These documents may play an important role in helping the TB community assess if the performance of these tools matches the needs and expectations of the TB community.

The application of NGS is becoming more affordable and is increasingly being used for high-resolution molecular epidemiology of TB and genotyping of drug resistance. This will further aid the diagnosis of MDR TB and extensively drug-resistant (XDR) TB and more accurately establish which genotypic markers can be used in DST assay development. The introduction of new anti-TB drugs or regimens needs to be paralleled with the ability to genotype for the molecular mechanisms of resistance that will emerge with scaled use of new drugs and to then use this information to develop appropriate genotypic assays to identify drug-resistant alleles to the new drugs.

RECENT POLICY AND RECOMMENDATION UPDATES FOR TB DIAGNOSTICS

Since late 2015, the following policies and associated recommendations have been published:^c

WHO policy on LF-LAM assay⁵

WHO published a policy statement for the use of the Alere Determine™ LAM assay in November 2015. This is an immunologic lateral flow (LF) strip-based immunodiagnostic test for the detection of LAM antigen in urine. The key recommendations are:

1. Except as specifically described below for PLHIV infection with low CD4 counts or who are seriously ill, LF-LAM should not be used for the diagnosis of TB (strong recommendation; low quality of evidence).
2. LF-LAM may be used to assist in the diagnosis of TB in HIV-positive adult *inpatients* with signs and symptoms of TB (pulmonary and/or extrapulmonary) who have a CD4 cell count less than or equal to 100 cells/ μ L, or HIV-positive patients who are seriously ill regardless of CD4 count or with unknown CD4 count (conditional recommendation; low quality of evidence).

Remarks:

- i. These recommendations also apply to HIV-positive adult *outpatients* with signs and symptom of TB (pulmonary and/or extrapulmonary) who have a CD4 cell count less than or equal to 100 cells/ μ L, or HIV-positive patients who are seriously ill^d regardless of CD4 count or with unknown CD4 count, based on the generalization of data from inpatients.
- ii. These recommendations also apply to HIV-positive children with signs and symptoms of TB (pulmonary and/or extrapulmonary) based on the generalization of data from adults, while acknowledging very limited data and concern regarding low specificity of the LF-LAM assay in children.

^c More detailed technical and performance descriptions are provided later in this landscape report.

^d As defined, “seriously ill” is based on four danger signs: respiratory rate >30/minute; temperature >39 °C; heart rate >120/minute; and unable to walk unaided. Source: Improving the diagnosis and treatment of smear-negative pulmonary and extrapulmonary tuberculosis among adults and adolescents: recommendations for HIV-prevalent and resource constrained settings. Geneva: World Health Organization; 2007 (http://www.ups.upenn.edu/bugdrug/antibiotic_manual/smear_neg_and_extrapulmTb.pdf).

- iii. LF-LAM should not be used as a screening test for TB (strong recommendation; low quality of evidence).

Based on the above, the existing commercial LAM assay has a very specific indication for use among severely immunosuppressed, HIV-infected patients only. Hospitalized patients with AIDS are the most likely setting for the use of this technology. Thus far, no NTP has adopted this technology. However, South Africa is considering it, given its TB-HIV co-infection burden, and data from a clinical trial that reported LAM-guided initiation of anti-TB treatment in HIV-positive hospital inpatients with presumed TB were associated with reduced 8-week mortality.²⁸ Implementation of LAM testing is likely to offer the greatest benefit where diagnostic resources are most scarce and where patients present with severe illness, advanced immunosuppression and an inability to self-expectorate sputum.

WHO policy on LAMP⁶

In August 2016, WHO issued a policy recommendation on the TB-LAMP *Mycobacterium tuberculosis* complex (MTBC) assay. This low-complexity NAAT technology is designed for use in microscopy centres and in higher-tiered test facilities. This recommendation is highly significant as this is the first recommendation for the use of a NAAT-based assay targeting the microscopy centre level. The two policy recommendations are:

1. TB-LAMP may be used as a replacement test for SSM for the diagnosis of PTB in adults with signs and symptoms consistent with TB (conditional recommendation; very low quality of evidence).
2. TB-LAMP may be used as a follow-on test to smear microscopy in adults with signs and symptoms consistent with PTB, especially when further testing of sputum smear-negative specimens is necessary (conditional recommendation; very low quality of evidence).

Remarks:

- i. These recommendations apply to settings where conventional SSM is able to be performed.
- ii. TB-LAMP should not replace the use of rapid molecular tests that detect TB and resistance to RIF, especially among populations at risk of MDR TB.
- iii. Due to limited evidence, it is unclear whether TB-LAMP has additional diagnostic value over SSM for the testing of PLHIV with signs and symptoms consistent with TB.
- iv. These recommendations apply only to the use of TB-LAMP in testing sputum specimens from patients with signs and symptoms consistent with PTB.

These recommendations are extrapolated to the use of the TB-LAMP assay in children, based on the generalization of data in adults, while acknowledging difficulties in the collection of sputum specimens from children.

WHO updated policy on the use of molecular LPAs for the detection of resistance to isoniazid (INH) and RIF⁷

In November 2015, WHO published an update to the first-line LPA policy, endorsing the Hain Lifescience (Germany) GenoType® MTBDR^{plus}v2.0 and the NIPRO Corporation (Japan) NTM+MDRTB Detection Kit 2. The key recommendation is:

- 1.** For persons with a sputum smear-positive specimen or a cultured isolate of MTBC, commercial molecular LPAs may be used as the initial test instead of phenotypic culture-based DST to detect resistance to RIF and INH (conditional recommendation; moderate certainty in the evidence for the test's accuracy).

Remarks:

- i.** This recommendation applies to the use of LPAs for testing sputum smear-positive specimens (direct testing) and cultured isolates of MTBC (indirect testing) from both pulmonary and extrapulmonary sites.
- ii.** LPAs are not recommended for the direct testing of sputum smear-negative specimens.
- iii.** This recommendation applies to the detection of MTBC and the diagnosis of MDR TB but acknowledge that the accuracy of detecting resistance to RIF and INH differs and, hence, the accuracy of a diagnosis of MDR TB is reduced overall.
- iv.** This recommendation does not eliminate the need for conventional culture-based DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance.
- v.** Conventional culture-based DST for INH may still be used to evaluate patients when the LPA result does not detect INH resistance. This is particularly important for populations with a high pre-test probability of resistance to INH.
- vi.** This recommendation applies to the use of LPA in children based on the generalization of data from adults.

Based on the above, either tool can be used to detect TB and also to genotype the alleles that are known to confer resistance to RIF and INH from either a sputum smear-

positive sample or from culture-based isolates. These tests are designed for reference and intermediate facilities. The test procedures, while requiring general equipment, can be performed manually or scaled with LPA-specific instrumentation to permit larger numbers of tests performed.

WHO policy guidelines on the use of molecular line-probe assays (LPAs) for the detection of resistance to second-line anti-TB drugs⁸

In May 2016, WHO issued a policy recommendation on second-line LPA (SL-LPA) assays (e.g. the Hain Lifescience GenoType[®] MTBDRs/v1.0 and v2.0). The key recommendations are:

- 1.** For patients with confirmed RR TB or MDR TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to FLQs (conditional recommendation; moderate certainty in the evidence for test accuracy for direct testing of sputum specimens; low certainty in the evidence for test accuracy for indirect testing of MTB cultures).
- 2.** For patients with confirmed RR TB or MDR TB, SL-LPA may be use as the initial test, instead of phenotypic culture-based DST, to detect resistance to SLIDs (conditional recommendation; low certainty in the evidence for test accuracy for direct testing of sputum specimens; very low certainty in the evidence for test accuracy for indirect testing of MTB cultures).

Remarks:

- i.** These recommendations apply to the use of SL-LPA for testing sputum specimens (direct testing) and cultured isolates of MTBC (indirect testing) from both pulmonary and extrapulmonary sites. Direct testing on sputum specimens allows for the earlier initiation of appropriate treatment.
- ii.** These recommendations apply to the direct testing of sputum specimens from RR TB or MDR TB, irrespective of the smear status, while acknowledging that the indeterminate rate is higher when testing sputum smear-negative specimens compared with sputum smear-positive specimens.
- iii.** These recommendations apply to the diagnosis of XDR TB, while acknowledging that the accuracy for detecting resistance to FLQs and to SLIDs differs and hence the accuracy of a diagnosis of XDR TB overall is reduced.

- iv. These recommendations do not eliminate the need for conventional phenotypic DST capacity, which will be necessary to confirm resistance to other drugs and to monitor the emergence of additional drug resistance.
- v. Conventional phenotypic DST can still be used in the evaluation of patients with a negative SL-LPA result, particularly in populations with a high pre-test probability for resistance to FLQs and/or SLIDs.
- vi. These recommendations apply to the use of SL-LPA in children with confirmed RR TB or MDR TB based on the generalization of data from adults.
- vii. Resistance-conferring mutations detected by SL-LPA are highly correlated with phenotypic resistance to ofloxacin (OFX) and levofloxacin; however, the correlation of these mutations with phenotypic resistance to moxifloxacin (MOX) and gatifloxacin is unclear and the inclusion of MOX or gatifloxacin in a MDR TB regimen is best guided by phenotypic DST results.
- viii. Resistance-conferring mutations detected by SL-LPA are highly correlated with phenotypic resistance to SLID and are an indication to use an MDR TB regimen that is appropriately strengthened.
- ix. Given high specificity for detecting resistance to FLQs and SLID, the positive results of SL-LPA could be used to guide the implementation of appropriate infection control precautions.

This test is designed to be a reflex test for samples testing positive for MDR TB, e.g. after using molecular LPA for the detection of INH and RIF. Similarly, this test is intended for use in reference and intermediate facilities. The test procedure, while requiring general laboratory equipment, can be performed either manually or scaled with LPA-specific instrumentation to permit high volume throughput.

WHO recommendation of GeneXpert® MTB/RIF Ultra⁹

In March 2017, WHO issued a report from the technical expert review of Ultra MTB/RIF. The dossier of evidence was prepared by FIND in collaboration with the Tuberculosis Clinical Diagnostics Research Consortium. The data was derived from a multi-country non-inferiority study. A total of 1520 patients with signs and symptoms of TB were enrolled in these countries for a direct comparison of the performance of Ultra MTB/RIF assay against the current GeneXpert® MTB/RIF assay (hereinafter Xpert® MTB/RIF) on the same specimen. The accuracy of these assays was assessed via with four cultures as the reference standard for TB detection (two liquid culture tubes + two Löwenstein–Jensen slants, performed on two specimens obtained on separate days). Phenotypic drug-susceptibility testing as well as sequencing was performed for rifampicin resistance

detection. The redevelopment of the cartridge and assay designs has resulted in Ultra having a limit of detection (LOD) of 16 bacterial colony forming units (cfu) per ml compared to 114 cfu/ml for Xpert® MTB/RIF.

The technical expert group agreed Ultra MTB/RIF was not inferior to the current Xpert® MTB/RIF for the detection of MTB and the detection of RR TB. The greatest benefit of the Ultra MTB/RIF was in the increased yield for the detection of MTB in smear-negative culture positive specimens, paediatric specimens, extra-pulmonary specimens (notably cerebrospinal fluid) and especially for HIV positive individuals whose specimens are frequently paucibacillary.

In the report, WHO listed a series of implementation considerations for the Ultra MTB/RIF:

1. The interpretation of Ultra MTB/RIF results for MTB detection are the same as for Xpert® MTB/RIF with the exception of “trace calls”.
2. Ultra MTB/RIF has high sensitivity for MTB detection and incorporates a new semiquantitative category “trace call” that corresponds to the lowest bacillary burden for MTB detection.
3. Ultra MTB/RIF has both high sensitivity and specificity for rifampicin resistance detection.
4. All persons with RR TB, identified by Ultra MTB/RIF should undergo further testing as per current WHO policy guidance to determine if there is additional resistance to the class of fluoroquinolones and/or the group of second-line injectable drugs.
5. Ultra MTB/RIF can be used on all GeneXpert® instrument platforms and is suitable for use at central or national reference laboratory level, regional and district levels. GeneXpert® has the potential to be used at the peripheral level, provided uninterrupted electricity supply and temperature conditions can be ensured.

The Ultra MTB/RIF cartridge is envisaged to eventually replace the current Xpert® MTB/RIF assay due to its greater sensitivity in detecting MTB. The new cartridge also has the potential to provide more accurate test data with its capability to discriminate silent mutations in *rpoB* that do not confer RIF drug resistance (see [Modular, cartridge-based, fully automated NAATs](#)).

SCREENING AND TRIAGE TOOLS

There are a number of TB disease screening triage tools available ranging from symptom-based screening checklists to automated digital chest X-rays (DCXR). The purpose of such tools is to triage patients most likely with active TB disease or TB infection, thereby reducing the need for laboratory testing and saving money with the intent of improving TB diagnosis. The next sections provide an update on such screening technologies in development.

DIGITAL CHEST X-RAYS

In 2016, WHO released a factsheet and a summary of current recommendations and guidance on programmatic approaches on chest radiography for TB detection. The factsheet highlighted the essential role of a chest X-ray (CXR) as a sensitive tool for screening active TB disease, diagnosing childhood TB (pulmonary and extrapulmonary) and excluding active TB before initiating treatment of latent TB.^{29,30} A recent survey in 14 TB high-burden countries (HBCs) reported that chest radiology was available at over 90% of district hospitals or community health centres and in 100% of reference or tertiary hospitals. At lower levels, only two countries noted that they offered CXR at local health posts or in the community and only three countries noted that CXR was broadly available at microscopy centres or primary health centres.³¹

As digital technology becomes more affordable and accessible, the use of DCXR is an area of increasing interest for clinicians and TB programmes. The potential of portable DCXR as a tool for TB screening in low-resource settings was first introduced in the 2014 landscape report.³² DCXR offers rapid and low-cost screening when compared with the older film-based method. In addition, the digital images can now be analysed via computer-based algorithms, which precludes the need for a radiographer to look for lesions and other indicators of TB disease thus reducing cost, offering greater flexibility and making more rapid diagnosis. As detailed in the fourth edition of this landscape report, the Computer Aided Detection for Tuberculosis (CAD4TB) automated analysis, commercialized by Delft Imaging Systems (Netherlands) with European Community (CE)-certification, can be used with digital images provided by DCXR equipment.

The use of DCXR may be used as a triage tool and for patients with abnormalities consistent with TB followed up with bacteriological tests. A recent study in South Africa demonstrated that prescreening by DCXR increased throughput of patient testing and decreased the amount of Xpert[®] MTB/RIF tests required with only a slight decrease in sensitivity.³³ The study noted the performance of the diagnostic algorithm among HIV-uninfected patients was considerably better than in HIV-infected patients, therefore consideration should be applied for different thresholds for both groups. In May 2016, the Ministry of Health of Ghana announced that it would roll out 52 digital X-ray systems with CAD4TB software and a platform for teleradiology. The Accelerating Tuberculosis

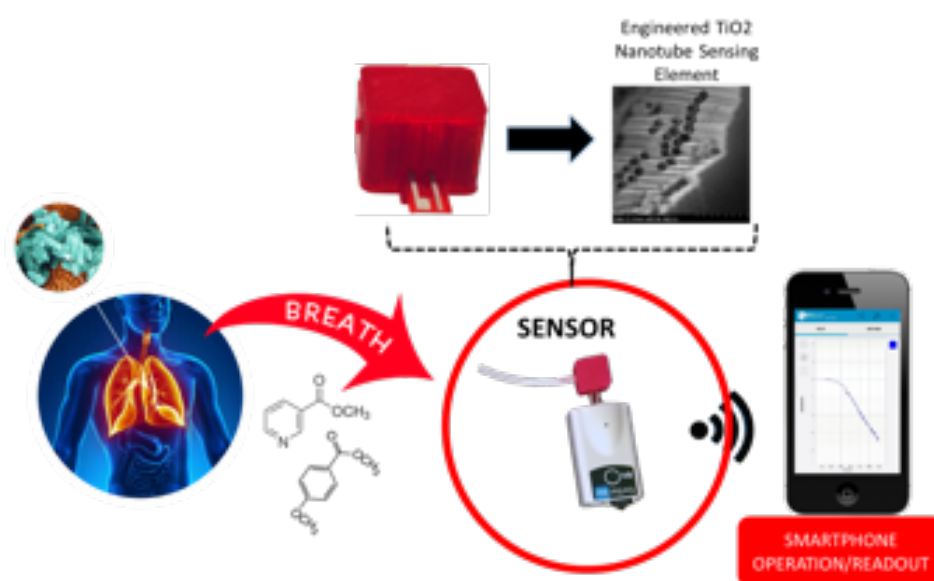
Case Detection in Ghana project is aimed at improving TB diagnosis in addition to providing improved health-care services for other general needs for X-rays, including traumatic injuries.

Meanwhile, Advenio (Chandigarh, India) is currently developing Ri-View TB, another automated image analysis software package for use with DCXR. The company is working with the National Institute for Research in Tuberculosis (Chennai, India) with support from the Bill & Melinda Gates Foundation but the current status of development is unknown.

VOLATILE ORGANIC COMPOUNDS (VOCS)

The rapid and non-invasive screening of PTB disease via metabolic compounds in breath and urine has been an area of research. Giant African pouched rats have accurately predicted active PTB disease by smelling sputum samples.³⁴⁻³⁶ The nematode *Caenorhabditis elegans* has a measurable chemotactic response upon exposure to MTB-specific VOCs.³⁷ A number of companies are developing instrumented VOC products via a range of methods, including gas chromatography (Menssana Research Inc., USA), metal-oxide sensors (Nanosynth Materials and Sensors, USA; and The eNose Company, Netherlands) and metabolite detection by chemical reaction (Metabolomx, USA). The Rapid Biosensor Systems Ltd (UK) has also developed a breath test but as it is antigen-based for the detection of MTB, it is represented in the “[Serologic and antigenic biomarkers of TB](#)” section below.

Figure 2. Prototype Nanosynth breath test and detector



Notes: The exhaled air is passed over the detection chip (red square) that is inserted in the test instrument. Bluetooth capability on the instrument then sends the test data to a smartphone that hosts the data analysis application and subsequently processes the test result.

Source: Image reproduced with permission of Nanosynth and the University of Utah.

Products are at varying stages in the development pipeline. While Menssana Research Inc. was one of the first companies in this space, the current status of its Breathscanner™ tool is unknown. Nanosynth has developed a prototype device that is currently undergoing pilot studies in Uganda and also in four districts of Mumbai (Figure 2). The Mumbai study intends to screen up to 1000 TB cases. In terms of time to market, Nanosynth expects to be ready in Q1 2019, with an initial target of India, South Africa and Uganda as its entry markets. This portable device involves a patient to breathe into a tube, and if the patient has TB then the VOCs, produced by MTB in the lungs, bind to the titanium oxide nanotubes present in the device. This binding results in an electrical current being produced that is detected and read by a hand-held electronic device such as a smartphone. Nanosynth claims this screening device takes 10 minutes to run a single test. Patients with a positive result are then recommended to undergo confirmatory testing to establish diagnosis and DST.

The eNose Company recently received ISO 13485 certification and its Aeonose™ device obtained CE registration on 1 December 2015 (Figure 3). Pilot and validation studies in several countries, including Bangladesh, Indonesia, Kenya, Paraguay, South Africa and Venezuela are ongoing. Data from Bangladesh have demonstrated a sensitivity and specificity (compared to SSM and solid culture) of 76.5% and 87.2%, respectively.³⁸ The intended population for Aeonose™ is adults and children older than 4 years of age. The environmental conditions for use include operational temperatures from 10–40 °C, storage temperature range of minus 40 °C to 70 °C and 10–85% non-condensing humidity. The device contains a rechargeable battery and is independent of mains electricity. Users of Aeonose™ should be qualified operators, and a nurse practitioner can easily be trained to operate the device.

Figure 3. Hand-held Aeonose™ device



Notes: The device contains three metal oxide sensors (left). Carbon fibre filters are placed onto the mouthpiece and in the device entry point (top centre). Test data are analysed by transferring data via an iOS system to a secure server in Amsterdam through a 3/4G connection via an application tool (lower centre) and returned data are presented as an interpretable test result (right).

Source: Images reproduced with permission of The eNose Company.

Carbon fibre filter discs are attached to both the Aeonose™ instrument and the mouthpiece to prevent risk of aerosolization of TB cells. A bacterial filter located inside the mouthpiece and a one-way valve system prevent contamination of the instrument by TB or by breath-associated microbes (Figure 3). A single test takes a minimum of 15 minutes to complete, including yielding results. The mouthpiece is gently inserted into the sample port on the top of the Aeonose™. The patient wears a disposable nose clip and slowly breathes into the device via a disposable mouthpiece for 5 minutes, then the device performs additional actions for another 10 minutes to clean the sensors. An audio signal indicates completed collection. The collected data are sent via Bluetooth to an iOS application hosted by an iPhone or iPad and then transmitted by the iOS device to a secure server to be analysed. The results are sent back immediately and displayed on the iOS application. The disposable mouthpiece and filters are removed and new ones inserted prior to further use. The device should be cleaned at the end of each day with 70% alcohol. The eNose Company currently recommends each device is returned yearly for a service and calibration check. Dedicated software to perform the service at the test location is close to finalization. While current costs are unknown, The eNose Company is reported to be working with larger organizations in order to reduce the currently high cost of production due to instrument complexity.

Finally, Metabolomx recently reported results from a small pilot study of its prototype array under a variety of urine test conditions, with 85.5% sensitivity and 79.5% specificity.³⁹ The prototype uses a colorimetric sensor array to indicate TB infection from VOCs in urine.

IMMUNE RESPONSE-BASED SCREENING TESTS FOR MTB EXPOSURE

Inhalation of MTB can lead a person's immune system to respond by eliminating the inhaled bacilli and controlling the multiplying inhaled bacilli. If the immune system successfully contains the bacilli, halts their multiplication and prevents further progression in the body, then LTBI is established. In this state, the person is infected with TB but is neither infectious nor symptomatic. However, if MTB overwhelms the immune system and continues to multiply, then the person progresses to TB disease. People who are infected with latent TB and who are not immunocompromised typically have a 10% lifetime risk of developing TB disease. If the person becomes immunocompromised, or is a child under the age of 5 years, then this risk can increase significantly, e.g. 20–37 times greater risk for PLHIV.⁴⁰ Therefore, as indicated in the WHO End TB Strategy, the diagnosis and treatment of LTBI are important elements in the control and elimination of TB disease worldwide.⁴¹

Detecting exposure to MTB can be currently performed using two immune response-based screening methods: the tuberculin skin test (TST); and interferon gamma release assays (IGRAs). However, both methods have shortcomings and are of insufficient performance to discriminate between active TB disease and LTBI.

Currently, the Mantoux TST is commonly used and is performed by an intradermal infection of a purified protein derivative from MTB into the skin of the forearm. The challenges that limit the performance of TST include patient age, immune status, recent Bacillus Calmette-

Guérin (BCG) vaccination history (BCG limits the specificity of TST), exposure to non-tuberculous mycobacteria (NTM) and the cut off for reading the test.⁴² In addition, shortages of supply of the TST antigens have been reported in Europe and the United States.^{43,44}

IGRAs are another immune response-based laboratory test used to indicate MTB exposure. The assays have higher specificity than the TST, have less cross-reactivity with BCG than the TST and correlate well with MTB exposure. Additionally, they require more complex infrastructure and highly skilled staff. This method requires CD(4)⁺ T-lymphocytes (T-cells) harvested from a patient's whole-blood sample to be incubated with MTB-specific antigens; the early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP 10). These antigens stimulate the release of interferon-gamma (IFN- γ) from the CD(4)⁺ T-cells, which is then quantified by the assay.

The QuantiFERON-TB (QFT) Gold In-Tube assay (Qiagen, USA) and the T-SPOT.TB[®] assay (Oxford Immunotec, UK) are two widely available IGRAs that have US Food and Drug Administration (FDA) and Health Canada clearance and are CE marked for use in Europe. The QFT assay also includes a third MTB antigen, the TB7.7 antigen. In addition to these, several other IGRA products are available from manufacturers from China (TB-IGRA, Beijing Wantai Biological Pharmacy Enterprise Co. Ltd; ASACIR TB, Haikou VTI Biological Institute), Republic of Korea (SD Biosensor Inc.) or India (TB Platinum, Immunoshop India Pvt Ltd). Qiagen has released the QFT gold plus, which also stimulates IFN- γ production from CD(8)⁺ cells, improving the identification of TB infection in immunodeficient individuals, including HIV-positive patients and small children (Figure 4). This assay involves larger MTB peptide antigens to stimulate CD(4)⁺ cells and shorter peptide antigens to stimulate the

Figure 4. QuantiFERON-TB Gold Plus kit from Qiagen: reagents and ELISA plates (left) and collection materials (right)



Source: Images reproduced with permission of Qiagen.

CD(8)+ cells. Qiagen claims greater sensitivity by its assay offering greater stimulation of IFN- γ release from two subsets of T-lymphocytes rather than just CD(4)+. Since the previous landscape report, there have been five studies to evaluate the performance of the newer test that note similar performance to the QFT gold.⁴⁵⁻⁴⁹

Meanwhile, the Statens Serum Institut in Denmark has developed a novel skin test named C-Tb for detecting LTBI. The basic concept behind the test is to combine the ease-of-use of the TST with the high specificity of the IGRAs. The test measures the body's immune response to two specific MTB antigens that are not contained in the BCG vaccine: ESAT-6; and CFP10. The C-Tb test is designed to be used in the exact same way as the TST for the end user. Contrary to the TST where the cut off of induration varies depending on local guidelines, age and immune status (from 5–15 mm), the C-Tb test has a fixed cut-off value of 5 mm of induration.⁵⁰ A double-blinded phase 3 study of C-Tb in 979 adults, ranging in TB status from presumed uninfected through intermediate and high risk of latent TB to active disease, was carried out by the Statens Serum Institut in Spain. Both C-Tb and IGRA had a specificity of 97% (Statens Serum Institut, unpublished information, 2016). C-Tb was strongly associated with MTB exposure and the C-Tb test was concordant to IGRA in 95% of study participants. A recent study in South Africa reported sensitivity of C-Tb to be similar to IGRA (73.9% and 75.1%, respectively) in patients with confirmed TB disease, with the sensitivity unaffected by HIV status. However, C-Tb sensitivity was significantly reduced for PLHIV with CD4 counts <100 cells/mm³.⁵¹

A recent independent review of TST, IGRAs and the C-Tb assay noted that while both IGRAs and C-Tb assays have improved specificity compared to the purified protein derivative TST, this came with a decrease in sensitivity.⁵² A similar review also noted that based on current technologies, the TST is suitable for use in resource-constrained settings as the test is low cost, relatively easy to perform and does not require facilities of highly skilled staff.⁴² The price of the new C-Tb test has yet to be determined but it is anticipated to be significantly lower than the IGRAs, making it a more cost-effective and simple-to-use test for LTBI. The C-Tb test will be manufactured under good manufacturing practice standards in compliance with both European and US guidelines. Thus, a high, consistent quality of the C-Tb product is ensured and, due to the simpler production process for recombinant production, supply will be stable avoiding the periodic shortages of TST that have been frequent in the past.^{43,44} The Statens Serum Institut is now in the process of submitting an application for marketing authorization to the European Medicines Agency. Other groups manufacturing or developing improved TSTs include Generium Pharmaceutical (the Diaskintest, Russian Federation), which is available commercially in Kazakhstan, the Russian Federation and Ukraine, and an ESAT-6-based skin test that has undergone phase II trials in China.⁵³

While these immune-response based screening tools can indicate if a person has been exposed to TB, challenges still remain to differentiate people with TB infection and TB disease,⁵⁴ predict and identify people beginning to progress from infection to active TB disease,^{55,56} and assess if a patient has fully recovered from TB disease (and no longer requires treatment).⁵⁷ (See also the “[Biomarkers to detect MTB exposure and TB disease](#)” section below).

SOLUTIONS FOR DIAGNOSTIC SAMPLE TRANSPORT

After the screening and triaging of patients at risk of TB, a diagnosis is necessary. Currently, PTB diagnostic tools require adequate amounts of quality sputum specimens collected from patients presumed to have TB. If a sputum sample is unable to be tested in the facility where the collection took place, it may be shipped to a different laboratory for diagnostic testing via culture, LPA or Xpert®. Certain tests are only available in larger facilities (e.g. Xpert®, culture or DST LPAs), therefore transportation of the specimen while maintaining its integrity is critical. As sputum is not sterile, prolonged storage at ambient temperatures encourages growth of commensal microflora, which can affect subsequent culture and also affect the quality of nucleic acids. Typically, samples are shipped via a courier under cold chain but this becomes more challenging in peri-urban or rural areas, thereby compromising quality of diagnosis and limiting access to care. FIND has drafted a preliminary target product profile (TPP) describing the key components and attributes required.⁵⁸ A systematic review of available solutions is under way and a technical consultation to review additional data is planned for June 2017. There are two companies that are offering products in this space.

DNA Genotek Inc. (Ottawa, Canada; a wholly owned subsidiary of OraSure Technologies Inc., USA) has developed the OMNIgene® SPUTUM (OM-S), to liquefy and decontaminate the sample and permit its transport without cold chain. The company claims viability for at least 8 days in temperatures as high as 40 °C. The OM-S treated sample may be used for microscopy, culture or nucleic acid testing; the primary diagnostic methods for TB. A pilot study in Nepal compared cold chain shipping with OM-S treated sputum and noted that the OM-S transport medium greatly reduce contamination of samples from 12% to 2% and increased TB diagnosis by 9% when using culture.⁵⁹ The smear microscopy results with both methods were identical. In another study, also in Nepal, OM-S treated sputum samples shipped at ambient temperatures gave identical results to samples shipped via cold chain when using Xpert®.⁶⁰ The study further demonstrated the OM-S transport medium may be suitable for use with Xpert® when using either the sediment or sputum protocols provided with the *Mycobacterium tuberculosis*/rifampicin resistance (MTB/RIF) assay. FIND is working with DNA Genotek Inc. to validate the performance of OM-S in a larger multisite study, after a small study demonstrated 100% concordance with high- and medium-characterized MTB loaded sputum panels stored in OM-S sputum.⁶¹

Longhorn Vaccines and Diagnostics LLC (USA), are offering the second product, the PrimeStore® Molecular Transport Medium (PS-MTM). Unlike OM-S, which liquefies and keeps MTB cells viable outside of the cold chain, PS-MTM liquefies the sample but also kills all viable microorganisms and stabilizes the nucleic acids in the sample.⁶² The nucleic acids remain stable over 28 days at ambient temperature in the medium and is compatible with a range of nucleic acid extraction systems. PS-MTM is intended to aid microscopy centres to send specimens to higher-tier laboratories for further NAAT analysis. An independent

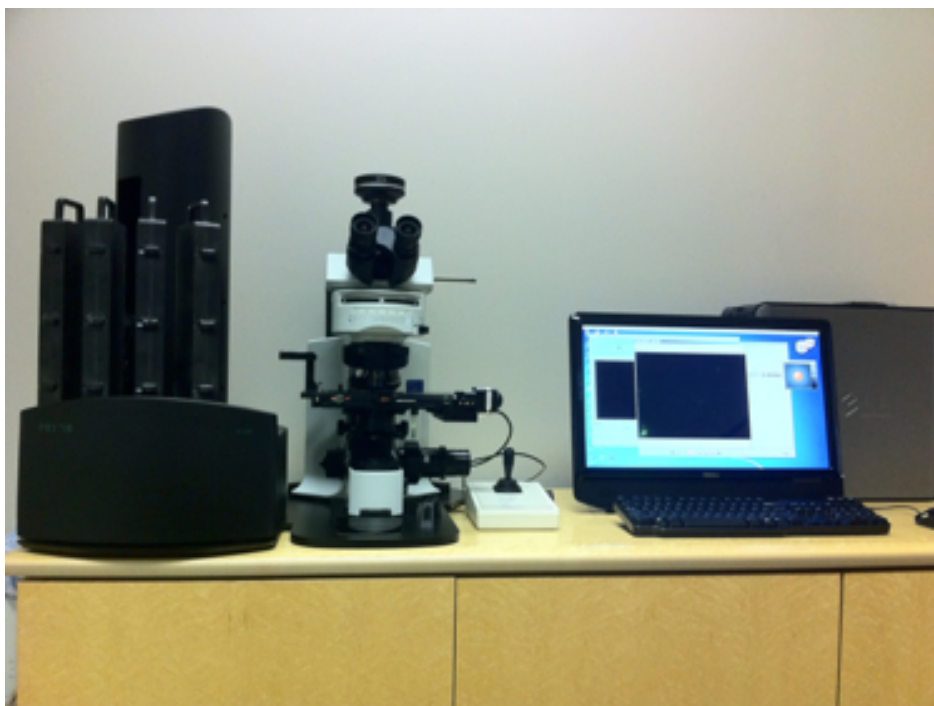
study using PS-MTM with real-time PCR targeting IS6110 on clinical sputum samples were compared with Xpert® and liquid culture data. PS-MTM with real-time PCR was concordant with culture at 82% (McNemar, P = 0.55) and 84% (McNemar, P = 0.05) for Xpert®.⁶³ A pilot study has demonstrated PS-MTM may also be used with the Xpert® method but further studies are necessary to build a stronger body of evidence to confirm this.⁶⁴ The company is currently working with manufacturers in the USA and Europe to produce its product at scale intend to seek European Conformity-in vitro diagnosis (CE-IVD) certification once these are established. While cost of the PS-MTM is unknown, the developer claims to have a pricing scheme for low- and middle-income countries (LMICs).

AUTOMATED MICROSCOPY

The simplicity and low costs associated with direct visual examination for acid-fast bacilli (AFB) SSM make it the most common diagnostics test used globally, especially in resource-constrained settings. Despite its widespread use, SSM is not ideal for the diagnosis of MTB as it has poor sensitivity and variability of results. The poor sensitivity of SSM is a challenge in diagnosing paediatric and PLHIV TB cases as the specimens are often paucibacillary, and thus not detected by this method. SSM is also time consuming, has low throughput and requires well-organized quality assurance programmes to maintain user performance. It also does not detect drug-resistant (DR) forms of TB. A market estimate of the number and cost of SSM in HBCs noted 77.6 million smears at a median cost of US\$ 109 million.⁶⁵

Previous landscape reports have documented the development of the Microimager, an automated reader that was in development by Becton Dickinson (USA; hereinafter BD) as a replacement tool for manual SSM. BD is no longer developing this tool and so it will not come onto the market. The TBDx system is an automated digital microscopy platform that is available from Signature Mapping Medical Sciences Inc. (a wholly owned subsidiary of Applied Visual Sciences Inc., USA). This platform consists of a high-quality microscope and imaging system that in conjunction with a slide holding carousel can

Figure 5. TBDx system



Note: Automated slide loader (left), fluorescence microscopy with digital camera and automated stage (centre) and laptop to operate the reader and employ the scoring algorithm (right).

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

read up to 200 prepared smears using fluorescent microscopy in a single run (Figure 5). The proprietary software reads the images to detect stained cells. SSM slides can be prepared via commercial automated or high-throughput staining platforms to permit scaled preparation of slides with more consistent staining and washing prior to reading – e.g. the RAL stainer (RAL Diagnostics, France) or Aerospray® TB Series 2 (ELITechGroup, France). Without the carousel, the system can automatically process 1–4 slides. The robotic carousel can host 50–200 slides with each slide being read in 5 minutes, meaning 200 slides would take about 16 hours to read. The developer notes the scanning algorithm can be changed to permit faster reading of strongly SSM positive samples to shorten the overall time of a run.

A pilot study in South Africa 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”.⁶⁶ One area of interest for the application of this product is where molecular testing is too expensive to routinely perform, for example, prescreening sputum samples prior to Xpert® analysis in order to reduce the number of Xpert® tests to screen negative or indeterminate samples only. This has been investigated in a study where over 1000 samples were screened using SSM, automated SSM, Xpert® and mycobacterial growth indicator tubes (MGIT™, liquid culture).⁶⁶ A primary finding was that using the TBDx to screen specimens prior to using Xpert® could detect 90% of patients with subsequent Xpert®-positive results. The cost-effectiveness of automated digital microscopy has been modelled to investigate the possible savings and impact on TB diagnosis.⁶⁷ The study investigated costs and effectiveness of different algorithms for automated digital microscopy, including as a standalone test and with confirmation of positive results with Xpert®. If screening more than 30 slides per day, the primary conclusion was that while universal Xpert® is the preferred diagnosis, when resources are scarce and MDR TB is not common, the application of automated digital microscopy can identify the majority of cases and halve the cost of diagnosis and treatment. There are no further cost updates since the previous landscape report, when the microscope and computer offered was available at US\$ 23 000 and the (optional) 200-slide robotic loader at US\$ 21 000.

QuantuMDx (UK) is developing a MTB cell enrichment device to improve the sensitivity of SSM beyond current basic concentration methods such as sputum sedimentation. The company has started to investigate the use of an MTB cell concentration technology as a standalone diagnostic called Capture-XT™. This technology is able to concentrate low numbers of cells into a 10 µL fluidic capture chamber from an initial much larger sample volume, therefore allowing the captured cells to then be visualized by either a microscopy technician or a device with a camera. The presence of cells in the viewing window will be positive for MTB as the proprietary capture mechanism is claimed to be specific to MTB cells. QuantuMDx also notes that they are developing a process where these concentrated cells could then be reflexed to the Q-POC™ molecular confirmatory and DST assay that they are also developing (see below the section on [NAATs for use in microscopy centres](#)). The potentially low-cost format of the Capture-XT™, which claims to have sensitivities close to that of culture, is seen as a replacement technology for SSM.

Including the reflex molecular test as a replacement for culture, this technology might potentially be implemented at the lower levels of the health-care system, including microscopy centres. The Capture-XT™ assay is projected to take 45 minutes from raw sputum. The current prototype processes samples individually but a higher-throughput device is under investigation. The device will be battery operated and will be able to operate for 8 hours on a single charge. Pilot testing of the technology has started. The Capture-XT™ diagnostic disposable assay has a target price of between US\$ 1–2 and QuantuMDx estimates the initial product release for Q4 2017–Q1 2018.

CULTURE-BASED TOOLS FOR THE DIAGNOSIS OF TB AND DST

There are no new updates regarding the culture-based diagnosis of MTB. Due to the risks associated with laboratory-acquired infection, culture of MTB requires higher biosafety requirements as opposed to methods that simply manipulate infected material (e.g. SSM). Therefore, most culture is performed in reference facilities or some intermediate laboratories dependent on appropriate biosafety facilities and trained staff. While culture can take a long time to generate a test result (typically up to 4 weeks), it is a highly sensitive assay and can also be used for the further phenotypic determination of drug resistance with culture-positive cases. Three manufacturers offer automated liquid culture-based systems: the BacT/ALERT® 3D instrument from bioMérieux (France); the BACTEC™ mycobacterial growth indicator tube (MGIT™) platform from BD (USA); and the Mycolor TK platform from Salubris (Turkey). While the Salubris product is new to the market, there has been a small comparative study of the performance of TK-SLC-L media and the MGIT™ platform.⁶⁸ The data showed very similar performance with a longer time for detection with TK-SLC-L (3 to 5 days); however, the group noted that contamination events were significantly reduced with the TK-SLC-L media (1.3% versus 13.7%). Pricing is not available for the bioMérieux and Salubris products, although FIND has negotiated preferential pricing with BD for HBCs for the procurement of the MGIT™ instruments, culture reagents and servicing.²⁰

In addition to commercial liquid culture systems, WHO has recommended an interim measure regarding the use of lower-cost and non-commercial methods such as microscopically observed drug susceptibility (MODS) assays, which can be used to simultaneously screen for MTB in addition to drug resistance in parallel culture assays.⁶⁹ Laboratories can procure individual reagents from local vendors to make their own test media or procure a MODS kit from Hardy Diagnostics (USA), which includes all key materials such as plates, sealed lids, media and drugs. This has been shown to have similar performance to MODS assays in Peru.⁷⁰ An advantage of MODS over conventional culture is that the algorithm typically screens for INH and RIF resistance as well as for growth of MTB; essentially speeding the time to a result for MDR TB from culturing sputum samples. One multicentre study assessed the performance of several methods to detect MDR TB or XDR TB from clinical samples specifically investigating resistance to INH, RIF, MOX, OFX, amikacin (AMK), capreomycin (CAP) and kanamycin (KAN).⁷¹ At 14.3 days, the mean time to result of the MODS assays was slower than LPAs or pyrosequencing (both 1.1 days) but faster than MGIT™ (24.7 days) and having the best performance in detecting RIF and KAN resistance.

BIOMARKERS TO DETECT MTB EXPOSURE AND TB DISEASE

The search for new biomarkers to indicate TB is ongoing.⁷²⁻⁷⁵ WHO convened a meeting in April 2014 to determine the priorities for research in the TB diagnostics field.¹⁰ Four TPPs for diagnostic tests were prioritized, including a rapid biomarker-based instrument-free POC test for non-sputum samples (that can also detect childhood and extrapulmonary TB). The identification of an appropriate biomarker is key to this need being realized with a good commercial product based on the TPP metrics. The following section notes the current commercial development work using serologic, immunologic, chemical and other methods to identify biosignatures of TB infection.

Saliva, urine, serum and whole blood biomarkers provide the advantage of easy sample collection, and have the potential to detect MTB exposure and differentiate between active TB disease and TB infection. There have been a number of new studies investigating the combination of genes or transcriptomes to uniquely identify and differentiate between TB infection, TB disease and other diseases. A recent study found a set of seven plasma inflammatory biomarkers that indicate TB infection.⁷⁶ Differences in biosignatures in saliva have also been found to potentially discriminate TB from other respiratory diseases and change with response to treatment.⁷⁷ Another study using serum has reported a set of six biomarkers strongly associated with active TB infection that also change after treatment, indicating a potential method to monitor treatment therapy.⁷⁸

Studies investigating miRNA signatures from serum and peripheral blood mononuclear cells may have diagnostic value.^{79,80} A recent meta-analysis using gene expression data from TB study cohorts indicated a three-gene signature may be sufficient to diagnose PTB.⁸¹ In contrast, one small study demonstrated these same three genes may have poor sensitivity to diagnose TB but could be used to rule out TB infection with very high confidence.⁸²

There have also been some developments in the search for TB pathogen-specific biomarker molecules.⁸³ A recent review article provides an excellent overview of the current methods and challenges faced in the use of “-omics” techniques to diagnose active TB.⁸⁴ One case control study identified a 27-transcript signature that distinguished active TB disease from LTBI and a 44-transcript signature distinguished active TB from other diseases from the whole blood of both HIV-infected and -uninfected adults.⁸⁵ The data were used to create disease risk scores to classify PTB from LTBI and other diseases with a sensitivity and specificity of 90% or more for each.

e “-omics” is a term used to describe of a range of molecular biology techniques including genomics, transcriptomics, proteomics and metabolomics.

A prospective cohort study of adolescents with LTBI were followed with regular transcriptomic profiling of target genes.⁸⁶ A 16-gene signature was found that predicted TB progression with a sensitivity of 66.1% and a specificity of 80.6% in the 12 months preceding TB disease diagnosis. When used with independent cohorts of unscreened adolescent and two cohorts of adults, the 16-gene signature had a sensitivity of 53.7% and a specificity of 82.8% in the 12 months preceding TB disease diagnosis. This signature may be of use to measure progression towards active TB while the patient is asymptomatic.

There is a potential for biomarkers to be employed in a direct and rapid diagnostic method using non-invasive or minimally invasive specimens (e.g. breath, urine, finger stick whole blood). This would be of particular benefit in a rapid triage test to rule out MTB infection. A three-gene signature could easily be translated onto the Xpert® platform and miRNA are often qualified by quantitative reverse transcription PCR, a method commonly used for viral HIV RNA measurement.⁸¹ The challenge to the measurement of these transcript signatures is that the cost of a rule-out assay would unlikely be less expensive on the Xpert® platform. Similarly, while the binary detection of analytes can use lateral flow strips, there is significant further complexity in pooling multiple protein biomarkers onto a single assay with qualitative readout via a device. These add great cost and complexity to assay development and it would be unrealistic for such technology to remain within cost for a triage test recommended by the recent WHO TPP.¹⁰

Further information on diagnostic methods for LTBI and developments in non-invasive specimens can be found above in section [Immune response-based screening tests for MTB exposure](#).

IMMUNE RESPONSE-BASED TESTS

Antrum Biotech (South Africa) has developed the InterGam Rapid Immuno Suspension Assay (IRISA™-TB), an enzyme linked immunosorbent assay (ELISA) designed for use in a laboratory to specifically diagnose extrapulmonary TB. The tool can detect the presence of extrapulmonary TB from samples, including pleural, pericardial, ascetic and cerebrospinal fluid. The 96-well plate format can host low or higher numbers of samples depending on test workloads. This level of test complexity makes it suitable for intermediate-level facilities (e.g. hospital laboratories) and requires instrumentation associated with ELISA assays (e.g. plate shaker, washer, reader). The IRISA™-TB is CE marked but pricing is currently not known.

ProteinLogic (UK) has developed ImmiPrint®, a complex immuno-array technology to detect soluble cellular differentiators (sCDs), produced by macrophages in response to an infection. Different infectious diseases can elicit unique sCD fingerprints, therefore the ImmiPrint® can be used to screen samples to identify sCD biomarker profiles to discriminate active TB infection from other infectious diseases. The company is now working to develop a low-cost platform to host the biomarker assays identified by

the ImmiPrint® technology. In 2015, ProteinLogic and partners were awarded a European Union Horizon 2020 Small- and Medium-sized Enterprises programme grant to develop a rapid POC test for the diagnosis of active TB disease. Its development partner, Biosensia (Ireland), is developing the RapiPlex platform, a multichannel fluidic chip, fluorescence immunoassay system, and an integrated optical reader that uses fluorescence detection to simultaneously measure up to 24 analytes using either quantitative and/or qualitative analysis. The test is claimed to require a single sample addition and testing is completed in 5–15 minutes. No further information is currently available.

SEROLOGIC AND ANTIGENIC BIOMARKERS OF TB

Significant progress in disease management has been made with rapid diagnostic tests (RDTs) for diseases such as malaria and HIV where disease-specific antigens or antibodies can be detected with a high degree of confidence via a simple and rapid test. In 2011, WHO released policy guidance on commercial serologic tests stating that they should not be used in the diagnosis of TB, despite being available in some markets.⁸⁷ However, the need for research on new/alternative POC tests for TB diagnosis, including serological tests with improved accuracy, was also highlighted. Research has focused on identifying new TB antigens and understanding the immuno-dominant domains of target antigens in order to increase specificity.^{88,89}

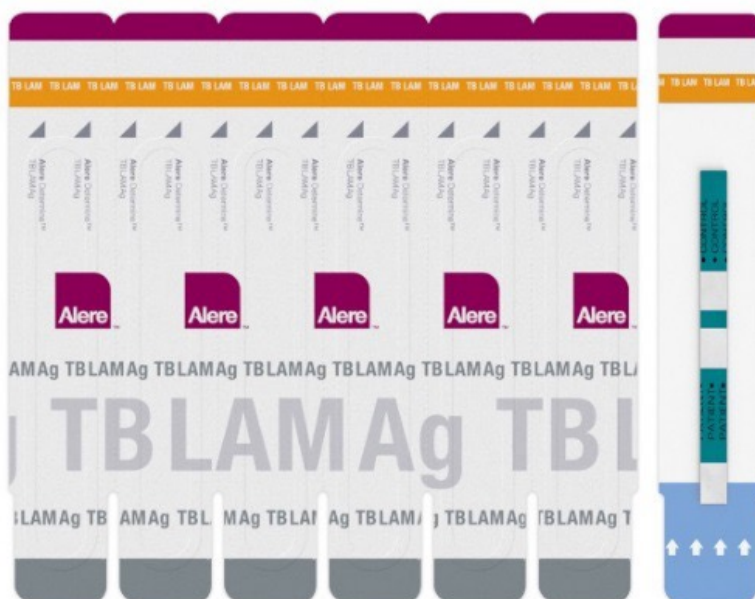
As such, several groups are developing serologic tests for use with blood specimens and *TB Biosciences* (USA) was developing a rapid LF immunoassay.⁸⁸ The product was initially scheduled for release in Q2 2015, however, development work has been stopped due to poor performance of the prototype assays. Instead, TB Biosciences is collaborating with Quidel (USA) to develop an LF assay to detect multiple TB biomarkers via a fluorescence reader with greater precision and accuracy. FIND and mBio (USA) have been engaged in a long-term project to develop a serologic assay for TB infection using over 50 different TB antigens for the interrogation of patient antibodies to TB. An update describing the development and performance of the prototype tool is expected to be published soon.

Rapid Biosensor Systems Ltd (UK) has developed the TB Breathalyzer, a tool to detect TB antigens from a breath specimen. Unlike VOCs, the company claims that antigen detection from breath sampling has greater specificity. The test detects the Ag85B antigen, which is MTBC specific and found in sputum or other fluids. The native antigen (Ag85B) can displace an antibody bound to a fluorescently labelled peptide on the sensor array. The decrease in fluorescence is measured by evanescent wave fluorimetry and the device then scores the result. The TB Breathalyzer uses a disposable collector and reaction tube that is read via a battery-powered reader after breath collection. The patient coughs into a collection tube and then depresses an internal concentric

plunger to collect the sample on the inside surfaces of the tube. The plunger is then twisted to smear the concentrated sample across the peptide-coated prism at the base of the tube. This coating is dry and no mixing of additional fluids is required. Each test takes 10 minutes to screen a patient. The limit of sensitivity of the device has not been determined but is anticipated to be in the range of 50–75 cfu.⁹⁰ A prototype device was piloted in a field trial in Ethiopia where it had a sensitivity of 79%.⁹⁰ Rapid Biosensor Systems Ltd has designed a unit with a production capacity of >500 000 per batch run, where the price per test would be close to US\$ 5.

Alere Inc. (USA) introduced the Determine™ TB Lipoarabinomannan (LAM) Ag rapid assay (Figure 6), in 2012. This is a LF immunochromatographic strip or RDT that targets the LAM antigen in urine via a polyclonal antibody capture and detection method on a nitrocellulose strip. LAM, a lipopolysaccharide, is a metabolite of mycobacterial species representing a key component of the cell wall and is produced by both growing cells and the degradation of the cell wall.⁹¹ As such, it should be noted that this assay is not only specific for MTB, but will also detect the presence of other NTMs. LAM from mycobacterial infection anywhere in the body is ultimately expelled from the body in urine and can be detected in it. The test requires a 60 µL aliquot of urine, with no other tools necessary. The test result is visually noted on the test stripe after 25 minutes incubation. The simple format of the assay does not require significant training for the user and the product is stable for 15 months at 30 °C.

Figure 6. Determine™ TB LAM Ag rapid assay, with strip ready for use shown on the right



Source: Image reproduced with permission of Alere Inc.

The Determine™ TB LAM Ag rapid assay has undergone significant independent evaluation of its potential use as a rapid screening tool for TB infection in high-income countries and HBCs in Africa, Asia and South America. These data were reviewed by WHO, resulting in the release of the WHO policy guidance described earlier.

The restricted use of the tool in only critically ill patients with HIV co-infection is necessary because earlier studies have shown that diagnostic performance of the assay improves as CD4 counts decrease.^{91,92} The reasons for this are not fully understood but it is hypothesized that critically ill HIV patients may have a disseminated TB infection that is very difficult to rapidly diagnose with current tools. The greater bacterial load associated with widespread infection and, therefore, antigen load, the greater likelihood of genitourinary tract TB and greater glomerular permeability to allow increased antigen levels in urine. Alere Inc. has identified Africa and Asia Pacific as the primary markets for sale of the Determine™ TB LAM Ag rapid assay. Other groups are currently investigating ways to improve the performance of the LAM in RDTs and other formats by using more specific antibodies, and improving to sample preparation and readers to improve test sensitivity.

OTHER BIOMARKER DEVELOPMENT NEWS

Earlier landscape reports highlighted the great interest around an assay that could specifically detect TB via the use of fluorescently labelled beta lactamase analogues that were enzymatically digested using a TB-specific β -lactamase.⁹⁴ GlobalBio Diagnostics Corp. (USA) began developing a POC diagnostic tool but due to a variety of development and funding challenges, the group is no longer developing the technology at this time.

NAATS AND SEQUENCING METHODS FOR TB DIAGNOSIS AND DST

The application of NAATs has revolutionized rapid and accurate diagnostic testing for most pathogens. The technology usually involves PCR to specifically amplify a targeted section of genomic DNA or cellular RNA (e.g. 16s RNA) of a pathogen. Detection methods, to reveal the presence of pathogenic genetic material, vary across products. Many use fluorescence, while simpler tests use luminescence or stripes on amplicon capture strips to visual detection. NAATs are frequently used to identify known genetic alleles that confer drug resistance.

NAAT-based diagnostics in TB can face a number of challenges, including the specimen type, the relatively low numbers of pathogens present in the specimen and the difficulty in lysing the TB cell wall to release the nucleic acids. Heterogeneous mixtures of drug-sensitive and drug-resistant bacteria in a sample can also confound genotyping of drug resistance. While NAAT methods are more sensitive than most other tests for TB, culture remains the gold standard for TB diagnosis as it offers the greatest sensitivity, with a limit of detection of ~10 cells/mL of sputum. However, it can take months to diagnose TB and thereafter MDR TB by culture-based means. The general accuracy and rapid time to detection make NAATs an essential tool for the TB community to rapidly and accurately diagnose TB and MDR TB. Its decentralization to lower levels of the health system, e.g. microscopy centres, will be necessary to replace or augment SSM methods and for the rapid detection MDR TB.¹⁴

Products to identify XDR TB are now in late development phases. Such tools need to be accurate, low cost, easy to perform and robust.⁹⁵ The introduction of new tools such as NGS will have a highly significant impact on improving the diagnosis of MDR TB and XDR TB, increase the understanding of emerging drug resistance mechanisms and permit molecular epidemiology to be performed at an unprecedented level as compared with current methods.

UPDATE TO THE NAAT PIPELINE

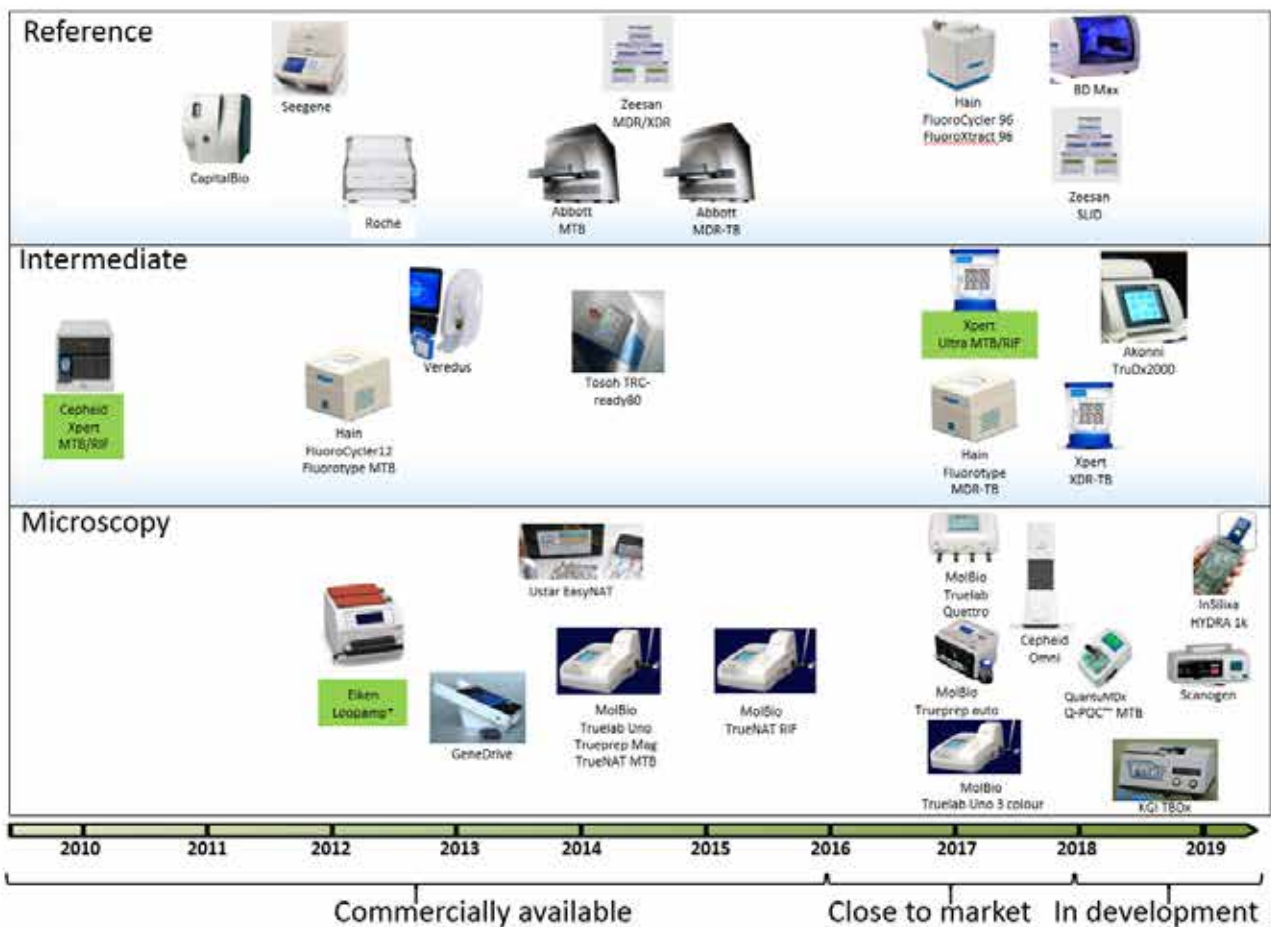
In the fourth edition of this landscape report,¹⁹ the pipeline of NAATs in development was presented as being highly promising, especially with molecular tests targeting intermediate laboratories and microscopy-level facilities. Unfortunately, the pipeline has somewhat contracted, with some product development effort being stopped entirely or postponed, or projects not demonstrating the performance initially anticipated. A summary of the status of current technologies on the market or close to market with revised release dates is shown in [Appendix 1](#). The pipeline for TB-related NAATs is shown in Figure 7. Other technologies and assays are described below but these are typically for use with open platforms or more generalized methods and equipment (e.g. NGS).

The Alere™ q assay and platform was originally planned to be released with CE-IVD marking in 2016, but all TB-related development of this tool has stopped. Enigma

Diagnostics Ltd (UK) were developing the Minilab platform, a standalone system that hosts a cartridge in which sample preparation and amplification were automatically performed in a process similar to the Xpert® MTB/RIF assay. It was recently announced that this group has entered liquidation and so development effort has now ceased. Several of the NAATs under development for use in microscopy facilities are delayed due to technical and funding challenges. Tangen Biosciences (USA) is delaying the development of TB assays to focus on other disease targets, while Qiagen (Germany) has noted that it is looking for external funding to drive the development of its POC tool for TB diagnosis if it passes early development milestones.

A recent independent evaluation of the Genedrive® MTBC assay (Epistem Ltd, UK) highlighted the need for independent evaluations of new assays soon after they become marketable.⁹⁶ The similar products in this class from Ustar Biotechnologies and Molbio Diagnostics have also undergone limited evaluation since their release.

Figure 7. Current and emerging automated, semi-modular or non-integrated TB NAATs; their intended laboratory location and release date (actual or anticipated)



Source: Author analysis

Green filled product labels indicate a WHO recommendation is available for the product.

*Eiken Loopamp™ was released in 2012 but endorsed in 2016

Stratification of diagnostic NAATs in the test continuum

While there are a number of NAAT-based assays and platforms available or in development (Figure 7), their most effective positioning in country TB programmes is dependent on the setting, test complexity and user requirements (Table 1). The number of facilities within an NTP is largely correlated to country income status. High-income and upper-middle-income countries generally have devoted more financial resources to TB at upper- and middle-tiered facilities, potentially offering more comprehensive TB diagnostic testing via NAATs. A survey of basic infrastructural and logistical capacities of microscopy centres in Brazil, Russian Federation, India, China and South Africa (BRICS) and LMICs highlighted the inequalities between these countries, observing that most LMICs have limited temperature control, limited access to mains electricity and a lack of general laboratory equipment.⁹⁷ In terms of implementation of NAATs for TB diagnosis, all countries noted a lack of skilled staff in level 1 facilities. Limited financial resources in LMICs have generally meant fewer resources invested in TB control with less dedicated laboratories and instead relying heavily on SSM in microscopy. A more recent study presented the data from surveys of 14 high-burden TB countries with regard to diagnostic testing and treatment options available across the four levels of health-care settings.¹⁴ No countries reported any NAAT testing being performed by community or village health workers or at local health posts (level 0). Only four countries offered

Table 1. Summary of NAATs relating their role in TB diagnosis in terms of intended location of use, throughput and other key factors

Test	Level	Throughput	Function	Complexity	Cost	Cost/test
POC assays	3, 2, 1	Moderate/low	TBDx, DST ^a	Low	Moderate/low	Low
Modular NAATs	3, 2, 1 ^b	Moderate	TBDx, DST	High	High	Moderate
Microarrays	3, 2	Moderate	MTB/NTM ^c Dx, DST ^a	High	High	High/moderate
LPAs	3, 2	Moderate	MTB/NTM ^c Dx, DST ^a	Medium	Medium	Medium
Automated batched PCR	3	High/moderate	MTB/NTM ^c Dx, DST ^a	High	High	Low
Open PCR platforms	3	Moderate	MTB Dx, DST ^a	High	High	Low
NGS	3	High	MTB/NTM ^c Dx, DST, mol epi	High	High	Moderate

^a DST is genotyping of drug resistance that may be multiplexed with TB diagnosis or applied as a reflexive test after MTB infection is confirmed. Level 1 microscopy centre or community care centre, Level 2 hospital or regional clinical facility, Level 3 reference or tertiary hospital.¹⁴

^b Testing at this level is performed in few LMICs.

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

routine NAAT or culture testing for DST at microscopy centres or primary health centres (level 1), while 12 countries offered this service at district hospitals or community health centres (level 3) and all countries did so at reference or tertiary hospitals (level 3).

While SSM is low cost and comparatively easy to implement, it is not as sensitive a test as NAATs, especially with PLHIV, nor for the diagnosis of paediatric TB. In addition, SSM does not detect drug resistance. It is, however, routinely used in most LMICs as the primary test for MTB at level 1 facilities.¹⁴ Therefore, to improve decentralized TB diagnosis and offer MDR TB testing, there is a pressing need for affordable high-performance NAATs that can be implemented at the microscopy centre level.

The following sections highlight the NAATs currently on the market or in development, and notes aspects of their intended use, performance (where data are available), cost and current placement in the development pipeline.

Application of NGS to TB diagnosis and control

The application of NGS or massively parallel sequencing is a new area of methodology that combines MTB diagnosis, drug resistance genotyping and molecular epidemiology from a “single” test or sample. The technique allows multiple individual DNA strands from MTB cells to be independently sequenced, thereby allowing for accurate discrimination of heterogenetic variation (e.g. heteroresistance) and quantitative analysis. NGS can be applied to the whole genome or to target regions within a genome after amplification of the specific target regions of genomic DNA.⁹⁸ A significant focus of NGS for clinical purposes has been on its application for the diagnosis and treatment of noncommunicable diseases such as cancers and screening for genetic inheritable diseases but the potential value of NGS has now been demonstrated with challenging infectious disease syndromes such as bacterial sepsis and MTB.

Although sequencing technology is currently not available in most resource-limited settings, reagent and equipment costs continue to fall and the potential to run sequencing assays directly from sputum (negating the need for culture) could dramatically reduce turnaround times. The methods to prepare and sequence samples can vary significantly and, in addition, the underlying mechanisms by which NGS systems operate are technically complex.^{99,100} There are a variety of NGS platforms on the market and in development, including tools for high and moderate throughputs, for example: Bio-Rad Laboratories (GnuBio); Illumina (MiSeq, HiSeq and NextSeq); Oxford Nanopore (minION, PromethION, GridION); PacBio (Sequel and RS2); Thermo Fisher (SOLiD, S5, personal genome machine [PGM], Proton); Qiagen (GeneReader); and the Vela Diagnostics NGS platform (Singapore). While most of these products are designed for use in a reference-level facility, often at great cost, smaller platforms with potential application in intermediate-level facilities are available (e.g. MiSeq, PGM and the minION). Currently, the Illumina platforms appear to be the most popular among research and clinical groups due to the low error rate, simplicity of sample preparation and ease of method development. Smaller platforms that are robust and

capable of use in smaller laboratories are currently in development, these include: Genalysis® from DNAE; Gene Electronic Nano-Integrated Ultra-Sensitive (GENIUS) from GenapSys; and the Genia sequencer from the Genia Corporation (all USA). These developing tools are intended to host small cartridge-based systems with integrated sample amplification and sequencing at a cost target of US\$ 100 or less, however, further details are limited. Currently, the cost of platforms and sequencing available on the market varies considerably (US\$ 80 000→750 000) and the cost per giga base (US\$ 41–2000).¹⁰¹

A recent prospective, multicentre and international pilot study assessed the real-time performance and cost of whole genome sequencing (WGS) for the laboratory diagnosis of mycobacterial infection, including drug-sensitive and -resistant forms of MTB.¹⁰² Key findings included that NGS showed high concordance with the current methods for both identification of mycobacteria and DST (93% for each), identification of an outbreak, the identification of an inter-regional cluster of INH-resistant TB and an undiagnosed case of MDR TB allowing an immediately change in treatment. Cost analysis of both the traditional (non-NGS) and the NGS algorithms showed a savings of 7% overall if NGS alone was to be used. The median time to perform NGS and receiving a full report was only 5 days, which is faster than current diagnostic algorithms.

Challenges of using NGS for TB diagnosis and control

While a highly promising new tool in the fight against MTB, NGS faces a variety of challenges to its broader implementation for clinical purposes.^{103,104} The first relates to acquiring enough MTB DNA to allow sufficient coverage or reads of the MTB genome targets for WGS. Peer-reviewed studies have used cultured MTB as the primary DNA source in clinical samples for WGS.^{102,105} Extracting sufficient MTB DNA can be challenging in terms of paucibacillary samples where there are only a few cells to extract DNA from. Results can be confounded by sequencing the genomic DNA from other commensal microflora, especially from samples such as stool. The MTB itself can be challenging in terms of its greater guanine cytosine (GC) nucleotide base pair content and repetitive regions that may affect some NGS methods.¹⁰⁵ Currently, the preparation of samples, processing and sequencing are a complex process requiring highly trained staff in a fully functional laboratory. Qiagen and Vela Diagnostics offer fully automated processes from DNA extraction to NGS via a suite of dedicated machines. Other challenges to NGS include identifying the best platform to a specific approach as different platforms offer different benefits for NGS in terms of read size, cost per read and error rates.¹⁰¹

While WGS cannot be reliably performed without culture of the targeted microbe to provide sufficient DNA, alternative methods that allow NGS to move beyond the reference laboratory have been described. Shotgun sequencing (random and incomplete sequencing of the MTB genome) from SSM positive sputum samples could enable molecular genotyping.¹⁰⁶ In addition, the targeted amplification of key drug resistance alleles by PCR prior to NGS is another method that does not first require culture. The PCR amplification stage compensates for the initial lack of target DNA

typically extracted from a sputum sample but sequencing is only performed on the PCR amplified DNAs. Pilot studies have demonstrated the feasibility to use sputum samples in different NGS platforms, and the use of PCR assays to target resistant genotypes to both first- and second-line drugs within a single test.^{98,107}

The massive amount of raw data generated by NGS and WGS presents a significant challenge to the user. NGS data management includes mapping or assembly of the genome, base variant calling and comparative phylogenetic analyses. Software and algorithms for each of these steps are typically highly complex and often unlinked to each other and thus it is unclear how data could be rapidly processed in laboratories with users unfamiliar with bioinformatics skills.¹⁰⁸ In one high-income country pilot study assessing the validity of NGS as a diagnostic tool, the group used a single centralized bioinformatics unit to review, parse, archive and process data into test results for return to the test laboratories in order to reduce costs.¹⁰² Several groups are developing software so that users without bioinformatics experience can use NGS data. Data storage and access are also major challenges to this as local computing infrastructures to support massive data files and the necessary software to support analysis are not cost effective and most likely unable to be supported by HBCs. An alternative is to leverage off existing cloud-based systems provided by large technology groups such as Amazon, Google and Microsoft (all USA), which offer flexibility, scalability, security and lower costs more than localized systems. Many US-based bioinformatics groups already take this approach to NGS data and its analysis.¹⁰⁹ FIND is reviewing current technologies and methods in order to identify where an end-to-end (from sample insertion to results) NGS system could be implemented into HBC laboratory settings.¹¹⁰

Other advantages of using NGS for TB diagnosis and control

NGS facilitates the creation of an accessible repository of assessed and fully annotated drug resistance alleles, therefore enabling the better understanding of drug resistance of MTB for current and emerging therapies. The breadth of the current pipeline of potential new TB drugs and regimens presents a challenge for diagnostic developers trying to proactively develop tools that can be deployed at the time of launch. The Critical Path to TB Drug Regimens (CPTR) programme led by the Critical Path Institute (USA) has recognized the need for an international data-sharing TB drug resistance platform to enable the accelerated development of safer, faster-acting and more efficacious drug regimens for TB treatment. The CPTR programme also supports the continuing and evolving need for public and industry access to high-quality, globally harmonized data regarding clinically relevant MTB drug resistance-conferring mutations to support next-generation rapid molecular assays.

The Relational Sequencing TB (ReSeqTB) platform, which was launched globally in April 2016, is a freely available resource to the TB community.^{111,112} Key elements of ReSeq include a validated regulatory-grade TB-specific WGS pipeline, implementation of standards, a robust and thorough curation process to accurately capture contributor data, a secure platform that has the flexibility to host and access data and meta-data,

including clinical outcomes. ReSeqTB is available through a cloud-based environment allowing individual laboratories to securely upload and analyse its own data in-house without the need for bioinformatic expertise (Figure 8). A list of mutations that define drug-resistant loci and polymorphisms derived through statistical analysis with input from TB sequencing and bioinformatic experts are freely available to download. These lists will be updated periodically and published as more data are contributed to the platform.

Figure 8. Workflow of ReSeqTB bridging targeted NGS platforms to provide rapid patient management decisions



Source: Image reproduced with permission of the Critical Path Institute.

In addition to a data browser with the ability to filter data, novel visualization tools to graphically display genome data with the locations of drug-resistant alleles have recently been released. Improvements and additional functionality are planned in the future to meet increasing demands for analysing WGS data. Additional regulatory compliant TB-specific targeted NGS pipelines and dedicated analytic tools, including a data mining tool to discover new single nucleotide polymorphisms associated with drug resistance, are expected to be available in 2017. Reporting language templates are being developed to standardize sequencing results that are generated and provided to clinicians. Clinical demonstration studies are needed to ensure that rapid patient-centred drug-resistant sequencing results are appropriately implemented and translated to improve patient outcomes.

Summary: The massive volume of actionable data produced by NGS methods will have a significant impact on TB diagnostics, patient treatment and control. Currently, many high-income countries are already devoting resources to NGS centres that can accurate sequence analysis of many pathogens, including MTB. As was seen with other NAATs in their infancy (e.g. PCR), the complexity and costs of the test processes initially limited their roll out to the smaller laboratory but technology developers are working on platforms and methods to make NGS a routine method in clinical laboratories. Part of this expansion has led to challenges in validation, quality control and data interpretation beyond what clinical laboratories have previously encountered.¹⁰⁴ However, as these technologies mature, improved quality assurance methods will also be developed to reduce barriers to implementation. NGS data provide information at a much higher resolution than that of traditional techniques, allowing fine-scale epidemiological investigations, while also offering improved diagnosis and surveillance of drug resistance to both first- and second-line drugs. One pilot study¹¹³ showed that DST via WGS genotyping is faster than conventional phenotypic methods and it is expected to become faster and cheaper in the future. Essentially, diagnosis, information on the optimal therapy and lineage of infection may all be available in one test method. The creation of the ReSeqTB database highlights how NGS data, when correctly curated, can be pooled to build a tool that will enable developers to customize assay development as new genotypes for drug resistance are identified. As with all large data collections, the information contained therein is most useful only if it is correct and stored in a unified format where it can be easily accessed and used by all.

Automated batched PCR

The combination of excellent sensitivity and specificity, low contamination risk and speed has made PCR technology an appealing alternative to culture- or immunoassay-based testing methods for diagnosing TB disease.¹¹⁴ Automated high-throughput screening provides the advantage for reproducible and accurate test results, decreased risk of cross-contamination and minimal manipulation. A reduced need for constant attention enables fewer trained staff to perform the necessary tests. For NAATs to be considered as high throughput, the technology must perform multiple tests within a short timeframe and at volume. There are currently three platforms that are available and two in development. The companies are listed in descending alphabetical order.

Abbott Molecular (USA; hereinafter Abbott) currently offers an automated DNA extraction platform (*m2000sp*) performed by the *m2000rt* real-time PCR platform to detect MTB from sputum, bronchial alveolar lavage or sediments derived from either specimen type. Abbott has developed an MTB inactivation buffer to reduce biohazard risk to the test operators. Abbott MaxRatio technology analyses test data and provides individual test results (Figure 9).¹¹⁵ The platforms are CE-IVD marked and can process up to 94 samples in a single run, taking 7 hours to complete from sample preparation (including inactivation) to interpretation of results.

Figure 9. Abbott Molecular platforms for automated sample preparation (*m2000sp*, left) and real-time PCR analysis (*m2000rt*, right) for MTBC and first-line DST



Note: Images are not to scale.

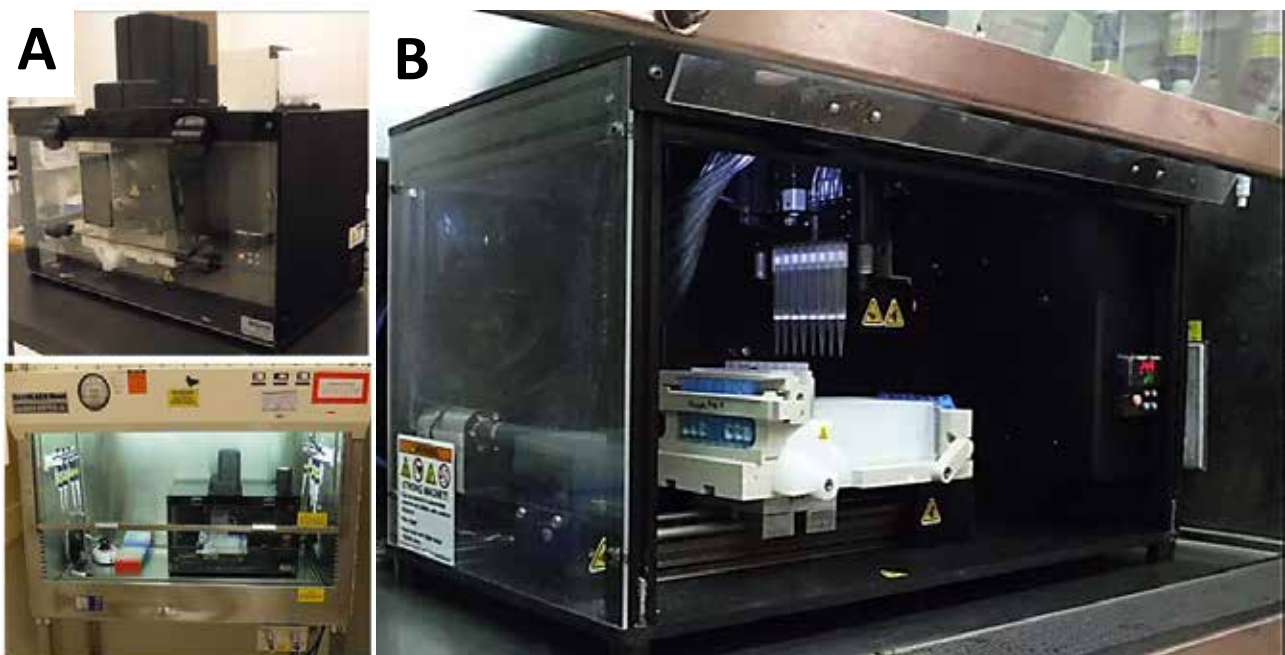
Source: Images reproduced with permission of Abbott Molecular.

Abbott's RealTime MTB assay for the detection of MTBC and RealTime MTB RIF/INH Resistance assay offer genotyping of resistance alleles, both with CE marking. The RealTime MTB assay detects MTB with a detection limit of 17 cfu/mL and a specificity of 100% when using a panel of known mycobacteria, viruses and other organisms.^{116,117} Abbott reports clinical sensitivity as 99% for SSM positive/culture positive samples and 83% with SSM negative/culture positive specimens.

The RealTime MTB RIF/INH Resistance assay can use previously extracted DNA of MTB-positive samples in an automated reflex mode. The detection limit is 60 cfu/mL with 100% specificity using the same 80 microorganism panel, and sensitivities of 94.8% (RIF) and 88.3% (INH); diagnostic specificity is listed as 100% (RIF) and 94.3% (INH). The equipment requires a working temperature range of 15–30 °C and the PCR reagents require storage at minus 25 °C to minus 15 °C. Recent peer-reviewed articles from independent and Abbott-sponsored studies have assessed the performance of the Abbott assays.¹¹⁶⁻¹²¹ The independent studies showed similar sensitivities and diagnostic performance features with the MTB assay^{118,121} and RIF/INH resistance assay.¹¹⁶ The RealTime MTB RIF/INH Resistance assay has been compared with other commercial TB diagnostic assays and platforms with comparable results, using both sputum and extrapulmonary specimens.^{116,118,120,121} While a broader range of specimens needs to be screened, in particular for drug resistance genotyping, the overall comments from these initial validation studies were positive with their data typically matching the performance data supplied by Abbott.¹¹⁷

Akonni Biosystems (USA; hereinafter Akonni) is developing products in two areas of the TB diagnostics space. The automated DNA/RNA extraction platform, TruTip® Automated Sample Prep Workstation (Figure 10) can process pulmonary specimens, in addition to all other clinical samples, including stool and tissue. The main design features include a homogenization subcomponent for difficult to liquefy/lyse samples, the ability to handle large viscous sample volumes, and highly competitive equipment pricing and unit extraction costs. The platform is intended to be used for and has been demonstrated with downstream diagnostic approaches such as microarrays, PCR, electrophoresis, isothermal amplification methods (LAMP, *helicase*-dependent amplification and transcription mediated amplification) and LPAs. The workstation leverages off the TruTip®, an Akonni product that uses a tuneable glass capture matrix (i.e. tuneable pore size, surface chemistry or covalent binding moieties) in a pipette tip to host DNA/RNA extraction and purification.^{122,123}

Figure 10. Akonni TruTip® Automated Sample Prep Workstation



Notes: HEPA, laminar flow enclosure for benchtop use (top picture, image A) or utilization in biosafety cabinet for secondary containment (bottom picture, image A). Front shield up to display consumables, including TruTip® sample tubes for bead beating, heating tubes, reagent tray and elution tubes (all automated) (image B).

Source: Images reproduced with permission of Akonni Biosystems.

In addition, the platform has a patented rotating magnetic arm that enables noncontact variable bead beating for difficult to liquefy/lyse samples. A heating module is also included for traditional chemical and/or heating lysis and decontamination. The robotic workstation runs up to eight samples in parallel to scale specimen processing.¹²⁴ Greater throughput can be achieved by using the TruTip® on 96 channel heads such as those featured on Hamilton and Tecan liquid handling platforms.¹²⁴ The unit comes in an enclosed cabinet with HEPA filtration for bench top use or is small enough to fit within a biosafety laminar flow hood for secondary containment. The Akonni platform is under internal design control and external design transfer to a contract manufacturer for scaled cGMP production. The product has been successfully shipped worldwide and is intended for use in HBCs. The platform is currently available to early adopters for research use only and is on track to gain US FDA Class I registration and CE-IVD certification by 2018. The product is expected to enter the WHO evaluation process when ready.

BD (USA) is developing a multiplexed real-time PCR assay for the detection of MTB and the BD MAX™ platform to genotype RIF and INH resistance. The platform has a 5-colour detection real-time PCR platform to expand its capacity to multiplex different PCR assays in the same reaction. The BD MAX™ is a fully integrated, single platform with CE-IVD marking and a capacity to test up to 24 sputum specimens in a single run. BD estimates that throughput could be as high as 72 samples in an 8-hour working day. As this product is in development, there are no performance data available yet. Equipment pricing is to be established and regulatory approvals will be sought once the product is readied for market release. BD anticipates the release date to be in Q3 2017.

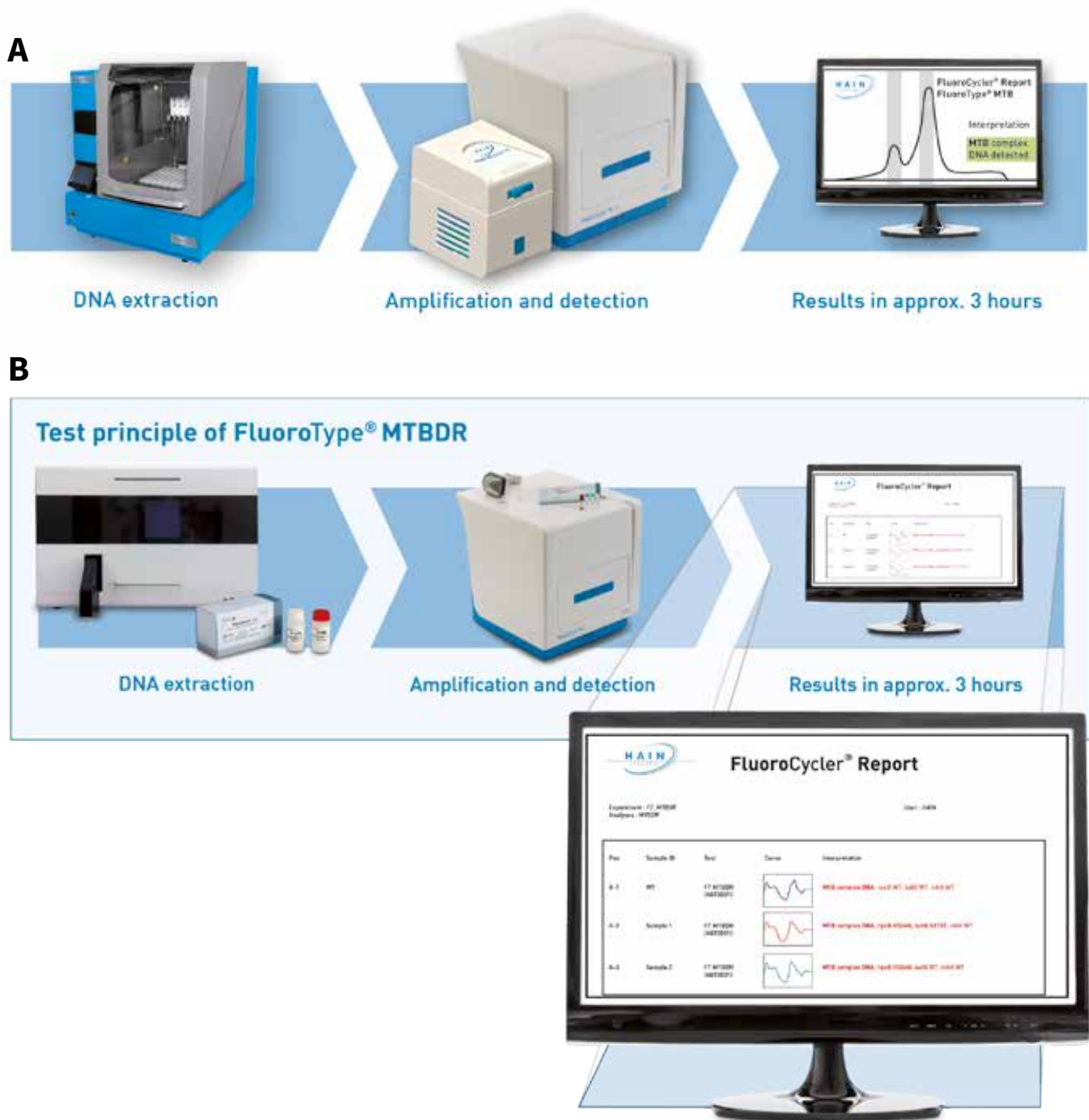
Hain Lifescience (Germany): The NAAT assays (excluding the GenoType® LPAs) were extensively described in the 2015 edition of this landscape report. The company's high-throughput platform includes a 96-well format for DNA extraction (GenoExtract® 96) and subsequent amplification and detection via real-time PCR (FluoroCycler® 96). The extraction platform processes sputum and extrapulmonary specimen types excluding whole blood using the GXT extraction kit. Also offered are a lower-throughput extraction platform and real-time PCR cycler, the GenoExtract® 12 and the FluoroCycler® 12, respectively. These platforms can process or amplify up to 12 samples at once.

Hain Lifescience currently offers a real-time PCR assay, the FluoroType® MTB to detect MTB DNA. A second assay, the FluoroType® MTBDR, which can detect TB in addition to genotyping for RIF and INH resistance alleles is to be released in Q2 2017. Both assays use novel amplification and probe technologies, Late-After-The-Exponential (LATE)-PCR coupled with "lights on/lights off" probes that tile side by side on the target region.¹²⁵⁻¹²⁷ Fluorescent melt curve analysis is used to measure the rate by which the probes dissociate from their target to create a highly specific and reproducible signal that confirms the correct target. A significant advantage to this approach is that much larger regions of target DNA can be interrogated than with the conventional method of using a single probe yet with similar accuracy. The analytical sensitivity of the Fluorotype® MTB assay is 15 cfu/mL, with a clinical performance of 99% specificity for MTBC. In SSM positive/culture positive samples the sensitivity is 100% and a reported

sensitivity of 90.2% for SSM negative/culture positive specimens. There are limited peer-reviewed data on the performance of the FluoroType® MTB assay but a direct comparison with the Abbott RealTime MTB assay showed good concordance of results in 97.3% of samples.¹¹⁶

The MTBDR assay sensitivity is 20 cfu/mL. In a low-MDR TB setting, Hain Lifescience

Figure 11. Hain Lifescience FluoroType® MTB [A] and FluoroType® MTBDR [B] processes



Source: Images reproduced with permission of Hain Lifescience.

suggests that the MTB assay be followed by the MTBDR assay as a reflex test. In regions with high MDR TB prevalence, the direct use of the MTBDR assay may save time and costs. Both assays are CE-IVD marked.

Hain Lifescience is developing an M(X)DR-TB assay and another assay to genotype pyrazinamide (PZA) resistance. The M(X)DR-TB assay targets RIF, INH, aminoglycosides (AMGs) and FLQs in a single reaction.¹²⁸ The assay can screen 36 different alleles associated with resistance to these drugs. The PZA assay uses lights on/lights off probes that tile the entire *pncA* gene target, presenting a real-time PCR assay that can detect mutations across a large target region, far beyond other currently used probe-based real-time PCR methods. From screening a panel of 654 isolates with 414 hosting *pncA* polymorphisms via the assay and using Sanger sequencing as a comparator, the analytical sensitivity was 94.4% and analytical specificity was 97.5%. These assays are planned for validation in 2017. Current pricing is not available.

Roche Diagnostics (Switzerland; hereinafter Roche) currently offers real-time PCR assays for MTB and *Mycobacterium avium* infection (MAI) that are CE-IVD marked, and are described in detail in the fourth edition of this landscape report. Currently, these assays require a DNA extraction platform to prepare a template for PCR and are aimed at high-income countries. Roche is developing three new high-throughput real-time PCR assays: the COBAS® MTB Test; the COBAS® MAI; and the COBAS® RIF/INH Test. These assays are being developed for their use with the COBAS® 6800/8800 Systems, which are fully automated from end-to-end, designed for very high throughput, e.g. the 8800 system can process 960 tests in an 8-hour period.

Summary: The initial costs associated with using these systems are expensive in terms of procuring equipment and preparing the appropriate infrastructure to host them. However, these automated product systems offer advantages in terms of test throughput, the ability to genotype drug resistance, while offering greater consistency of test performance in a high-throughput processing algorithm. Each of the systems has individual advantages, e.g. the sensitivity of the Abbott platform or the potential of the Hain Lifescience assays to detect multiple drug resistance genotypes in a single test. The BD and Roche platforms are wholly user free once specimens are prepared for an assay run, and the throughput of the larger Roche platforms could meet demand for large volumes of tests. Both the Abbot and Roche platforms can be used for other disease targets thus procurement and operating costs could be defrayed across different programmes. The Akonni platform only addresses sample preparation and does not include detection of MTB by PCR or other nucleic acid amplification methods. There is also the need for further validation work to be published in order to understand not only the performance of the tools in diagnosing TB, but also in the cost savings and associated value from offering more rapid diagnosis than with culture when used in a centralized laboratory. None of these products has yet been endorsed by WHO.

Autonomous NAAT reagents for use in open systems

While many of the NAAT products listed in this landscape report represent specific assays with dedicated equipment, there are manufacturers that produce only the assay reagents as kits that can be hosted on a variety of real-time PCR platforms in general, on platforms recommended by the manufacturer. These reagents are manufactured under quality conditions, have undergone validation and have some performance data (e.g. analytical and clinical performance) and so have less risk associated than with “in-house” PCR assays, which are prone to error and risk, creating reduced or variable test performance.¹²⁹ Also, unlike the commercial assays noted in this landscape report, extensive performance assessment before use of the assay reagents is typically not performed with sufficient rigour. There are a large number of assay kits available on the market, with many having CE-IVD registration, but most do not have any peer-reviewed data to inform on their performance in clinical settings. The assay kits described in this section were chosen because they offer scaled genotyping of MDR TB and XDR TB or have very high sensitivity, and in most cases have regulatory approval from a national administrative body.

Seegene Inc. (Republic of Korea) offers several highly multiplexed NAAT assay kits that use real-time PCR or capillary electrophoresis for amplicon detection. The Seeplex assay uses 10-colour detection using melt curve analysis after PCR amplification. The company has developed the proprietary software MuDT to discriminate between the 10 channels on the Bio-Rad Laboratories (USA) CFX96 real-time PCR platform. The viewer software analyses the raw data to generate test results from its assays. Sample preparation is not supplied by the company thus a different product must be used to first prepare MTB DNA for real-time PCR analysis. The Seegene Anyplex™ series of assays kits 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 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 (seven alleles for FLQs and six alleles for injectable drugs). The time to generate results is under 3 hours (excluding DNA extraction) and 94 samples can be tested in one run. Sample types include sputum or bronchial washes and the assay reagents are stable for 12 months at -20 °C.

Since the last landscape report, there have been four independent peer-reviewed articles describing the performance of Seegene Inc. assays on either culture isolates or from patient specimens, predominantly sputum. One study compared the Anyplex™ II MTB/MDR/XDR assay performance to phenotypic DST and other molecular methods to address discordant results. Overall, the Anyplex™ assay sensitivity and specificity were 93.3% and 100%, respectively.¹³⁰ Two studies from South Africa have compared the performance of the Anyplex™ MTB/NTM/MDR TB against the Xpert® MTB/RIF assay and the Hain Lifescience MTBDR*plus* LPA.^{123,131} When the three methods were screened using qualified culture isolates, the assays had identical performance for the detection of RIF (but the discordances were not the same) with 96.7% sensitivity and 98.1% specificity.¹³² With INH,

both the LPA and Anyplex™ assay had the same sensitivity (100%) but the Anyplex™ assay had reduced specificity (82.4%), detecting several wild-type isolates as INH resistant. The second study compared the three assays on isolates and SSM positive and SSM negative culture confirmed TB. All three assays had similar performance with SSM positive sputum but the Anyplex™ assay had the lowest sensitivity at 65.5% with SSM negative samples.¹³¹

A multicentre independent evaluation of the Anyplex™ MTB/NTM/MDR TB assay was performed in the Republic of Korea, where the researchers noted comparable performance with the Roche COBAS® TaqMan® MTB assay when using respiratory specimens with culture confirmation. Overall, the diagnostic sensitivity and specificity of this Anyplex™ assay was 87.5% and 98.2%, respectively; 95.2% sensitivity for SSM positive with a drop in sensitivity to 69.2% with SSM negative specimens.¹³³ A smaller clinical study in the United Kingdom using the Anyplex™ MTB/NTM showed high sensitivity and specificity in the diagnosis of MTB and NTMs using this assay.¹³⁴ A recent study evaluated the performance of the Anyplex™ II MTB/MDR/XDR assays using cultured isolates that were phenotyped for drug resistance. The study compared the performance of this assay with two LPAs from Hain Lifescience to detect MTB TB (GenotypeMDRTB*plus*v2.0) and XDR TB (Genotype MDRTBs/v2.0). Overall, the study found that all three molecular assays had similar performance in genotyping their targeted drug resistance alleles with the exception of INH where the researchers noted poor specificity of 61–62% for both assays.¹³⁵ All of the Seegene Inc. TB products are CE-IVD marked and are approved by the Korean FDA. The company currently markets in over 50 countries.

Xiamen Zeesan Biotech Co. Ltd (China) has developed the MeltPro® Drug-Resistant TB Molecular Diagnostics System that includes a real-time PCR instrument and a series of real-time PCR assays to genotype drug resistance alleles (RIF, INH, EMB, streptomycin [STR], FLQ and SLID resistance). The assays use real-time PCR and multicolour melting curve analysis to indicate allelic variations associated with drug resistance.¹³⁶ The company notes that the assays can be used with its real-time PCR machine or with PCR machines offered by Bio-Rad Laboratories (CFX96), Qiagen (Rotor-Gene 6000) or Roche (LC480 II), and claims each assay has a limit of detection of 1000 cfu/mL and is 100% specific to MTBC only. Up to 94 samples can be processed in a 96-well thermocycler and notes that results are generated in under 4 hours. An evaluation of 1000 phenotypically scored specimens in China noted that the INH assay (that targets 30 genotypes) showed clinical sensitivity and specificity at 90.8% and 96.4%, respectively.¹³⁷ The lowest heteroresistance (e.g. two different genotypes in one sample) level that the assay can detect was 40%. A similar study investigated the performance of the STR assay wherein clinical sensitivity and specificity were 88.8% and 95.5%, respectively; the lowest level of heteroresistance with this assay was 20%.¹³⁸ The assays for RIF, INH, EMB, STR and FLQ have received China Food and Drug Administration (CFDA) approval.

A prospective study assessed the performance of the MeltPro® RIF, INH, FLQ and SLID (AMK and KAN) assays using SSM positive sputum samples that also underwent MGIT™-based DST.¹³⁹ The RIF assay had a sensitivity and specificity of 94.2% and 97.5%, respectively, and the FLQ assay had a sensitivity and specificity of 83.3% and 98.1%, respectively. Data for injectable drugs varied depending on the drug, with AMK resistance detection at 75% (sensitivity) and with 98.7% specificity. While highly specific with KAN

resistance (99.2%), the sensitivity of the assay was much less (63.5%) and considered unacceptable. Therefore, the assay is being further developed and is anticipated for CFDA approval in 2018. Xiamen Zeesan Biotech Co. Ltd currently markets its products only in China. The reagents are stable for 1 year at -20 °C.

While not yet manufactured, the totally optimized PCR TB (TOP TB) assay developed by Thisis (USA) has recently been shown to have very high performance in its first pilot evaluation.¹⁴⁰ The assay has an analytical sensitivity of 1–4 cfu/mL. This may be of particular importance in the case of PLHIV where assay sensitivity is key with typically paucibacillary samples being presented. Initial performance data were derived from a convenience sample of 261 HIV-infected PTB suspects. Liquid culture, Xpert® MTB/RIF, TOP TB and a composite reference PCR assay were each used to detect MTB. Using culture as the reference, TOP TB had 100% sensitivity but only 35% specificity. Using DNA sequencing data from the composite PCR assay as the reference standard, the sensitivity of culture (27%) and Xpert® MTB/RIF (27%) was lower than TOP TB (99%) but each had similar specificity (100%, 98% and 87%, respectively). The composite reference assay targets the same gene as the TOP TB assay but in a completely different region. Thisis is currently working with diagnostic companies to host its assay.

Summary: The use of assay reagent kits permits greater application of existing equipment within a laboratory, creating cost savings as no purchase of new hardware is required. The commercially available assay kits described above are designed with an emphasis on genotypic identification of first- and second-line drug resistance. The primary advantage of such kits is that they are manufactured under good manufacturing practice. Given that these assays have a relatively low sensitivity, their application could be used for batched screening of culture-positive isolates for first- and second-line drug resistance. The prevalence of MDR TB and XDR TB in addition to the specific genotypes circulating within local populations needs to be carefully considered. Logistically, the use of such assays requires highly skilled staff and dedicated spaces to ensure the correct operation of testing and to limit DNA/amplicon contamination. There is 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. The WHO has not yet endorsed any of these products.

LPAs

LPAs are a relatively low-cost NAAT and a variety of assays have been developed for detection of mycobacterial species, TB diagnosis, speciation of NTMs and to genotype common alleles to first- or second-line drug resistance alleles (Table 2). Due to their relatively low sensitivity, the use of LPAs is restricted to either with SSM positive specimens or to culture isolates to confirm TB infection, and in most cases rapid genotyping for RIF/INH and possibly other drugs for MDR TB. While a relatively simple assay in principle, the appropriate use of LPAs needs dedicated areas and equipment for the various processes used in LPA methodology (DNA extraction, PCR amplification, hybridization and data interpretation). Based on the comments above, LPAs are used only in the upper- and middle-tier facilities where the conditions can be met and there are trained staff for their use.

Table 2. Current LPA products and associated equipment marketed for MTBC diagnosis, mycobacterial speciation and genotypic DST

Developer	Test name	Myc/MTB/Spec	DST	CE-IVD	Automated hybridization	Test reader	Released
AID	TB Resistance Module INH/RIF	Y/Y/N	RIF/INH	Y	N/A	AID scanner	Q2 2013
AID	TB Resistance Module FLQ	Y/Y/N	FLQ/EMB	Y	N/A	AID scanner	Q2 2014
AID	TB Resistance Module AMG	Y/Y/N	AMG	Y	N/A	AID scanner	Q2 2015
Hain Lifescience	GenoQuick® MTB	N/Y/N	No	Y	GT-Blot 48	GenoScan®	2010
Hain Lifescience	GenoType® MTBDRplusv2.0	Y/Y/N	RIF/INH	Y	GT-Blot 48	GenoScan®	2012
Hain Lifescience	GenoType® MTBDRslv1.0	Y/Y/N	FLQ/AMG/EMB	Y	GT-Blot 48	GenoScan®	2012
Hain Lifescience	GenoType® MTBDRslv2.0	Y/Y/N	FLQ/AMG	Y	GT-Blot 48	GenoScan®	2015
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 Assay	Y/Y/Y	No	N/A	Genoline station	Genoblot	2006
LG Life Sciences	AdvanSure MDR TB Assay	Y/Y/N	RIF/INH	N/A	Genoline station	Genoblot	2006
NIPRO Corp.	NTM/MDR-TB	Y/Y/N	RIF/INH	N/A	Name unknown	Name unknown	2012
NIPRO Corp.	INH	Y/Y/N	INH	N/A	Name unknown	Name unknown	2012
NIPRO Corp.	PZA	Y/Y/N	PZA	N/A	Name unknown	Name unknown	2012
NIPRO Corp.	FLQ	Y/Y/N	FLQ	N/A	Name unknown	Name unknown	2012
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

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

Improvements to test sites and protocols have reduced concerns over amplicon contamination of the test area and user variation in result determination. Throughput of LPA testing can be increased via using automated instrumentation for DNA extraction (generic or developer specific, e.g. Hain Lifescience GenoLyse® platforms). Most developers now offer associated equipment for use with the assays, including PCR machines, wash/hybridization platforms, digital scanners and the software to score test results from processed strips. These advances in processing have improved test throughput and also simplified the implementation of LPA testing in many upper-tier laboratories and reduced risks around incorrect use or interpretation. The time to process up to 48 samples after DNA extraction is typically under 4 hours if using the automated systems described in Table 2.

As noted earlier in this document, WHO has approved the use of several LPAs in 2016; two for the diagnosis of TB in addition to the genotyping of RIF and INH,⁷ and a third LPA to genotype resistance to SLIDs and FLQs for XDR TB.⁸ Specifically, the Hain Lifescience GenoType® MTBDR*plus*v2.0 assay and the NIPRO Corporation NTM+MDRTB detection kit can be used to genotype RIF/INH. FIND recently published the data from a multicentre non-inferiority study where the performance for both of the new assays was compared to the original GenoType® MTBDR*plus*v1.0, which had been previously endorsed by WHO in 2008, and concluded that both of the new assays had comparable performance to the existing test.¹⁴¹

An independent study in South Africa assessed the performance of the MTBDR*plus*v2.0 and the GenoType® MTBDR*sl* as a follow-on rapid screening test for MDR TB and XDR TB directly for sputum specimens. This study indicated that the test performed well with SSM positive/culture positive sputum but that performance significantly dropped in SSM negative/culture positive samples.¹⁴² The pricing of the NIPRO Corporation assay is not known but FIND has negotiated preferential pricing for the MTBDR*plus*v2.0 for HBCs. The MTBDR*plus*v2.0 assay retails at €720 (96 tests) and the other ancillary equipment needed to perform this assay are also available under this pricing initiative.²⁰

Hain Lifescience has two versions of the GenoType® MTBDR*sl* assay. Both assays detect MTBC and have internal controls to inform on adequate amplification of the sample by PCR and for hybridization. The first version was developed to genotype resistance to FLQ via *gryA*, SLID resistance (SLID including KAN, AMK and CAP) via *rrs* and ethambutol (EMB) resistance via *embB*. The MTBDR*sl*v2.0 assay targets *gyrA* but includes assays for *gyrB* mutations that are also associated with FLQ resistance. Furthermore, the assay incorporates further SLID resistance genotypes via the *eis* promoter region. The *embB* resistance component is not used in the v2.0 of the assay.

The WHO endorsement of the MTBDR*sl* assays is significant in terms of the application of new shorter treatment regimens for MDR TB for both adults and children older than 4 years of age as there is a need to first screen RR TB patients for resistance to these second-line drugs.^{15,18} Patients can be screened in a matter of days and those not resistant to second-line drugs are eligible for short-term treatments. WHO also noted that among patients prescribed a conventional MDR TB regimen, these tests would also help decide from the start who would benefit from adding one of the new drugs

to strengthen the regimen.⁸ Performance data from a variety of evaluations are now available, including a multicentre evaluation study,¹⁴³ and performance evaluations in both European and South African laboratories using both phenotyped culture isolates and clinical specimens.^{142,144,145} This assay uses the same processing equipment offered by Hain Lifescience and currently, while negotiated pricing is available for the hardware,²⁰ the price per test is €7.50 with volume discounts available.

The MolecuTech REBA MTB-MDR[®] LPA offered by YD Diagnostics has undergone evaluation in a study led by FIND and a manuscript describing its performance is being drafted.

Summary: The ability to rapidly detect MDR TB or XDR TB has a great impact on patient care and in selecting the correct treatment from the start. LPAs can offer actionable results within 1-2 days and can be applied to SSM positive sputum samples. Allied with culture methodology, LPAs offer a much faster time to result for DST than by using subsequent phenotypic testing. Test sensitivity is more challenging with SSM negative/culture positive samples but Hain Lifescience has been improving its PCR assay reagents to increase sensitivity using SSM negative/culture positive samples with the MTBDRs/v2.0 assay. In 2015, WHO noted that the increase in reported MDR TB cases was due to expansion of molecular genotypic tests such as LPAs and Xpert[®] MTB/RIF.¹¹ Further key benefits to the use of these tests are that genotyping can be scaled and at a lower cost than compared to phenotypic testing. It should be noted that the complexity of using LPAs does limit their application to laboratories in reference laboratories or tertiary or secondary hospitals that are at the upper tiers of health-care systems. LPAs are also challenged by the limited number of allelic targets that can be screened on a single strip. This is demonstrated with PZA due to the higher number of alleles associated with resistance that led to a 2-strip method using 49 probes to be investigated or with INH where other rare alleles associated with INH resistance are not included in the assay.^{146,147} Recently, there were concerns around false-positive FLQ results with the MTBDRs/l assay due to amplified target DNA not binding to either the wild-type or to the resistant allele probes leading to the assumption that as neither the wild type nor mutant were not positive, a novel mutation may be present and thus likely to be FLQ resistant.¹⁴⁸ A detailed analysis of this problem using DNA sequencing and phenotypic testing for FLQ resistance concluded that the absence of wild-type probe hybridization without mutation probe hybridization was the result of a failure of the mutation probe not hybridizing correctly and not the result of novel or rare mutations within the target sequence.¹⁴⁹

Microarrays

Microarrays may be used as an alternative method to rapidly diagnose and speciate TB and genotype for MDR TB. Microarrays require the purification and amplification of TB DNA with target-specific primers to enable asymmetric PCR to generate labelled DNAs.

As with LPAs, this now labelled ssDNA is subsequently bound to their complementary capture probe that represents a specific allele (e.g. wild type or drug resistant) via hybridization. The principle difference is that each probe is printed in a small discrete spot, as opposed to a comparatively large stripe with LPA. This decrease in area allows more probes to be printed in a geometric array offering two advantages. First, more controls or probes to other resistance alleles may be incorporated into a test. Second, probes are typically printed in duplicate or triplicate on the array thereby providing greater accuracy when scoring a test result. However, the interpretation of microarray data does require a dedicated instrument as the probe spots are not visible to the naked eye and typically detection uses fluorophores or electrochemical detection. Microarray kits offer primers to amplify the target DNA regions in addition to the arrays and other materials required for performing the test. There are several microarray products on the market or in development.

Beijing Capital Biotechnology Ltd (China) offers two microarray products (GeneChip) directly related to TB: the Mycobacterial Species Identification Array Kit; and the *M. tuberculosis* Drug Resistance Detection Array Kit. The first product speciates MTB and other NTMs from a specimen, while the second genotypes for resistance to RIF via *rpoB* and INH via *katG* and the *inhA* promoter region. There are a significant number of operator steps to extract and purify DNA, prepare and perform PCR followed by hybridization of PCR product to the array and the subsequent washing before reading each array on a microarray scanner. Many of these processes are automated but the user is required to transfer the test materials between the necessary equipment. Published evaluation reports noted the entire process takes 6 hours.^{150,151} The company offers the ancillary equipment related to these tests. As with LPAs, assay sensitivity is more limited than most NAAT diagnostics and the company notes that the limit of detection for either assay is 1000 cfu/mL.

MTB identified through the use of microarrays was identified using clinical specimens and culture-positive samples, with 100% specificity.¹⁵⁰ Recently, one study also showed good performance in the correct identification of culture isolates, while using sputum with 100% concordance with culture (18/18) but more limited with pleural fluid (16.7%; 2/12) and bronchiolar lavages (10%; 1/10).¹⁵¹ The initial evaluation of the assay compared phenotypic culture testing with the MDR TB assay where the researchers noted 91.8% for isolates and 94.6% for sputum samples for RMP resistance, and 70.2% for isolates and 78.1% for sputum samples for INH resistance.¹⁵² A multicentre evaluation of the assay versus phenotypic cultured-based testing gave similar data with the sensitivity and specificity of the GeneChip of 87.56% and 97.95%, respectively, for RIF resistance and 80.34% and 95.82%, respectively, for INH resistance.¹⁵³ The GeneChip was also used to investigate MDR TB in naive cases and with patients already enrolled on treatment. This study was in a city in China where there is a high prevalence of MDR TB. In an analysis, the mean cost of diagnosing a single case of MDR TB using the GeneChip test, in China, was US\$ 22.38 as opposed to US\$ 53.03 using the phenotypic algorithm. The company concluded that a decrease in market price and improvements in the performance of the GeneChip would influence its wider application. Both arrays have received CFDA

approval for use in China and have CE-IVD marking. Prices in 2015 were US\$ 25 per MTB/NTM array with the MDR TB assay listed at US\$ 21.

Other array developers are developing microarray platforms that have more integrated steps to automate more key processes and, therefore, limit the need for user input to complete a test. The technologies also combine MTB diagnosis and genotyping of drug resistance within a single-test unit. Sample preparation is still required to extract and purify TB DNA from sputum specimens. Greater automation may permit their use at the intermediate level due to reduced need for skilled user input but the equipment still requires reliable main power and reagents stored under cold chain.

Akonnii continues to develop the microarray platform (TruDx®2000) based on its proprietary gel array drops (TruArray®), which are claimed to improve DNA/RNA hybridization kinetics (Figure 12). This is achieved by encapsulating the capture probes in a porous gel matrix that is 99% water rather than simply printed on a solid surface that can compromise hybridization kinetics and efficiency due to the immediate proximity of printed probes to the surface. The assay is designed to be used with DNA from sputum that is prepared via the TruTip® extraction tool (Figure 12), a pipette tip that contains a nucleic acid binding matrix with volume transfer and reagent mixing/washing mixing being performed via a hand-held battery-powered automatic pipettor that hosts the TruTip®. In developing its array, Akonnii has employed several innovative components in the design of the chip, including an all plastic microfluidic design that is valve-less and pulls the sample and other reagents into the reaction detection chamber via capillary flow.

Figure 12. Akonnii TruDx®2000 platform with TruTip® extraction pipette, TruArray® processor and TruArray® scanner (left); TruArray® test, microfluidic valve-less design for simultaneous on-slide PCR and microarray hybridization in a closed format (right)



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Asymmetric PCR amplification of targets is simultaneously coupled to probe-hybridization events on the array in a single chamber so that the array “sees” the total sample (no sample splitting).^{154,155} Waste wash fluids are wicked into an absorbent chamber, therefore limiting the risk for amplicon contamination of the test area (Figure 12). The all plastic design and plastic film-based substrate significantly reduce manufacturing costs versus traditional glass, silicon wafers or microelectronic chips with surface functionalization coatings. Akonni is pursuing a very low-cost reel-to-reel manufacturing approach to making the TruArray® tests. The company labels amplified DNA with a fluorescent dye that is used to detect the bound probe to its respective complementary target probe and then detected by a charged-coupled device reader that employs a light-emitting diode to excite the fluorophore. The processor and application are hosted on the reader so that an attendant computer to operate the process and analyse the raw data is not necessary.

The prototype array is designed to identify MTB and *M. avium* complexes via *IS6110* and *IS1245* in addition to genotyping resistance alleles to RIF, INH, EMB and STR.^{156,157} An internal control to indicate the presence of confounding materials is also included to qualify each assay’s performance. The analytical sensitivity limit of detection is estimated to be 25–110 genome copies per amplification reaction. In a blinded analysis of 153 clinical isolates, microarray sensitivity for first-line drugs relative to phenotypic DST was 100% for RIF (14/14), 90.0% for INH (36/40), 70% for EMB (7/10), and 89.1% (57/64) combined. Overall, microarray specificity for RIF, INH, and EMB combined was 97.2% (384/395). For the second-line drug STR, overall sensitivity was 34.8% (8/23) because of limited microarray coverage for STR-conferring mutations, and specificity was 99.2% (129/130). All false-susceptible discrepant results were a consequence of DNA mutations that are not represented by a specific probe on the microarray. There were zero invalid results from 220 total tests. The test was performed in 6 hours and can be run in batches of 1–16 samples at a time. The simplified microarray system is suitable for detecting resistance-conferring mutations in clinical MTB isolates and can now be used for prospective trials or integrated into an all-in-one, closed-amplicon consumable.^{156,157}

The combined platform (TruTip® workstation and TruDx®2000) has shown promising results with sediment and raw sputum under National Institutes of Health (NIH) programmes with Harvard University and the University California San Diego (manuscripts pending). The platform is currently undergoing retrospective and prospective trials in China, Mexico, Peru and the Republic of Moldova. Preliminary data on non-sputum samples such as stool and gastric aspirate are promising. Studies done in Peru resulted in an analytical limit of detection of 32 cfu/mL. Also, the TruTip® workstation was retrospectively tested with MTB-positive paired raw sputum and sediment samples (extracts tested via real-time PCR) resulting in 99.2% MTB detection (114/114 SSM positive and 9/10 SSM negative). The TruTip® microarray specificity is 91.3% (63/69) for RIF and 98.2% (56/57) for INH. Sequencing analysis of discordants is in progress for discrepant analysis. Two new MDR arrays are in production and prototype testing: a PZA mini-sequencing array; and an XDR array inclusive of markers for FLQs and SLIDs in addition to broadening the coverage for RIF and INH resistance. The release date for this product is also unknown but the developer is intending to submit the tool for CE-IVD marking in addition to aiming for WHO endorsement in the coming years. Akonni intends to offer the tool globally once these are obtained and in production.

Figure 13. Veredus Laboratories VerePLEX™ Biosystem and VereMTB™ Detection Kit



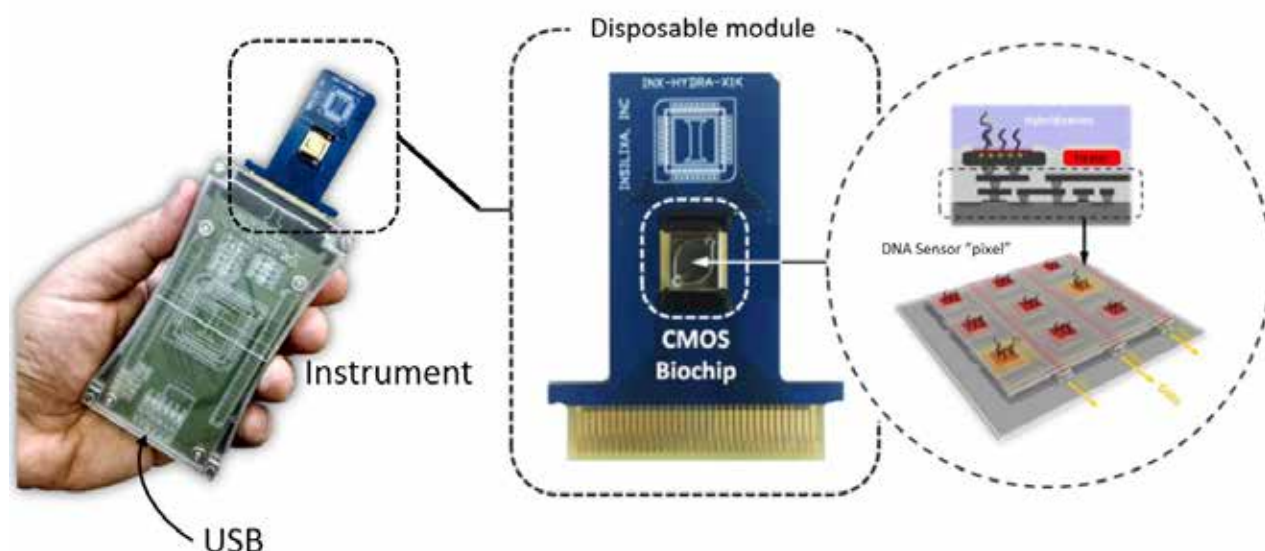
Notes: Both PCR amplification and microarray hybridization are performed in the VereMTB™ Detection chip (image A). The VerePLEX™ Biosystem and reader includes a five-unit processing station for the on-chip PCR and the subsequent hybridization and washing of the chip (image B). For analysis each chip is manually inserted into the reader that interrogates the array (far right).

Source: Images reproduced with permission of Veredus Laboratories.

Veredus Laboratories (Singapore) market the VerePLEX™ Biosystem platform, developed by ST Microelectronics (Switzerland). This platform can host several disease-specific microarrays, including the VereMTB™ Detection chip. The VereMTB™ chip is designed to identify MTB as well as speciate common NTMs.¹⁵⁸ The array can also genotype for resistance to both RIF and INH. The system first involves independent extraction of TB DNA from samples. The extracted DNA solution is then added to two chambers on the array chip that is inserted into a processing module on the VerePLEX™ Biosystem where target amplification occurs via PCR with subsequent binding of amplified DNAs to their complementary probes. This platform can independently host up to five test arrays for PCR at one time (Figure 13). After PCR, each chip is individually read in a reader and the raw data are analysed by VereID software to generate results on speciation and resistance to RIF and/or INH. The time to result is around 2 hours and the assay may be used with culture or SSM positive samples.

One evaluation study has examined the performance of the assay in both low- and high-burden settings. The assay correctly identified MTBC isolates from negative samples. Drug resistance was assessed via culture DST, the MTBDRplus1.0 LPA, the Xpert® MTB/RIF assay, DNA sequencing and the VereMTB™ Detection chip. The sensitivity and specificity of the chip for genotyping resistance to RIF were both 100% as compared to MTBDRplus and DST. With INH, the specificity was lower depending on the target gene or the qualifying assay used.¹⁵⁸ In the fourth edition of this landscape report, Veredus Laboratories had noted that the product had been on the market as a research use only product since 2012 and that it would be entered for CE-IVD certification in 2016. It has not been possible to confirm if this has occurred.

Figure 14. 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 of InSilixa Inc.

InSilixa Inc (USA) and *Stanford University* (USA) have developed the Hydra-1K microarray platform. This technology employs complementary metal-oxide semiconductor (CMOS) technology for lens free digital imaging and uses heating within 1024 individual spots on the array to enable on-chip PCR of the specific MTB target amplicons (Figure 14). The array can detect the binding of complementary DNAs to their arrayed probes on DNA sensor pixels and, in addition, the assay uses melt curve analyses after amplification and hybridization to measure the rate of dissociation of amplicons from their capture probes as the temperature is increased. This creates specific fingerprints that can more accurately interrogate specific genotypes as compared to LPAs or other microarrays. *InSilixa Inc.* also claims to have improved the detection of alleles that are challenging to detect due to complex secondary structure of these targets preventing hybridization.^{159,160} The company has completed, tested and validated the multiplex PCR assay and capture probes as these pertain to the Hydra-1K CMOS biochip for the detection of MTB and 120 mutations that confer resistance to INH, RIF and FLQs. *InSilixa Inc.* is currently working to incorporate a DNA extraction component into the device with a prototype completed in Q1 2017 and projects that a functional prototype will be ready for pilot testing in field settings in 2 years.

Summary: Microarrays can offer rapid genotyping and with greater confidence than LPAs based upon multiple probes per array targeting the same allele to confirm a binding event. In principle, arrays can also interrogate more allelic targets than LPAs in a single test but they have to be first amplified by multiplexed PCR, which may be a rate-limiting step if many different gene targets are required. While the processing of LPAs can be automated to enable higher throughput, each of the steps is independent. With arrays, some developers

are focusing their efforts on creating chips where PCR, and other subsequent steps, are performed without user intervention, essentially creating platforms that maintain a high test quality but with limited user input. Historically, microarrays have been considered relatively expensive but developers are looking into using novel substrates to significantly reduce manufacturing costs (e.g. Akonni) and in some cases using the substrate for measurement of probe binding (e.g. InSilixa Inc. or Veredus Laboratories). In addition, new detection methods permit low-cost instrumentation due to reduced complexity as opposed to traditional instruments using confocal microscopy and fluorescence detection of bound probe spots. There are very limited peer-reviewed data on these products and performance needs to be more rigorously assessed, especially by independent groups since several pilot studies in this section were performed by the developers.

Modular, cartridge-based, fully automated NAATs

The scaled adoption of the Cepheid Inc. Xpert® MTB/RIF assay across many HBCs has led to other technology developers following its lead in developing cartridge-based assays for the diagnosis of MTB and for genotyping of drug resistance. The advantage to such platforms is that a high-quality test can be offered but without requiring significant skilled user input. In principle, the result can be obtained on the same day. A further key advantage to most cartridge-based platforms is that diagnostic tests for other key diseases can be hosted on the same platform, e.g. HIV or hepatitis C virus. In this section, the Cepheid Inc. Xpert® and the Tosoh Bioscience (a subsidiary of Tosoh Corporation, hereinafter Tosoh) TRC.80 instruments and associated assays are described.

The Xpert® system fully integrates and automates sample extraction, amplification and detection in one cartridge. The sample reagent is supplied with the test so sputum specimens are liquefied and MTB cells inactivated. The processed sample is then transferred to the test cartridge. After the cartridge barcode is scanned, it is placed into the Xpert® system. The processing of the sample, DNA extraction, semi-nested PCR amplification and determination of real-time data analysis are all fully automated in a process that takes less than 2 hours. The Xpert® system is available in a one-, two-, four- or up to a 16-module configuration. The Infinity is an automated, multimode molecular diagnostic analyser that uses exclusive “load and go” technology for total walkaway operation with complete random access availability. Infinity systems allow configurations in sets of eight modules from 16 to 80 modules. All instrument configurations use the same patented cartridge technology for every Xpert® test. Most laboratories in the emerging markets use the GX-4 with four independent test modules for processing test cartridges (Figure 15). In 2012, Unitaid, the President’s Emergency Plan for AIDS Relief (PEPFAR), the United States Agency for International Development (USAID) and the Bill & Melinda Gates Foundation finalized an agreement with Cepheid Inc. to further reduce the negotiated price of the Xpert® MTB/RIF test for eligible customers on the FIND country list to US\$ 9.98 per test, ex-works and prepaid. As of December 2016, eligible procurers bought 6.9 million cartridges at the concessional price. A recent publication reviews experiences with implementation of the Xpert® MTB/RIF assay.²¹

A growing number of countries have already adopted national algorithms positioning the Xpert® MTB/RIF as the initial diagnostic test for all people with signs and symptoms of pulmonary TB.¹ Nigeria, with the fourth largest TB burden in the world, has become the latest country to issue a national directive calling for the use of Xpert® MTB/RIF as the initial diagnostic test for all presumptive TB cases. During phased implementation of this directive, smear microscopy will remain the initial diagnostic with referral for Xpert® MTB/RIF testing for high-risk groups in areas where access to Xpert® remains a challenge. The directive was issued on 16 March 2016 by the Nigeria National TB and Leprosy Control Programme.

Uptake by countries appears to be accelerating with over 23 million Xpert® MTB/RIF cartridges and almost 30 000 Xpert® systems procured between 2010 and 2016. Significantly, India recently announced the procurement of 500 Xpert® systems. However, with the exception of South Africa, the number of procured cartridges in 2015 compared to the total number of instrument modules reflected an average ratio of only 1.0 test per module per working day.¹ A recent review of the Xpert® MTB/RIF assay noted that more than 16 million tests have been performed in 122 countries since 2011.²¹ However, work remains to be done to realise the full potential of the installed capacity. New data suggest that Xpert® is very highly priced with mark-ups of three to four times purchasing costs at the point of care primarily driven by factors such as transport, custom fees and local distribution. In the private sector in high-burden countries access is quite limited, with notable exceptions, such as the Initiative for Promoting Affordable and Quality TB Tests (IPAQT) programme in India.

Figure 15. Cepheid Inc. GeneXpert® IV System (GX-4) with four independent modules for processing test cartridges (left) and the Xpert® MTB/RIF cartridge (right)



Source: Images reproduced with permission of Cepheid Inc.

Cepheid Inc. has been developing two new TB cartridges to be hosted on the current GeneXpert® platform. The design goal of the Ultra MTB/RIF assay is that it is as sensitive as culture-based methods and can also genotype RIF resistance as a surrogate for MDR TB diagnosis (Figure 16).¹⁶³ The key modifications include a larger reaction volume, nested PCR, faster PCR cycling, multicopy MTB specific targets (*IS6110* and *IS1081*), and melt curve analysis to more accurately discriminate alleles in *rpoB* that are associated with RIF resistance. Whilst both Ultra MTB/RIF and Xpert® MTB/RIF assays had similar performance in identifying RIF resistance, as compared with sequencing of *rpoB*, the use of melt curve analysis to genotype RIF resistance in the Ultra MTB/RIF allows for better discrimination of silent and active mutations in the *rpoB* target. The current Xpert® MTB/RIF assay cannot discriminate between silent and active mutations in *rpoB* and therefore any mutation is flagged as being RIF resistant.

Initial analytical data collected using Ultra MTB/RIF method on an open platform (i.e. not in the cartridge) showed high sensitivity and specificity for both RIF-resistant and sensitive strains. The Ultra MTB/RIF assay was also able to detect mixed alleles (heteroresistance) when one of the alleles represented 40% of the total MTB DNA.

An MTB positive sample is identified when either or both *IS* targets are detected before 37 cycle thresholds (Cts) of amplification and at least two of the *rpoB* targets are amplified before 40 Cts. Conversely MTB is reported as not detected if neither of the multi-copy target probes are positive and the sample processing control is positive with a Ct of less than 35 cycles. A further result definition, the “trace result”, has been created for this platform. A “trace result” is defined as when either or both *IS* targets are amplified before 37 cycle thresholds (Cts) and only one of the *rpoB* targets are amplified before 40 Cts. If MTB is detected by a “trace call” then no result can be determined for RIF resistance and the result is reported as MTB detected, trace, RIF indeterminate.

Figure 16. Cepheid Inc. Xpert® MTB/RIF Ultra cartridge



Source: Images reproduced with permission of Cepheid Inc.

WHO conducted a technical expert meeting in January 2017 in order to assess the results of the primary multicentre clinical evaluation study. The conclusion was that Ultra MTB/RIF is non-inferior to Xpert® MTB/RIF. The greater sensitivity of the Ultra MTB/RIF assay is anticipated to improve diagnosis in cases where paucibacillary specimens are typical or clients challenging to diagnose, e.g. in PLHIV, paediatric and extra pulmonary TB (notably cerebrospinal fluid). Cepheid Inc. projects that the Ultra MTB/RIF assay will be on the market as a CE-IVD registered diagnostic test in 2017. The Ultra MTB/RIF assay will be available to eligible customers at US\$ 9.98 per test, ex-works and prepaid, the same price as the current Xpert® MTB/RIF assay.

The second assay in development is intended to detect XDR TB as a reflex assay to Xpert® MTB/RIF or Ultra MTB/RIF positive tests for TB. The XDR TB assay is designed to detect mutations for resistance to INH, FLQ and AMG with further use of asymmetric PCR in conjunction with sloppy molecular beacons and fluorescent melt curve analysis to genotype for resistance alleles to these drugs. A further development with this product is the expansion to a 10-channel fluorescent detection spectrum rather than the current 6-channel spectrum. The expansion of the range of fluorophores in a multiplexed reaction permits the use of further sloppy molecular beacon designs to interrogate yet more alleles in a single-test reaction.¹⁶³ The XDR TB assay is currently undergoing pilot studies and the release date and price is not known. While these assays utilize novel attributes as compared with the Xpert® MTB/RIF cartridge, existing Xpert® systems can be modified by optical recalibration and software updates to operate the new XDR TB assay. Cepheid Inc. was recently acquired by the Danaher Corporation but it is not expected to affect the current role of Cepheid Inc. in production and supply of Xpert® systems and Xpert® assays to the global TB community.

Tosoh Bioscience (Japan) has developed a standalone device for integrated molecular diagnostics, the TRCReady® 80 that can process up to eight samples. The platform requires a computer for operation and two test units can be used. Tosoh markets the TRCRapid® M.TB kit for use on this platform to permit fully automated sample purification, amplification and detection. Sample preparation uses liquefied sputum to which lysis buffer is added, with heating to inactivate MTB cells and to release 16S RNA. The assay uses transcription reverse-transcription concerted reaction (TRCR) amplification technology to amplify the MTBC-specific target within 16S RNA and also uses an internal control. The amplification reactions are measured in real-time via total fluorescence and take only 30 minutes to complete. The internal control is used to indicate the presence of confounders that may inhibit TRCR amplification. The entire process takes 40 minutes if sample preparation is included. One multicentre evaluation of an earlier TRCR MTB assay noted an overall sensitivity and specificity of 87% and 98%, respectively, with a detection limit of 30–50 cfu/mL.¹⁶⁴ Currently, there are no data available on reagent stability, cost, regulatory certification or intended markets for this device. However, the device has been described in clinical settings in both Europe and Japan.¹⁶⁴⁻¹⁶⁶ *Summary:* Although there are other standalone automated NAAT platforms, there are currently few developers other than Cepheid Inc. in this space who are offering products now or in the near future in terms of sufficient evidence to enable WHO recommendations as to their performance and use. Xpert® has changed approaches to TB diagnosis, especially with PLHIV and by improving the rates of early diagnosis of MDR TB. The recent recommendation of the

more sensitive Xpert® Ultra MTB/RIF cartridge will permit the rapid testing for MTB but with the a similar sensitivity to culture and also inform on RR TB.¹⁶⁷⁻¹⁶⁹ Similarly, the use of the XDR TB assay will provide more rapid diagnosis of XDR TB and, in addition, as this assay also detects FLQs and SLIDs, it may be used for screening eligible patients for shorter-term MDR TB treatment.¹⁵ Since the Xpert® MTB/RIF assay was approved over 6 years ago it has seen a dramatic uptake in global terms, a process that has highlighted the gaps and challenges that are described in a 2016 review.²¹ A primary challenge is to better measure the impact of Xpert® in terms of its use in improving patient outcomes; while it is clearly evident that the assay is more sensitive than SSM and can identify MDR TB, other gaps in the TB test and treat continuum have been shown to obscure the impact of improved diagnosis. These include the empiric treatment of TB cases that were Xpert® or SSM negative or that studies on improved outcomes were statistically underpowered to detect important effects on mortality.^{21,169,170} The cost of Xpert® remains a challenge to widespread use and there currently appears to be limited competition in terms of other automated platforms. Tosoh described above, has yet to be submitted for WHO review and release dates are unknown.

NAATs for use at peripheral centres

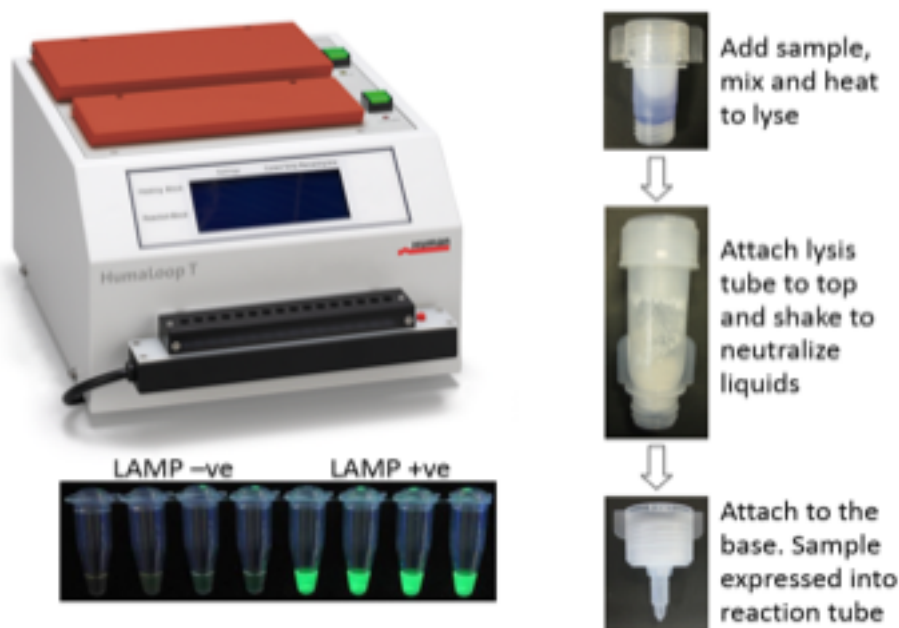
While the majority of the above technologies represents some advances in the diagnosis and treatment of TB, these are typically for centralized facilities and usually not available to the majority of the population initially seeking care. The reality is that most access to diagnosis and care for TB infection is via a vast network of decentralized, peripheral microscopy centres often located within primary or community health centres.¹⁷¹ There are a variety of clinical, analytical and logistical challenges associated with this thus while microscopy remains the lowest cost assay for the diagnosis of PTB, there is a need to significantly improve the rates of diagnosis, especially with populations challenging to diagnose via SSM e.g. PLHIV and paediatric groups. There have been efforts to better define microscopy centres in terms of the infrastructure, staff skills and resources available in HBCs,⁹⁷ in conjunction with efforts to estimate the cost per SSM event in order to better define the potential available markets in BRICS and HBCs so that test developers can better understand the market.^{65,172} A survey of microscopy centres in HBCs and BRICS revealed that most countries faced challenges in terms of extreme environmental conditions (e.g. humidity and dust), infrastructure needs (mains electrical power), available equipment and skills.⁹⁷ These factors, and others such as cost, create significant challenges to developers as tools for use in these settings must be robust, operate independently of mains power, easy to use, scalable, more accurate than SSM and offered at an acceptable cost.^{95,97,173} Allied to this is the need for a low-cost molecular diagnostic test to inform on drug resistance as most current methods are often inadequate in terms of a rapid turnaround time.¹⁷⁴

There are currently some products that have been marketed for several years with an intended use in peripheral settings. However, of these only one technology has been sufficiently evaluated in a variety of settings in order to now be approved by WHO, the Loopamp™ MTBC Detection kit from *Eiken Chemical Corp.* (Japan).⁶ Since 2012, this test has undergone 20 evaluation studies in 17 countries. This is the first NAAT product specifically designed for use in microscopy-level facilities to receive WHO endorsement, which had

two conditional recommendations for the use of the assay instead of conventional SSM where patients present with symptoms of TB or as a follow-on test where follow-on testing of conventional SSM results is necessary.

The first version of the assay was released in 2011 by the company and has undergone some modifications since in terms of specimen and reaction volumes and the protocol used for the assay. The assay is well suited to resource-limited settings as the equipment is relatively simple and several user steps are added to reduce instrumentation complexity, including sample preparation and the interpretation of test results (Figure 17). The assays can be batched and up to 14 samples can be screened in a single run with control reactions. The assay uses 60 μ L of raw sputum that is transferred to the sample preparation tube (Figure 17). MTB cells are inactivated and lysed via a combination exposure to highly alkaline conditions and temperature (90 °C) for 5 minutes. The sample preparation tube interlocks with a sample neutralization tube where the pH of the heated sample is neutralized. The final step is the addition of an applicator tube whereby ~30 μ L of the liquid treated sputum contents can be expressed into a reaction tube. The assay reagents are stored as a glassified pellet inside each reaction tube lid. The DNA extracts are added to the tube strip, the caps are then closed and the tube strips inverted for 2 minutes to wet the reagents and then mixed to permit their introduction to the samples.

Figure 17. HumaLoopT instrument (from HUMAN Diagnostics Worldwide, top left) and the Pure DNA Extraction kit



Notes: The images show the Extraction kit components that are sequentially integrated during the sample preparation process (right top to lower) and the fluorescent signal generated by MTB DNA positive reactions (bottom left).

Source: Images reproduced with permission of HUMAN Diagnostics Worldwide.

The assay uses LAMP, an isothermal manual DNA amplification method that takes 40 minutes to perform at 67 °C in this assay. After incubation, the reactions are terminated by briefly heating at an elevated temperature. The results of each reaction are scored visually by the user via fluorescence, which is generated when DNA is amplified by the TB-LAMP reaction (Figure 17). The light source is supplied with the instrument and the user compares the green fluorescence of the positive control to each test. A negative control provides further user input to score any negative tests. The summary data from the 2016 WHO policy guidance document noted the pooled sensitivity of TB-LAMP was higher than for SSM, ranging from 77.7% to 80.3%. The pooled sensitivity for the TB-LAMP among SSM positive patients ranged from 95.2% to 96.6% across studies, depending on the reference standard used.⁶ The pooled specificity of the assays was also slightly variant depending on the reference methods used to qualify results with ranges from 97.7% to 98.1%. Eiken Chemical Corp. has partnered with HUMAN Diagnostics Worldwide (Germany) to globally distribute and market the assay and instrumentation necessary to perform the Loopamp™ MTBC Detection kit. Training requirements for LAMP is similar to the amount of training for smear microscopy.¹⁷⁵ These products have been available since Q4 2016 and eligible countries and negotiated pricing can be accessed on the FIND website.²⁰ The Loopamp™ Pure DNA Extraction kit costs €298.20 (90 extractions), the Loopamp™ MTB Detection Kit is €352.50 (2 x 48 reactions) and the instrument is €2450.00.

Epistem Ltd (UK) offers the Genedrive® MTB/RIF assay for use with its Genedrive® PCR instrument. This assay can test one sputum sample at a time and requires some manual preparation of the test material. The Genedrive® MTB/RIF cartridge assays can screen for three individual components: an MTBC assay; a genotyping assay targeting *rpoB* to indicate RIF resistance; and a control assay to indicate inhibitory compounds in the sample. The assay reagents are provided pre-dried in each reaction tube and the user rehydrates these to 20 µL with sterile water. Small volumes of sputum samples (e.g. 20–50 µL) that are smeared onto a paper-based substrate, left to dry and then 1 mm discs are removed via a circular punch and one disc is transferred to each rehydrated test reaction well. The test cartridge is then inserted into the Genedrive® PCR instrument for PCR analysis, and the results automatically scored after melt curve analysis. Excluding cartridge preparation, the process cartridge insertion to results delivery is approximately a 1-hour process. The technology has been CE-IVD certified and in April 2015 the Drug Controller General of India (DCGI) issued a 3-year import license to the company's distributor, Xcelris Labs. The MTB/RIF product was released in May 2016 in India only.

Peer-reviewed data on the performance of this product are very limited. However, one independent study has reported analytical and clinical performance of the assay to detect MTB but did not investigate the RIF resistance component.⁹⁶ The analytical sensitivity of the MTB assay was 100 genome equivalents when using MTB DNA. Using quantified MTB cells spiked into distilled water or non-infected sputum, the limit of detection increased to 2.5×10^4 and 2.5×10^5 cfu/mL, respectively. In screening specificity with NTMs and other bacterial species, all were negative via the MTB-specific assay but several NTMs were occasionally positive via the *rpoB* assay and indeed several negative controls (9.4%) also gave a false-positive result upon *rpoB* melt curve analysis.

To evaluate clinical performance the Genedrive® MTB/RIF assay was compared to culture, SSM and the Xpert® MTB/RIF assay. The sensitivity and specificity of the assay were

assessed using pre- and post-homogenized sputum and from a second sputum sediment pellet. With SSM positive/culture positive samples, the sensitivity of the Genedrive® sequentially improved from pre-homogenization (58.7%), post-homogenization (74.7%) and then with pellets (85.1%); specificity remained similar for all at ~97.5% on all three specimen types. With the SSM negative/culture positive samples prepared in the same way, sensitivity was very low at either 0% pre-homogenized or ~4.5% for post-homogenized and sediment samples. The sensitivity of Xpert® on SSM positive/culture positive and SSM negative/culture positive samples was 98.4% and 68.2%, respectively. The sensitivity and specificity of SSM for the first sputum sample (used for the pre- and post-homogenized samples) was 77.3% and 100%, respectively. Therefore, in this study, the researchers noted that sensitivity of the Genedrive® was inferior to Xpert® and also poorer than SSM, the assay it is intended to replace. If samples are first concentrated, then sensitivity increases but the need for liquefaction and sedimentation detract from the inherent simplicity intended with this assay. The study also highlighted that MTB cells are not fully inactivated on the sample preparation paper and so the need for biosafety also affects the use setting of the assay. The researchers recommended a biosafety level 2 laboratory in addition to a safety cabinet as a minimum requirement for use of the assay. As a result of these findings, civil society groups have written to the Drug Controller General of India to ask that this technology not be used in India until the performance of the test is significantly improved.¹⁷⁶

Epistem Ltd is marketing the product in India but it has noted that it is recommending its use in appropriate biosafety settings and it is working to redevelop the assay sample preparation to increase sensitivity. The company is generating more performance data to better inform on the assays performance regarding the concerns of civil society groups. Since the recent assessment of its technology, Epistem Ltd has noted that the lack of specificity of the *rpoB* assay when challenged by some NTMs has been resolved via a subsequent refinement to the data logic used in processing test results. It also offers post-market data on test performance in a white paper located on its website.¹⁷⁷

Figure 18. Molbio Diagnostics technologies: current and pending products for NAAT-based detection of MTBC and drug resistance



Notes: Trueprep™ AUTO, an automated and cartridge-based sample preparation extraction tool (image A). Truelab™ Uno and Truelab™ Quattro, single and 4x scaled test reactors for amplification and analysis of test data (image B) from the Truenat™ reaction chip (image C).

Source: Images reproduced with permission of Molbio Diagnostics.

Molbio Diagnostics (India) represents a joint venture of the Tulip Group and Bigtec Labs (both India), which has developed several platforms for the rapid diagnosis of infectious diseases, including MTB using the Truenat™, an innovative on-chip real-time PCR assay (Figure 18). The chips are hosted by the Truelab™ Uno RealTime micro PCR System, a battery-powered processing unit that is portable and has data storage, processing power, a global positioning system, Bluetooth connectivity and WiFi by virtue of incorporating an android phone to operate the technology. Two newer Truelab™ devices are soon to be available: the Truelab™ Uno Dx in which the system has increased fluorescence detection from two to three channels for further multiplexing of assays (release scheduled for Q2 2017); and the Truelab™ Quattro where four PCR chips can be independently processed rather than one with the Uno systems (release scheduled for Q3 2017, Figure 18). Molbio Diagnostics also offers an independent and semi-automated device to prepare DNA for analysis, the Trueprep™ MAG, which is also battery-powered and the company offers extraction kits for both PTB and extrapulmonary TB using this device (Figure 18). MTB DNA is detected via real-time PCR on the Truenat™ MTB chip which detects MTB and also an internal control to indicate adequate sample integrity for PCR. A second assay, the Truenat™ MTB/RIF, has been developed to genotype for RIF resistance in addition to detecting MTB. As noted previously, the company is developing a fully automated extraction platform, the Trueprep™ AUTO, which can process up to 48 samples in an 8-hour period. The time from sample to result using these platforms is 1 hour and its release is anticipated in Q2 2017.

Evidence of acceptable performance via the independent evaluation of the Molbio Diagnostics TB platforms is still needed with only two peer-reviewed articles regarding the performance of the Truenat™ platform for diagnosis of TB.^{178,179} The first study noted that the MTB assay had high sensitivity and specificity with SSM positive/culture positive of 99% and 100%, respectively. With SSM negative/culture positive specimens the sensitivity and specificity was found to be of 76% and 100%.¹⁷⁹ The second study also included the Xpert® MTB/RIF assay as a comparator and both assays had very similar performance in terms of specificity and sensitivity with both SSM positive/culture positive and SSM negative/culture positive specimens.¹⁷⁸

In 2016, six TB-related Molbio Diagnostics products received CE-IVD marking, including devices (Truenat™ Uno and Trueprep™ MAG), sample preparation kits (Trueprep™ MAG sputum and Trueprep™ MAG EPTB) and diagnostic assays (Truenat™ MTB and Truenat™ MTB-RIF). In addition, these products have also been approved for use in India. Ongoing evaluations include a multicentric study for including the Truelab™ platform in the Revised National Tuberculosis Control Program led by the Indian Council of Medical Research is nearing completion. If the performance metrics from this study are successful, then the products may be procured by the Indian public sector. The Molbio Diagnostics TB products are currently being evaluated by FIND, which intends to perform an accuracy study at three sites, and is planning an operational assessment of the technology at the microscopy level. Current prices offered by the company for the private market to procure the Trueprep™ and Uno Systems are US\$ 7000 and US\$ 14 per assay and it is intended that public sectors will receive a further discount.

Ustar Biotechnologies (China) is producing the EasyNAT™ TB assay to detect MTB DNA from sputum (Figure 19). Unlike the earlier described technologies, the company does not provide a platform but instead relies upon manual processing for the extraction of DNA and testing of sputum samples with a requirement for a uniform heat source to amplify

DNA via a water bath, PCR machine or similar. The assay uses an isothermal amplification method, cross-priming amplification, to amplify and label the *IS6110* target of MTBC. The cross-priming amplification amplicons are then detected by an immunochromatographic strip that detects the hapten labelled amplicons but within a sealed cassette to prevent amplicon contamination of the test site. Using batched processing the company estimates up to 40 samples can be tested in an 8-hour working day. Independent data on the performance of the assay are limited to two studies in China and the United Republic of Tanzania, respectively.^{180,181} A multicentre evaluation of the EasyNAT™ TB assay in China had sensitivity and specificity with SSM positive/culture positive samples of 84.1% and 97.8%, respectively. For SSM negative/culture positive samples, the sensitivity was reduced to 59.8%. In the much smaller cohort assessed in the United Republic of Tanzania, the assay performance was assessed on a population with a high HIV comorbidity (46.2%). The sensitivity and specificity of the assay were 81.6% and 100% with SSM positive/culture positive samples, respectively. However, with SSM negative/culture positive samples from PLHIV the sensitivity was reported to be only 10% (only 10 patients were SSM negative/culture positive). The assay has received CE-IVD marking and was approved by the CFDA in 2014. The approvals have also been granted in Indonesia and the Philippines. Approvals for use in India were expected in December 2016. Ustar Biotechnologies is targeting markets in both private and public systems with a price point of US\$ 6–8 per test. The company is currently also in the early development phase of a fully integrated diagnostic tool for MTB diagnosis.

Other NAAT-based platforms in early or mid-development: In earlier landscape reports, this area was shown as being very promising with 13 groups noted as developing products

Figure 19. Ustar Biotechnologies EasyNAT™ TB assay



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targeting the microscopy centre as their primary area of use. Since the 2015 landscape report, several companies have stopped development, postponed development or have been removed from this list due to a lack of any development information.

Tangen Biosciences (USA) is developing a small modular platform to diagnosis TB and other infectious diseases but they have currently stopped development of an MTB assay in order to focus development efforts on other disease targets. The North Western Global Health Foundation has developed a modular assay to be integrated onto the Savannah platform being developed by Quidel. Currently, Quidel is reviewing its development of this platform and, once again, development work in terms of a TB assay has been delayed to de-risk the business case by focusing on more incentivized products. Similarly, Qiagen is considering if there is a business case to develop its point-of-need technology for TB diagnosis. Fluorosentric Inc. (USA), GenePOC™ (Canada) and Wave80 Biosciences (USA) have platforms on which they claim to be developing MTB assays but there is no available evidence to support this. Updates on the products and development work by Tosoh Bioscience and Thisis are noted earlier in this landscape report. Development work under way by Cepheid Inc., QuantuMDx (UK), KGI (USA) and Scanogen (USA) is described below.

Cepheid Inc. (USA), in 2015, announced they are developing a true POC molecular system, the GeneXpert® Omni (hereinafter Omni) (Figure 20). This is a single standalone system that is capable of processing Xpert® cartridges in more austere settings than the current Xpert® instruments, which are not designed for extreme conditions such as elevated temperatures and humidity above 30 °C.^{181,182} This system will run both the current Xpert® MTB/RIF assay and Xpert® Ultra MTB/RIF, in addition to Xpert® HIV-1 Qual, Xpert® HIV-1 Viral Load and Xpert® HCV Viral Load assays. These assays will be available after Q3 2017. Over time, it is intended that the majority of the Xpert® menu will be available on the Omni, which will utilize the same fully automated sample preparation and processing as the original Xpert® systems. The Omni is small (23.1 x 7.6 x 10.6 centimetres) and portable, weighing only 1.0 kilograms. The system can be powered via mains electricity but also includes an internal rechargeable battery to prevent a test run being aborted due to a sudden power outage. Paired with an external battery, the Xpert® Omni will have 16 hours of power (i.e. 2 days of operation), without mains electricity – a common feature in many microscopy centres.⁹⁷

While the Omni uses the same core technology as the modular Xpert® systems, Cepheid Inc. has replaced key components with solid state technology that enables miniaturization and also adds robustness to delicate electrical systems. The Omni is computer independent and instead houses an internal processor to operate the system via a dedicated mobile device, thus no computer interface is required for operation. The mobile device is designed to permit secure connectivity that integrates real-time data streams for improved monitoring of productivity and performance for external quality assurance purposes. The device can store data for more than 20 000 tests if connectivity is absent or sporadic. The projected release of the Omni in emerging markets is after Q2 2018. At the original press release (July 2015) the price per unit was listed as US\$ 2895 but it is not known if this has changed.

Figure 20. GeneXpert® Omni

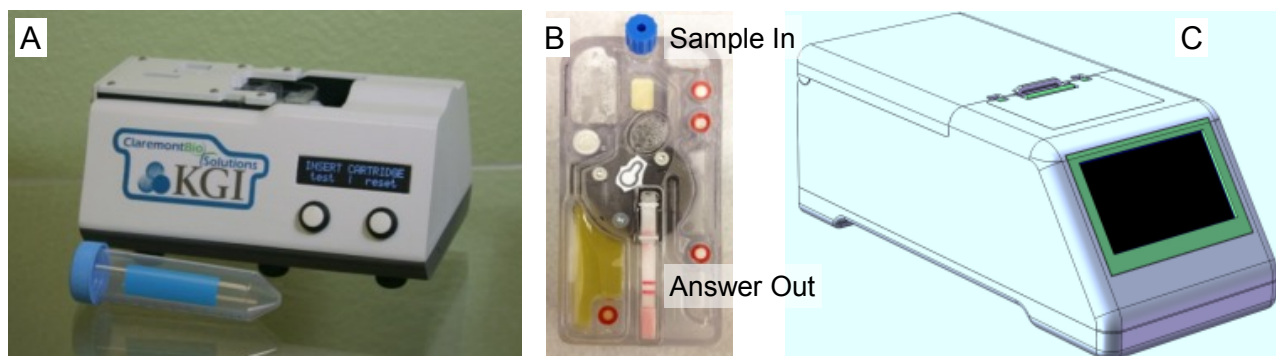


Notes: The Omni is a true POC molecular system, currently in development, for the independent processing of the Xpert® test cartridges for use in resource-limited settings.

Source: Image reproduced with permission of Cepheid Inc.

QuantuMDx Group (UK) is developing the Q-POC™ platform. The device is presently in a small benchtop format and at alpha prototype stage. The company intends on moving towards a portable hand-held, battery-powered device, although this may be a v2 product. The Q-POC™ is intended to provide extremely sensitive detection of MTB, similar to that of culture, and achieves this through a novel sample preparation technology that concentrates MTB from sputum samples from at least 1 mL (essentially harvesting the MTB cells from as much sputum as the patient can produce). The concentrated MTB cells are then lysed, amplified and detected by the Q-POC™. The initial assays will use real-time PCR for DNA amplification and detection with future assays, including subsequent microarray detection for a comprehensive and integrated DST. All reagents are on board with a target shelf life of 18 months, the Capture-XT™ assay is projected to take 45 minutes from raw sputum, while the molecular assay (including many drug susceptibility markers) takes <20 minutes. Both devices will be battery operated and will be able to operate for 8 hours on a single charge. As the instrument is fully automated, user training will be less than 1 day. Software updates will be updated via USB and units replaced if a fault occurs. The company is working with strategic partners and third party manufacturers to determine the costs with rigour to ensure that meeting cost expectations of donors and NTPs match the pricing. The technology entered the clinical testing phase in Q4 2016 and the estimated release date for the Q-POC™ MTB assay is Q4 2017–Q1 2018. The Q-POC™ device has a target price of ~US\$ 2000, with the molecular test at US\$ 4–7 with distribution to the global TB market. QuantuMDx Group recently received funding from the Bill & Melinda Gates Foundation to further develop this product.

Figure 21. KGI TBDx system



Notes: The alpha prototype instrument (image A) and cartridge (image B). Design concept for the beta prototype (image C).

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Keck Graduate Institute (USA; hereinafter KGI) is developing the TBDx system (Figure 21), in collaboration with this fully integrated nucleic acid testing device is designed to be compact, simple, inexpensive and robust to enable TB diagnosis in peripheral sites of HBCs. Proof of principle has been established through an alpha prototype cartridge and instrument that can perform all processing steps in the analysis of liquefied and disinfected sputum on board a single disposable cartridge. MTBC cells are lysed in a microbead beater, followed by DNA capture on the beads, wash and elution using a derivative of Claremont BioSolutions's PureLyse® technology. This novel solid-phase extraction method does not require chaotropic salts or organic solvents, therefore significantly simplifying nucleic acid preparation.

The DNA-containing eluate solubilizes dry thermostable master-mix reagents that are uniquely packaged and integrated into the cartridge. In this system, isothermal DNA amplification is performed either with LAMP or cross-priming amplification. After amplification, the test reaction is interrogated for MTBC amplicons via an immunochromatographic strip with a visual readout for the alpha prototype, and electronic readout for future instrument iterations. The cartridge contains all reagents on board, and liquid handling processes within the cartridge are operated through inexpensive electrolytic pumps (epumps). The alpha prototype can detect MTB spiked into sputum with a limit of detection of ~4800 cfu/mL based on PROBIT analysis. Further optimization is ongoing to improve the sensitivity. A preliminary clinical evaluation with sputum specimens from TB patients and controls revealed a sensitivity of 90% and specificity of 96% relative to qPCR. Efforts are ongoing to refine the system into a beta prototype that is suitable for scale-up and comprehensive validation. KGI and its partners intend to complete development of this beta prototype 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\$ 400 for the instrument and US\$ 8 per test cartridge. The test is targeted at HBC markets.

Scanogen (USA) are developing a product that uses DNA to detect the presence of MTB in a sample but does this directly from the available DNA rather than via its specific amplification and so, in principle, offers a faster time to result. Its core technology uses single-molecule detection to identify target molecules under force of DNA-tethered micrometer beads.^{183,184} Scanogen claims that this technology enables single-molecule detection with extremely low background noise and high sequence specificity. In the context of limited-resource setting TB diagnosis, single-molecule detection may offer important advantages over the available amplification-based molecular platforms in that the core reagents are low cost and are less prone to stability issues outside of cold chain (e.g. proteins and oligonucleotides). In addition, the equipment consists of inexpensive and low-power equipment comprised of light-emitting diode ring illumination, a lens and a digital complementary metal-oxide semiconductor camera. A further advantage of this technology is that the assay does not require temperature cycling and so instrumentation complexity and the need for electrical power is greatly reduced. The device will be hand held and the intended use is by a health-care worker in a microscopy centre setting or similar (Figure 22). The assay will detect TB cells in a sputum sample collected directly in the disposable cartridge with no manual step for sample preparation required. Sputum digestion as well as TB cell inactivation and lysis will take place inside the disposable cartridge. The test is aimed to take 30 minutes to perform and preliminary performance targets are a limit of detection of 25 cfu/mL with >98% specificity. The intended cost per test is US\$ 6 and US\$ 2000 for the instrument. The time to product release has been projected to be in 2019.

Summary: It is disappointing to note the reduction in peripheral diagnostic technologies in development since the 2015 landscape report. However, the development challenges facing diagnostics intended for use in such settings are great and varied. While efforts have been made to identify the value of these markets (e.g. SSM in BRICS), some companies still cite insufficient commercial incentive or lack of market visibility as

Figure 22. Scanogen reader and test cartridge for use in microscopy centres



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deterrents to commercialisation. The cost of developing an existing platform and new assay can be too high. For example, despite significant grant funding, the Alere™ q MTB and DST assay was unable to be commercialized at an affordable price to the end user. A challenge observed with the Eiken Chemical Corp. and Genedrive® tools is the relatively small amount of sputum sample applied to an assay, which will have a bearing on sensitivity in screening SSM negative samples where the number of MTB cells may be very low and likely undetectable by either method.

The Eiken Chemical Corp. Loopamp™ MTBC assay is the first NAAT product intended for use in microscopy centres that has received WHO endorsement. Further information is needed to better understand the performance of this assay and where it offers the greatest benefit. Inclusion in WHO guidelines may trigger country uptake, and thus, experience and data with implementation. Additionally, the Molbio Diagnostics products are undergoing field evaluation. The Cepheid Inc. and Molbio Diagnostics products can offer screening for MDR TB and, with the Cepheid Inc. Omni, possibly XDR TB at the microscopy-centre level. While most TB programmes remain as separate components within the public health-care systems in many HBCs, the Cepheid Inc. and Molbio Diagnostics platforms can also host other disease-specific assays, many of which are highly relevant to the burden of other diseases, including HIV-1, dengue, chikungunya and hepatitis C viruses. In addition to these, the Eiken Chemical Corp. also offers a malarial diagnostic assay using its Loopamp™ platform. Further performance data are needed to realise the potential of new technologies in development.

APPENDIX

Status update on NAAT-based technologies for reference and intermediate laboratories

Developer	Technology type	Product name	Stage in pipeline	CE or CE-IVD marked	Expected release date	WHO endorsed
Abbott	Platform	<i>m2000sp/ m2000rt</i>	In production	Yes	On market	No
Abbott	Diagnostic assay	RealTime MTB	In production	Yes	On market	No
Abbott	DST Assay	RealTime MTB-DR	In production	Yes	On market	No
Akonni	Platform	TruDx [®] 2000	Late development	No	Unknown	No
Akonni	Diagnostic assay	TruDx [®] array	Late development	No	Unknown	No
BD	Platform	BD MAX [™]	In production	Yes	On market	No
BD	Diagnostic/ DST assay	Unnamed assay	Early development	No	Unknown	No
Cepheid Inc.	Platform	GeneXpert [®]	In production	Yes	On market	Yes
Cepheid Inc.	Diagnostic/ DST assay	Xpert [®] MTB/RIF assay	In production	Yes	On market	Yes
Cepheid Inc.	Diagnostic/ DST assay	Xpert [®] Ultra MTB/ RIF	Validation	No	Q3 2017	Yes
Cepheid Inc.	DST Assay	Xpert [®] XDR	Late development	No	2017	No
Hain Lifescience	Diagnostic assay	FluoroType [®] MTB	In production	Yes	On market	No
Hain Lifescience	Diagnostic/ DST assay	FluoroType [®] MTBDR	In production	Yes	Q1 2017	No
Hain Lifescience	Platform	FluoroCycler [®] 96	In production	Yes	Q1 2017	No
Hain Lifescience	Platform	GenoXtract [®] 96	In production	Yes	On market	No
Hain Lifescience	Platform	FluoroCycler [®] 12	In production	Yes	On market	No
Hain Lifescience	Diagnostic/ DST assay	GenoType [®] MTBDRv2.0	In production	Yes	On market	Yes
Hain Lifescience	Diagnostic/ DST assay	GenoType [®] MTBDRs/	In production	Yes	On market	Yes
NIPRO Corp.	Diagnostic/ DST assay	NTM+MDRTB Kit 2	In production	Yes	On market	Yes
Quidel/ NWGHF	Platform	Savanna	Paused	No	Unknown	No

Developer	Technology type	Product name	Stage in pipeline	CE or CE-IVD marked	Expected release date	WHO endorsed
Quidel/ NWGHF	Diagnostic assay	Not named	Paused	No	Unknown	No
Roche	Platform	COBAS®	In production	Yes	On market	No
Roche	Diagnostic assay	COBAS® TaqMan® MTB	In production	Yes	On market	No
Roche	Diagnostic assay	COBAS® TaqMan® MAI	In production	Yes	On market	No
Roche	Diagnostic assay	COBAS® TaqMan® MTB	Early development	No	Unknown	No
Roche	Diagnostic assay	COBAS® TaqMan® MDR	Early development	No	Unknown	No
Tosoh	Diagnostic assay	TRC-80	In production	Unknown	Unknown	No
Tosoh	Diagnostic assay	TRCRapid® M.TB	In production	Unknown	Unknown	No
Veredus Laboratories	Platform	VerePLEX™ Biosystem	In production	In process	Unknown	No
Veredus Laboratories	Diagnostic/DST assay	VereMTB™	In production	Q2 2017	2016 (RUO)	No
Veredus Laboratories	Platform	VerePLEX™ Biosystem	In production	In process	Unknown	No
Veredus Laboratories	Diagnostic/DST assay	VereMTB™	In production	Q2 2017	2016 (RUO)	No

RUO; research use only

Update on technology status of NAAT-based technologies intended for use in microscopy centres

Developer	Technology type	Product name	Stage in pipeline	CE or CE-IVD marked	Expected release date	WHO endorsed
Cepheid Inc.	Platform	Omni	Late development	No	Q2 2018	No
Molbio Diagnostics	Platform	Truelab™ Uno 2 channel	In production	Yes	On market	No
Molbio Diagnostics	Dx assay	Truenat™ MTB	In production	Yes	On market	No
Molbio Diagnostics	DST assay	Truenat™ RIF	In production	Yes	On market	No
Molbio Diagnostics	Platform	Trueprep™ Mag	In production	Yes	On market	No
Molbio Diagnostics	Platform	Truelab™ Uno Dx 3 channel	In production	No	Q2 2017	No
Molbio Diagnostics	Platform	Trueprep™ Auto	In production	No	Q2 2017	No
Molbio Diagnostics	Platform	Truelab™ Quattro	Ready for production	No	Q3 2017	No
Eiken Chemical Corp.	Dx assay	Loopamp™ MTBC	In production	Yes	On market	Yes
Eiken Chemical Corp.	Platform	LF-120	In production	Yes	On market	Yes
Ustar Biotechnologies	Dx assay	EasyNAT	In production	Yes	On market	No
InSilixa Inc.	Platform	HYDRA	Early development	No	2018	No
InSilixa inc.	Dx/DST assay	Not named	Early development	No	2018	No
KGI	Platform	TBDx system	Mid-development	No	Unknown	No
KGI	Dx assay	TBDx system	Mid-development	No	Unknown	No
Qiagen	Platform	PON (point of need)	Paused	No	Unknown	No
Qiagen	Dx assay	PON (point of need)	Paused	No	Unknown	No
QuantuMDx	Platform	Q-POC™	Early development	No	Unknown	No
QuantuMDx	Dx/DST assay	Not named	Early development	No	Unknown	No
Scanogen	Platform	Not named	Early development	No	2019	No
Scanogen	Dx assay	Not named	Early development	No	2019	No
Tangen Biosciences	Platform	Not named	Paused	No	Unknown	No
Tangen Biosciences	Dx assay	Not named	Paused	No	Unknown	No

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