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TUBERCULOSIS

Diagnostics Technology and Market Landscape

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List of abbreviations

AIDS	Acquired immune deficiency syndrome		**NEAR**	Nicking enzyme amplification reaction
AMTD	Amplified mycobacterium tuberculosis direct		**NTM**	Non-tuberculous mycobacteria
BMGF	Bill & Melinda Gates Foundation		**NWGAF**	Northwestern Global Health Foundation
CDC	Centers for Disease Control and Prevention (United States)		**PATH**	Program for Appropriate Technology in Health
CE-IVD	Conformité Européenne-*in vitro* diagnostic		**PCR**	Polymerase chain reaction
CFDA	China Food and Drug Administration (Previously SFDA, State Food and Drug Administration)		**PEPFAR**	U.S. President's Emergency Plan for AIDS Relief
			POC	Point of care
CPA	Cross-priming amplification		**PZA**	Pyrazinamide
DFA	Dynamic flux amplification		**QA**	Quality assurance
DNA	Deoxyribonucleic acid		**QC**	Quality control
DPO	Dual Priming Oligonucleotide		**qPCR**	Real time polymerase chain reaction
DST	Drug susceptibility testing		**RIF**	Rifampicin
FDA	United States Food and Drug Administration		**RNA**	Ribonucleic acid
			Rs	Rupees
FIND	Foundation for Innovative New Diagnostics		**rRNA**	Ribosomal RNA
FQ	Fluoroquinolone		**RNTCP**	Revised National Tuberculosis Control Programme (India)
GLI	Global Laboratory Initiative		**SDA**	Strand displacement amplification
HPA	Hybridization protection assay		**SML**	Surrogate marker locus
HIV	Human immunodeficiency virus		**STAG-TB**	Strategic and Technical Advisory Group for Tuberculosis
IRD	Interactive Research and Development		**TB**	Tuberculosis
IAC	Internal amplification control		**TDR**	Special Programme for Research and Training in Tropical Diseases (WHO)
INH	Isoniazid		**TMA**	Transcription mediated amplification
IPAQT	Initiative for Promoting Affordable, Quality TB tests		**TOCE**	Tagging Oligonucleotide Cleavage Extension
LAMP	Loop-mediated amplification		**TPP(s)**	Target product profile(s)
LED	Light-emitting diode		**TRC**	Transcription-reverse transcription concerted
LPA	Line probe assay			
MDR	Multi-drug resistant		**UK**	United Kingdom
MGIT	Mycobacterial growth indicator tube		**USA**	United States of America
MTB	Mycobacterium tuberculosis		**USAID**	United States Agency for International Development
MTBC	Mycobacterium tuberculosis complex		**WHO**	World Health Organization
NAAT	Nucleic acid amplification test		**XDR**	Extensively drug resistant
NALF	Nucleic acid lateral flow			

Foreword

Although most cases of tuberculosis (TB) are curable, there were 1.4 million deaths from TB in 2011. Effective and rapid diagnosis is critical for timely initiation of appropriate treatment, but many patients—roughly one-third of new cases—do not have access to appropriate TB diagnostics. Multidrug-resistant (MDR) TB, in particular, presents diagnostic-related challenges. The World Health Organization (WHO) reports that of an estimated 8.7 million cases in 2011, only 6.2 million cases were diagnosed and notified to national TB programmes. Lack of access to TB diagnostics can be traced, in part, to market shortcomings, including unavailability of appropriate diagnostic tools, high prices, and tools that are ill-adapted to resource-limited settings.

Basic tools such as smear microscopy and culture still often form the mainstay of TB diagnosis, especially in resource-limited settings. However, these tools have notable shortcomings (e.g., long time to results for culture, intensive labour requirements, and low sensitivity of smears), and recently have seen only incremental improvements. In contrast, more rapid change and innovation are evident in the area of nucleic acid amplification technologies (NAAT). Developments have been particularly pronounced since 2010, when WHO endorsed Xpert® MTB/RIF. While a major advance, Xpert MTB/RIF is still relatively expensive, and tools adapted for use at the point of patient care in resource-limited settings are still needed. Improved market function is needed to increase TB case finding and, subsequently, access to appropriate TB care—especially for MDR and paediatric TB.

Review of the landscape of TB diagnostics—considering current and expected future technologies, as well as critical market issues—highlights potential market-based approaches to address shortcomings and improve market function. For example, opportunities for TB diagnostics market interventions may include efforts to accelerate market entry for innovative TB diagnostics to be used at the point of patient care, and innovative means of engaging with the private sector to increase uptake of existing WHO-endorsed tests.

Executive summary

The public health problem of TB and access issues related to TB diagnostics

Although curable, TB caused 1.4 million deaths in 2011 alone. Tuberculosis remains a leading cause of death among children, women of reproductive age, and people co-infected with HIV. Effective diagnosis is essential for TB care and control, but many people with active TB do not have access to initial diagnostics.

WHO reports that overall TB case detection is still less than 60 % in low-income countries (LICs) and only 66 % globally. That is, of an estimated 8.7 million people who became ill with TB in 2011, 2.9 million with active disease were not diagnosed and notified to national TB control programs. In addition, only 19 % of MDR-TB cases were appropriately diagnosed and notified. Drug susceptibility testing (DST) can be used to assess drug resistance and guide appropriate treatment, but fewer than 1 in 20 new TB patients has access. Many existing diagnostics are inadequate for people living with HIV (PLHIV), patients with extra-pulmonary TB, and children.

TB diagnostics technology landscape

The technology landscape highlights current and emerging tools for improved diagnosis of TB. The emphasis of this report is on NAAT products, where the most significant recent development has been seen. A variety of options, either commercially available or in late-stage development, are designed for detection of TB, first and/or second-line drug resistance, or for TB diagnosis and drug resistance combined. Commercialized technologies and those in late-stage development do hold promise in expanding the potential for TB diagnosis via NAATs. However, GeneXpert remains the leading technology in this area and is the last product endorsed by WHO in 2010. While a growing portfolio of TB NAAT assays are commercialized or in late-stage development, none is expected to be endorsed by WHO in 2013, and few tests are anticipated to have the necessary evidence base for endorsement over the next two to three years.

TB diagnostics market landscape

The market landscape notes critical information gaps related to the TB diagnostic market size and dynamics, and describes ongoing efforts to quantify the current TB diagnostics market. This section also describes unique attributes of private-sector markets, and new initiatives to engage with private-sector purchasers to improve access to appropriate TB diagnostics.

Market shortcomings related to TB diagnostics

Market shortcomings related to TB diagnostics include issues of availability, affordability, quality, acceptability/adaptability, and delivery. For example, there is no true, instrument-free point-of-care (POC) TB diagnostic test for use in peripheral settings. While Xpert® MTB/RIF offers rapid diagnosis in decentralized settings, the test is still expensive. Current diagnostics are not adapted for specific patient groups or decentralized healthcare settings, and limited (or no) information on the quality of diagnostics is available to guide procurement. Inappropriate tests are commonly used, particularly in the unregulated private sector.

While new technologies—particularly NAAT products—may improve diagnosis, there is currently a monopolistic market, and barriers to adoption of novel innovative technologies may threaten their uptake.

Potential opportunities for TB diagnostics market interventions
The need for a biomarker-based, simple, low-cost, instrument-free rapid test remains a key priority. Potential market-based interventions may include efforts to accelerate market entry for innovative POC TB diagnostics, including any with comprehensive DST capability and ability to use specimens other than sputum.

1. Introduction

The *UNITAID Tuberculosis Diagnostics Landscape* is published as part of a broad and on-going effort to understand the technology landscape for tuberculosis (TB) diagnostics. The first edition of the *UNITAID Tuberculosis Diagnostics Technology Landscape (July 2012)* outlined established TB diagnostic technologies, including smear microscopy and culture, as well as emerging tools, such as NAAT. The *UNITAID Tuberculosis Diagnostics Technology Landscape: Semi-annual Update (December 2012)* focused on GeneXpert® MTB/RIF and four of the most advanced NAAT products. These reports are available at:

http://www.unitaid.eu/resources/publications/technical-reports.

This report is intended to complement these earlier reports, stimulating discussion and informing potential opportunities for market intervention that could improve access to TB diagnostics, and, ultimately, public health outcomes related to TB.

To serve this purpose, this report:

- Reviews the public health problem of TB, and critical access issues related to TB diagnostics (Sections 4 and 5);
- Assesses the technology landscape, including Xpert® MTB/RIF evidence, roll-out, and future plans, as well as newer NAAT technologies and technology-related information gaps hindering market entry (Section 6);
- Analyses the market landscape, providing a high-level overview of efforts to characterize the market for TB diagnostics and a review of market approaches to improve access to WHO-endorsed tools in the private sector (Section 7); and
- Summarizes market shortcomings related to TB diagnostics, providing the context for next steps and areas of potential intervention (Section 8).

Nucleic acid amplification test (NAAT) products are the focus of this report as most new evidence and technological development have been related to this class of diagnostics. Alternative technologies (e.g., improved diagnostic tools utilizing microscopy, serology, biomarkers, etc.) are also in development, but the technology pipeline and market have shown relatively less movement in these areas.[1] The most significant recent advance in TB diagnosis has been the development, WHO endorsement, and roll-out of Xpert® MTB/RIF (Cepheid Inc., California, USA), a rapid molecular assay that can be implemented outside of traditional reference laboratories and can detect TB as well as resistance to rifampicin. The accuracy of Xpert® MTB/RIF is substantially higher than conventional sputum microscopy, and the evidence base on this technology has rapidly grown over the past year. The focus on NAATs is also relevant because of the rapid emergence of newer products that offer the promise of point-of-care (POC) deployment in peripheral microscopy laboratories and health centres; that is, such NAATs could be used both to test and treat in the same encounter, the key objective of POC testing programs. Furthermore, NAATs now offer the best hope

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1 UNITAID monitors development in these and other areas, using alternative diagnostic technologies. If progress were to accelerate in these areas, UNITAID would plan to undertake more detailed landscaping efforts to include these.

of increasing access to drug susceptibility testing (DST) to the scale required, especially in resource-limited settings with limited culture and biosafety level-3 laboratory facilities.

This report reviews Xpert® MTB/RIF evidence, roll-out, and future plans, provides an update on WHO policy reviews of newer NAAT products, and describes all TB NAATs on the market and those currently under development. This report also provides an update on the on-going work to assess the market size for TB diagnostics and the private sector business models for roll-out of TB diagnostics. The material in this report is current through May 2013.

2. Methodology

The *Tuberculosis Diagnostic Landscape: June 2013* report was compiled by David Boyle (Program for Appropriate Technology in Health [PATH], Seattle] and Madhukar Pai (McGill University, Montreal) with support from UNITAID. Additional assistance was provided by Carole Jefferson.

The material for the technology and market landscape portions of this report was gathered by the authors from publicly available information, published and unpublished reports and articles, and interviews with test developers and manufacturers. All images have been reproduced with permissions from the respective companies, publishers or agencies.

Sections on the public health problem and commodity access issues were adapted from UNITAID materials including the *UNITAID Strategy 2013-2016*.

3. Acknowledgements and conflicts of interest

David Boyle holds a grant unrelated to TB in which Ustar Biotechnologies (China) is a collaborator (Bill & Melinda Gates Foundation [BMGF] OPP 1044825). He has no other commercial/financial conflicts pertaining to information described in this document.

Madhukar Pai has no commercial/financial conflicts. He has received grant funding for TB diagnostics research from Grand Challenges Canada and BMGF. He previously served as Co-Chair of the Stop TB Partnership's New Diagnostics Working Group and as a consultant for the Foundation for Innovative New Diagnostics (FIND). He is currently serving as a consultant for the BMGF. BMGF had no involvement in the production of this report.

4. Public health problem

When appropriately diagnosed, TB is largely curable with currently available medicines. But enrolment on appropriate TB medicines is impossible without timely access to the right diagnostic tools to diagnose both TB infection and drug resistance. Without a diagnosis of TB infection, a patient is unlikely to receive treatment. If untreated, nearly 70 % of patients with pulmonary TB die within 10 years. Without DST to assess drug resistance, a patient with multidrug-resistant (MDR) TB may receive inappropriate treatment—leading to a risk of treatment failure in the individual, and drug resistance in the broader population.

5. Commodity access issue

In much of the world, lack of access to appropriate diagnostic commodities remains a barrier to treatment. Many people with active TB do not have access to initial diagnostics: despite modest improvements, WHO reports that overall case detection is still less than 60 % in low-income countries (LICs) and only 66 % globally. This means that, of an estimated 8.7 million people who became ill with TB in 2011, 2.9 million with active disease were not diagnosed and notified to national TB control programs. In addition, only 19 % of MDR-TB cases were appropriately diagnosed and notified. Even for previously treated patients, the highest-risk patient group, testing for MDR-TB was performed for only 6 %.

DST can be used to assess drug resistance and guide appropriate treatment, but access is extremely limited: DST is available to less than 4 % of new bacteriologically-positive cases. Even among notified and confirmed MDR-TB cases, second-line DST for common second-line TB drugs—fluoroquinolones and injectables—was performed for only 23 % in 2011, according to figures reported in the World Health Organization's *Global Tuberculosis Report 2012.*

Many existing diagnostics are inadequate for people living with HIV (PLHIV), patients with extrapulmonary TB, and children. People living with HIV and patients with extrapulmonary TB often have a low bacterial load in their lungs, making it difficult to detect TB using traditional diagnostic tools such as smear microscopy. Children often have difficulty producing sputum—the most common sample type for TB diagnosis—and can also be more susceptible to forms of TB disease outside the lungs.

New evidence and new tools hold promise in addressing some of these commodity access issues. In particular, NAAT products offer technological advances in diagnosing TB and drug resistance quickly, accurately, and at or near the point of patient care in resource-limited settings.

6. Technology landscape

6.1. Xpert® MTB/RIF evidence, roll-out, and future plans

6.1.1. Xpert® MTB/RIF guidance and evidence

The Xpert® MTB/RIF test (Cepheid Inc., Sunnyvale, CA; http://www.cepheid.com) was endorsed by WHO in 2010.[1] The WHO policy recommended that Xpert® MTB/RIF should be used as an initial diagnostic test in individuals suspected of MDR or HIV-associated TB. It should be used as an add-on test to smear microscopy in settings where MDR or HIV are of lesser concern, especially in smear-negative specimens. The policy process that led to WHO endorsement of Xpert® MTB/RIF has been reviewed elsewhere.[2] WHO recently published an information note to provide guidance on algorithms for management of PLHIV and presumed to have TB.[3]

A Cochrane systematic review by Steingart and colleagues, published in January 2013, included 18 published studies on the accuracy of Xpert® MTB/RIF for pulmonary TB and rifampicin resistance in adults.[4] The meta-analysis showed high accuracy of the test, reinforcing WHO's endorsement of the technology.

When used as an initial test replacing smear microscopy (15 studies, 7517 participants), Xpert® MTB/RIF achieved a pooled sensitivity of 88% (95% credible interval [CrI] 83% to 92%) and pooled specificity of 98% (95% CrI 97% to 99%). The pooled sensitivity was 98% (95% CrI 97% to 99%) for smear-positive, culture-positive TB and 68% (95% CrI 59% to 75%) for smear-negative, culture-positive TB (15 studies); the pooled sensitivity was 80% (95% CrI 67% to 88%) in people living with HIV and 89% (95% CrI 81% to 94%) in people without HIV infection (4 studies).[4] These findings show high accuracy in smear-positive samples, and modest accuracy in smear-negative samples. Accuracy is not significantly impacted by HIV-infection.

For the detection of rifampicin resistance (11 studies, 2340 participants), Xpert® MTB/RIF achieved a pooled sensitivity of 94% (95% CrI 87% to 97%) and pooled specificity of 98% (95% CrI 97% to 99%).[4] Thus, an Xpert® MTB/RIF result that is positive for rifampicin resistance should be carefully interpreted and take into consideration the risk of MDR-TB in a given patient and the expected prevalence of MDR-TB in a given setting.

Since publication of the Cochrane review, many new studies have emerged; an updated version of the Cochrane review is expected to be published in autumn 2013.

6.1.2. Update on Xpert® MTB/RIF roll-out

According to WHO, as of 31 March 2013, a total of 1123 GeneXpert instruments and 2,315,380 Xpert® MTB/RIF cartridges have been procured worldwide in the public sector in 83 of the 145 countries eligible for concessional pricing (Figure 1).[5] Updated quarterly sales figures are publicly available via the WHO website for monitoring the roll-out of Xpert® MTB/RIF.[5]

Figure 1. GeneXpert instruments and Xpert® MTB/RIF cartridge sales

Cumulative number of GeneXpert instrument modules and Xpert MTB/RIF cartridges procured under concessional pricing

Data provided by FIND
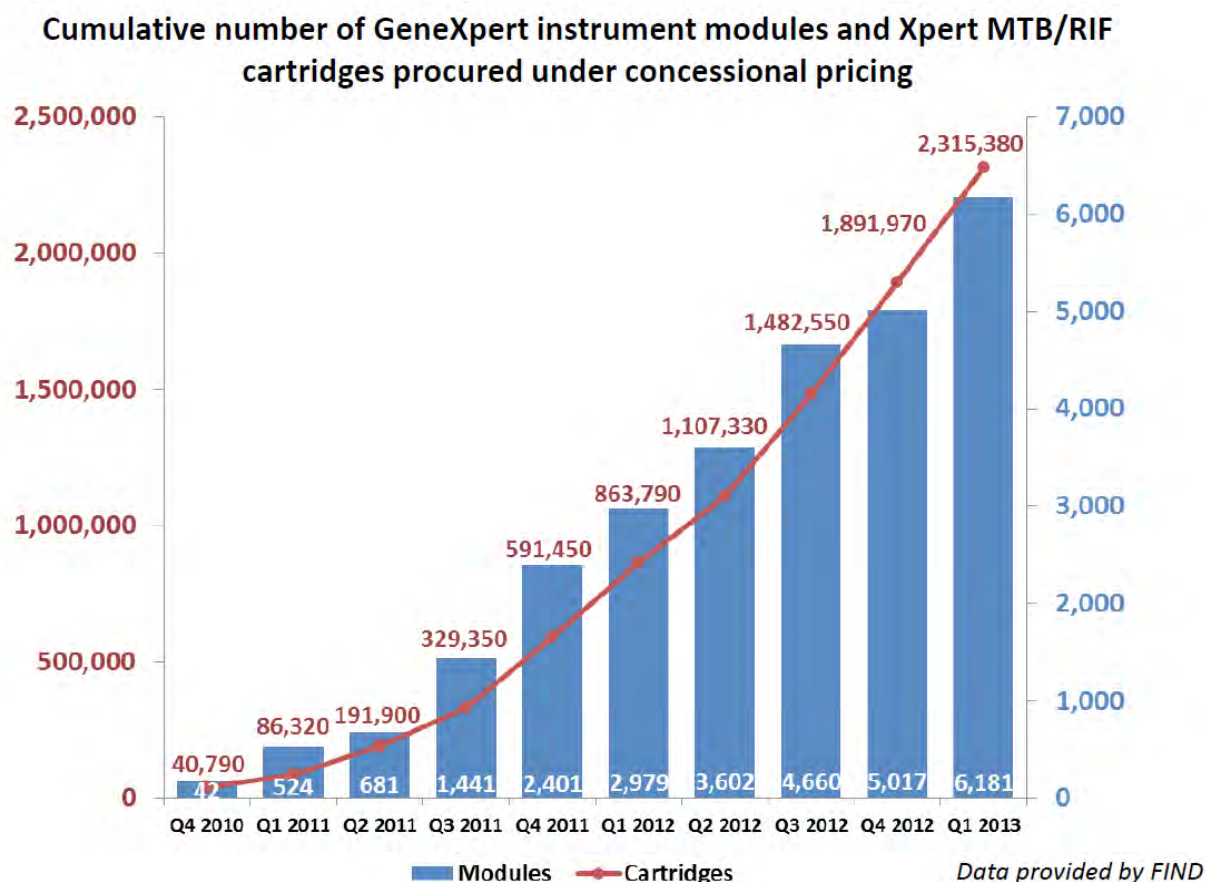
Note: Data as of 31 March, 2013. Source: WHO & FIND, Geneva[5]

In March 2011, South Africa began a phased implementation program to replace smear microscopy with Xpert® MTB/RIF as the initial test for persons with suspected TB. Since then, 1,388,450 cartridges (60% of global total) have been procured for use in the country (as of 31 March 2013). During this time, South Africa has seen significant increases in TB case detection (8 to 16% in year 1, and 14% in year 2), and detection of drug resistance (7%).[6]

As of 31 March 2013, India had procured 110,110 cartridges. The Revised National TB Control Programme (RNTCP) and FIND are currently implementing two projects.[7] First, a feasibility and impact study of Xpert® MTB/RIF in 18 decentralized treatment units, testing nearly 5000 persons with suspected TB every month. Preliminary results show significant increase in the numbers of TB and MDR-TB cases identified. Second, Xpert® MTB/RIF is being implemented via the EXPANDx TB Cartridge-based Nucleic Acid Amplification Test project to increase capacity for DST and supplement the existing reference lab network for DST. The goal is to conduct more than 24,000 rapid DST across the 12 labs where Xpert® MTB/RIF are being implemented.

As of 31 March 2013, Brazil had procured 34,260 cartridges. Based on a pilot roll-out of Xpert® MTB/RIF in two municipalities, Brazil has made plans to replace all diagnostic smear microscopy with Xpert® MTB/RIF.[8] Priority will be given to cities with more than 200 new TB cases notified in 2011. 66 cities have been identified as targets for the roll-out and a total of 120 GX instruments and 400,000 cartridges are expected to be procured.

In June 2012, UNITAID, BMGF, the US Agency for International Development (USAID), and U.S. President's Emergency Plan for AIDS Relief (PEPFAR) announced an agreement with Cepheid Inc. to reduce the cost of the test to US $9.98 per cartridge (from the original price of US $16.86). This purchase price is applicable to over 145 purchasers in low- and middle-income countries. As part of the agreement, the TBXpert Project is supporting scale-up through an accelerated roll-out of the test in high-burden countries. This project will provide approximately 1.4 million Xpert® MTB/RIF test cartridges and over 200 GeneXpert instruments for the rapid detection of TB and rifampicin resistance in 21 recipient countries[2] in 2013-2015. The TBXpert Project is funded by UNITAID and executed by the WHO Stop TB Department and the Stop TB Partnership. To ensure country absorptive capacity and effective use of the technology, the TBXpert Project links a broad network of partners and existing initiatives for TB laboratory strengthening and innovative approaches to expand access to vulnerable populations in both the public and private sector. TBXpert Project partners include the Global Laboratory Initiative (GLI), TB REACH, the EXPAND-TB Project, Interactive Research and Development (IRD), and the African Society for Laboratory Medicine. The project was initiated on 28 January 2013, and the first tranche of funding to implementers has been disbursed to fund delivery of commodities.

TB REACH, an initiative by the Stop TB Partnership supported by the Canadian International Development Agency, promotes new ways of detecting and treating TB cases. In its first Wave of grants, TB REACH supported a project in Tanzania using Xpert® MTB/RIF on a mobile van in Tanzania before it was endorsed by WHO. In Wave 2, 30 of 44 projects in 18 countries used Xpert® MTB/RIF as part of case finding activities. In total, these projects planned to use over 250,000 test cartridges by placing 152 instruments. The projects employed a wide variety of testing algorithms and all projects tested people with suspected TB rather than using the test for DST of already-confirmed TB patients. The projects sought to bring the test as close to the patient as possible: a number were placed on mobile units and in lower-level facilities, employing local solutions such as truck batteries, generators, and solar panels to address power issues. As of 31 December 2012, just over 120,000 tests had been conducted in the 33 projects, identifying just over 16,000 MTB-positive individuals. In Wave 3, TB REACH is supporting another nine projects in nine countries, using 18 instruments and 53,244 cartridges. In addition, TB REACH has partnered with UNITAID to provide support to a number of partners that are using Xpert testing in its most recent funding wave. Under UNITAID's TBXpert Project, TB REACH will be supporting an additional 20 projects in 12 countries. The projects plan to use 539,542 test cartridges and 133 instruments.

In addition to these developments, efforts are underway (described in Section 7) to enhance uptake of the Xpert technology in the private sector in certain high-burden countries. Currently, the private sector in high TB burden countries is excluded from the negotiated pricing agreement and the US $9.98 price does not apply.

Production and supply chain issues have been seen during initial roll-out of Xpert, with several reports of cartridge shortages in late 2012 and early 2013. According to a media release from Cepheid in April 2013 [http://www.cepheidcares.com/tb/index.php/resources/commitment], however, many planned manufacturing enhancements are now fully operational. Cepheid predicts that the supply of cartridges will increase substantially in upcoming months, and expects to have considerably reduced or eliminated any product shortages by the end of June 2013. In addition, Cepheid is building an inventory buffer to ensure timely delivery of orders.

6.1.3. Planned technology refinements for Xpert® MTB/RIF
Recent technology developments for Xpert® MTB/RIF include:

1) Development of 10 colour channel detection with high-resolution melt capability, a software upgrade scheduled for release next year. This will expand the multiplexing capability of the existing installed base of GeneXpert systems without the need for hardware upgrades or replacement. A prototype assay for

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2 TBXpert project countries where Xpert® MTB/RIF implementation is supported by UNITAID include: Bangladesh,* Belarus,* Cambodia, Congo, Ethiopia,* India,* Indonesia,* Kenya,* Kyrgyzstan,* Malawi, Moldova,* Mozambique,* Myanmar,* Nepal, Pakistan, Philippines, Swaziland,* Tanzania,* Uganda,* Uzbekistan,* Viet Nam.* Countries marked with an asterisk are also EXPAND TB project countries.

detection of MDR-TB has been developed with the new dyes and quenchers that enable expansion from 6 to 10 colours.

2) Remote calibration, released late in 2012 and used in over 40 countries to date. Remote calibration allows for GeneXpert modules to be calibrated without the need for an expensive service technician call; over 90% of modules can be calibrated over the internet.

3) GeneXpert Data Management initiatives to allow for real-time aggregation of de-identified geo-positioned test data. Proof-of-concept studies are being conducted in South Africa and soon the United States which assess the ability to monitor and track disease incidence and drug resistance.

4) HIV cartridges for use with the GeneXpert platform. The GeneXpert platform can be used to detect a number of infectious diseases. Of relevance to settings with high TB and HIV prevalence, Cepheid is expected to release a separate cartridge for HIV viral load - qualitative (whole blood) and quantitative (plasma) in the first half of 2014.[9] These tests are based on detection of the HIV long terminal repeat for consistent detection of HIV-1 variants.

6.1.4. Upcoming policy revisions related to Xpert® MTB/RIF
To account for the rapidly expanding evidence base on Xpert® MTB/RIF, WHO has initiated a process for updating policy guidance on the use of this assay. This process will update the evidence on the use of Xpert® MTB/RIF for pulmonary TB in adults and review new evidence for use on non-respiratory samples (extrapulmonary TB) and for the detection of TB and rifampicin resistance in children. An Expert Group meeting was held on 20 and 21 May 2013, and recommendations from the Expert Group meeting were submitted to Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) in June 2013. The revised policy guidance on the use of Xpert® MTB/RIF would serve as an update to the 2011 WHO Policy Statement, with an expanded scope to also include recommendations for use in extra-pulmonary and paediatric TB.

6.2. Update on WHO review of newer NAAT technologies
Since the WHO endorsement of Xpert® MTB/RIF in 2010, evidence on two other molecular tests has been reviewed by WHO Expert Groups: a simple, manual NAAT that could potentially be implemented in peripheral microscopy centres, and a new line probe assay (LPA) for second-line DST that can be used for the diagnosis of extensively drug-resistant TB (XDR-TB). The manual NAAT for microscopy centres is relevant because Xpert® MTB/RIF is intended for use at the district or subdistrict level and not peripheral microscopy centres. The LPA for second-line DST is important in the context of numerous reports of XDR-TB and the need for rapid diagnosis of serious forms of drug-resistance.

6.2.1. Genotype® MTBDRsl test
In 2013, WHO published a report of the Expert Group Meeting (held on 21st March, 2012) that was held to review the evidence on Genotype® MTBDR*sl* by Hain Lifescience (Germany) for policy recommendation.[10] The Genotype® MTBDR*sl* test is a NAAT, specifically an LPA designed for the rapid molecular detection of the predominant genetic alleles associated with resistance to aminoglycosides (kanamycin, amikacin), cyclic peptides (capreomycin), ethambutol, fluoroquinolone, and streptomycin. These are second-line drugs used in the treatment of MDR-TB.[11] The principle behind this test is similar to another Hain LPA, the Genotype® MTBDRplus® (v1.0), which was endorsed by WHO in 2008. Either assay can utilize sputum or culture isolates and produce test results in less than 24 hours. The Genotype® MTBDR*sl* is intended as a lower-cost and rapid tool for DST and specifically to identify XDR-TB from MDR-TB positive specimens at the reference laboratory level. It is aimed at supplanting the current, conventional culture-based DST methods which can take several weeks to generate results.

The data used in the meta-analysis were derived from 18 studies. Based on their review of the evidence, the WHO Expert Group did not endorse the replacement of culture-based DST with the Genotype® MTBDR*sl* test.[10] They recommended that "the Genotype® MTBDR*sl* assay cannot be used as a replacement test

for conventional phenotypic DST" [Strong recommendation - Very Low Quality of Evidence], with the following comments:[10]

- The Genotype MTBDR*sl* may be used as a rule-in test for XDR-TB but cannot be used to define XDR-TB for surveillance purposes.
- As cross-resistance between the second-line injectables is incomplete, the Genotype MTBDR*sl* cannot be used to identify individual drugs to be used for treatment.
- The Genotype MTBDR*sl* may be used to guide infection control precautions while awaiting confirmatory results from conventional phenotypic testing.

6.2.2. Loopamp™ MTBC Detection Kit

Evidence on this manual NAAT, developed by Eiken Chemical Corp. (Japan) and FIND, was also reviewed in 2012 by a WHO Expert Group (20th April, 2012). The loop-mediated isothermal amplification (LAMP) platform has been developed as a replacement to smear microscopy.[12] The Loopamp™ MTBC Detection Kit has several features that make it attractive as a diagnostic platform for resource-limited settings, including reduced reliance on key laboratory infrastructure, relative simplicity of use, rapid time to result, and a result format that is visually scored. Published performance data from a reference laboratory in Japan is available.[13] The new, as yet unpublished, performance data were derived from 11 evaluation studies (rural or simple urban microscopy centres) performed in three countries and presented to the WHO Expert Group. The Expert Group noted that "while the LAMP technology has potential as a rapid TB diagnostic tool the body of evidence presented to the Group was insufficient to make a recommendation either in favour of, or against the use of TB-LAMP as a replacement test for [acid-fast bacilli] microscopy." The Expert Group Meeting report is expected to be published shortly.

In light of the findings from this Expert Group Meeting, FIND recently released a request for applications for TB diagnostic groups currently evaluating other TB assays to further evaluate the performance of the TB-LAMP assay in microscopy centres using light-emitting diode (LED) microscopy, with further comparative testing using GeneXpert MTB/RIF and liquid and solid culture. Countries or regions with high HIV comorbidity were of particular interest for this next round of evaluations.

6.3. Technology review of NAAT technologies on the market

NAAT-based TB tests are high-performance assays that can rapidly and accurately detect the presence of TB-derived RNA or DNA in sputum and other clinical samples, allowing the timely identification of active TB.[12,14] Some NAATs are also designed to rapidly detect drug-resistance. Since conventional liquid culture and DST can take up to two to three weeks,[15] the rapid turn-around of NAATs (one to two days) allows for rapid DST and initiation of second-line drug therapy, while culture and full DST results are pending.

A screening of the NAAT technology market has identified 14 commercially available NAATs and associated platforms that can diagnose TB and/or drug resistance for first- and second-line drugs (Table 1). Included in the table are the core amplification technologies used to amplify the TB target nucleic acids and the subsequent method used to detect amplified products. The level of integration of these tests is also presented in Table 1; the devices vary in complexity from fully or partially integrated to being minimally dependant on instrumentation. Throughput is an important part of the test algorithm, and so the scale of testing at the intended site for use is also reflected in Table 1. Interestingly, many of the developers are including the capacity to multiplex testing and therefore have introduced screens for common alleles associated with MDR or XDR TB.

Some of these technologies are already endorsed by WHO, while many others are not or have not yet been reviewed by the Expert Review Group. These late stage/commercialized technologies will be discussed briefly, highlighting the perceived or proposed advantages of each. NAATs at an earlier stage of development are discussed in a later section.

Table 1. Commercially available NAAT assays for the diagnosis of pulmonary TB from sputum samples
Manufacturer and details of diagnostic assays that are commercially available, including those with WHO endorsement

Manufacturer, Country	Test	Amplification, detection technology	Fully integrated	Dedicated instrument	Multi-plexed	Throughput	MDR detection	Intended setting	Market release	WHO-endorsed	Regulatory approvals
Becton Dickinson, USA	BD ProbeTec ET Direct TB Assay	SDA	No	Yes	Yes	150 tests, 8 hrs.	No	Reference	1999	No	CE-IVD
CapitalBio, China	Mycobacteria Real-time PCR	qPCR	No	No	Yes	36 tests, 4 hrs.	No	Reference	2011	No	CE-IVD, CFDA
CapitalBio, China	Drug Resistance Detection Kit	PCR, microarray	No	Yes	Yes	24-48 tests, 8 hrs.	Yes, XDR	Reference	2009	No	CE-IVD, CFDA
Cepheid, USA	Xpert MTB/RIF® assay	qPCR	Yes	Yes	Yes	90 minutes per sample module	Yes	Intermediate/peripheral	2009	Yes	CE-IVD, Health Canada
Eiken, Japan	Loopamp™ MTBC Detection Kit	LAMP, fluorescence	No	Yes*	No	14 tests, 2 hrs.	No	Peripheral	2012	No	CE-IVD, Japan
Epistem, UK	Genedrive™ Mycobacterium iD® Test-kit	qPCR	No	Yes	No†	90 minutes per test device	Yes	Peripheral	2013	No	CE-IVD
Hologic Genprobe, USA	AMTD test	TMA, HPA luminescence	No	Yes	No	50 tests, 5.5 hrs.	No	Reference	1993	No	FDA, CE-IVD
Hain Lifescience, Germany	GenoType® MTBDRplus (v1.0)‡	PCR, LPA	No§	Yes	Yes	96 tests per day	Yes	Reference	2008	Yes	CE-IVD, CFDA
Hain Lifescience, Germany	GenoType® MTBDRsl	PCR, LPA	No§	Yes	Yes	96 tests per day	Yes, XDR	Reference	2009	No	CE-IVD
Molbio, India	Truelab™ TB Assay	qPCR	No§	Yes	No	12 tests per test device in 8 hrs.	No	Peripheral	2013	No	CE-IVD, Indian DCGI
Seegene, South Korea	Anyplex II™ MTB/MDR/XDR	qPCR	No§	Yes	Yes	90 tests, 6 hrs.	Yes, XDR	Reference	2012	No	CE-IVD, Korean FDA
Roche, USA	Cobas® TaqMan® MTB Test	qPCR	No§	Yes	Yes	44 tests, 3 hrs.	No	Reference	2009	No	CE-IVD, Health Canada, FDA?
Tosoh Bioscience, Japan	TRC Rapid M.TB	TRC	No	Yes	No	N/A	No	Reference	Unknown	No	N/A
Ustar Biotechnologies, China	EasyNAT™ TB	CPA, enclosed lateral flow strip	No	No	No	30 tests per day	No	Peripheral	2009	No	CE-IVD, CFDA pending

* The Loopamp assay may be used without the dedicated equipment supplied by Eiken but this has not been evaluated.
† The Epistem Genedrive has three single PCR chambers that when combined gives multiplexed result for TB and Rifampicin resistance.
‡ The Hain MTBDRplus assay has been redeveloped; however, the v2.0 assay has not yet been endorsed by WHO.
§ Automated DNA extraction platforms are available.

Although significant efforts are being made to develop NAATs for use in peripheral laboratory settings, most technologies listed in Table 1 are suited for the reference/centralized laboratory. In settings where large numbers of specimens must be tested, accurate yet high throughput screening tools are necessary to cope with the volume and demand for testing. Many technologies used in reference laboratories are technically complex and involve dedicated equipment, separate staged rooms, and user expertise. Therefore, test requirements include adequate infrastructure for appropriate storage of test equipment and reagents, and proficient laboratory staff to perform the tests. Most technologies listed in Table 1 are CE-IVD marked, indicating that the manufacturer declares full compliance with European Union directives and has completed an appropriate conformity assessment. In addition, many have approval from in-country regulatory bodies such as the United States Food and Drug Administration (FDA) or the China Food and Drug Administration (CFDA); however, it should be noted that national regulatory bodies' requirements can vary.

The use of nucleic acid-based technologies follows several core principles. First, specimens (MTBC cells and other material therein) are lysed by either chemical, temperature, physical disruption or a combination of these processes. This releases the nucleic acids (DNA and RNA) which are then extracted from the lysed sample, washed free of proteins (and other inhibitory compounds that may affect amplification), and concentrated. Specific targets relating to MTBC nucleic acid sequence are then amplified to grossly increase their numbers and facilitate detection. Amplification uses a variety of methods including: complex automated readers that measure fluorescence or luminescence, simple visual assessment of fluorescence, or banding on specific test strips. These processes can be either separate, or fully or partially integrated. Detailed description of these formats is included in the following examples.

Although not reviewed for endorsement by WHO, the Hologic Genprobe (USA) Amplified Mycobacterium Tuberculosis Direct (AMTD) assay has been FDA approved since 1995. The AMTD test is widely used in high-income countries and can be used for batch testing of large volumes of samples and can identify MTBC from either culture or sputum samples. The assay uses a single tube system in which samples are first lysed, and then subjected to isothermal amplification and finally amplicon detection via luminescence with GenProbe's proprietary amplicon detection method, the hybridization protection assay (HPA). The test uses transcription mediated amplification (TMA) to amplify single-stranded RNA from ribosomal RNA. The amplicons generated by TMA bind to a fluorescent probe and prevent its decay. The luminescence is detected by an instrument (Figure 2A). If no amplicon is generated, then subsequently the probe is decayed and the test reaction does not luminesce.[16] The test does require a dedicated luminescence reader, sonicating water bath, heat block and other consumables. Several studies evaluating the AMTD assay have been synthesized in published systematic reviews.[17,18]

Figure 2A and 2B. Reference laboratory-based equipment for MTBC assays. A: The Hologic Gen-probe Luminometer used with the AMTD assay. B: The COBAS® TaqMan® 48 Analyzer, a real-time polymerase chain reaction (qPCR) platform, for use with the TaqMan® MTB Test.
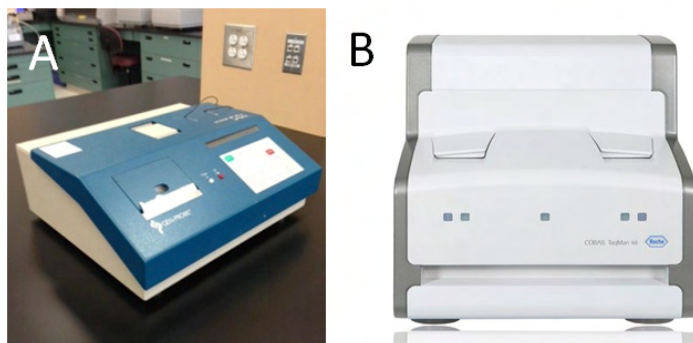
Images used with permission from Hologic Gen-probe and Roche Diagnostics.

The Roche (Switzerland) COBAS® TaqMan® MTB Test is an *in vitro* nucleic acid amplification test for the qualitative detection of MTBC DNA in liquefied, decontaminated and concentrated human respiratory specimens, including sputum and bronchial alveolar lavages.[19] The predecessor test, the AMPLICOR® MTB Test, was FDA approved (1996) but the COBAS® TaqMan® MTB is not. The current test is not fully automated and utilizes the AMPLICOR® Respiratory Specimen Preparation Kit for manual specimen preparation and then the COBAS® TaqMan® 48 Analyzer is used for automated amplification and detection of TB via qPCR (Figure 2B).[20] This test allows for high throughput but also requires a dedicated polymerase chain reaction (PCR) platform in addition to materials and equipment to process the samples prior to amplification. An added utility of the COBAS® TaqMan® 48 Analyzer is that it can also be used with other Roche Cobas assays such as for HIV viral load. The performance of the assay has been evaluated and published in peer reviewed articles.[21,22] Several studies evaluating the previous versions of the Roche assay have been synthesized in published systematic reviews.[17,23]

The Becton Dickinson ProbeTec ET Direct TB Assay is a partially automated tool with which to diagnose MTB. After mycobacterial inactivation of sputum samples, which occurs during the initial processing of the specimen, the remaining procedure can be completed without containment. Importantly, initial specimen processing and amplification may be carried out in the same room, and all the reagents can be stored at room temperature. Sample preparation is the most labor intensive and represents the main shortcoming of the system; thereafter, the assay is almost completely automated. The assay uses strand displacement amplification (SDA), an exothermal amplification assay, and this is used to amplify two different target regions of MTBC increasing the sensitivity of the test. Detection is via fluorescence detection of amplicons generated with automated result scoring via the reader. An internal amplification control (IAC) aids the user in identifying samples that inhibit amplification and therefore also increases sensitivity.[23] This technology has been subjected to multiple evaluations.[24-26]

More recently, other diagnostic manufacturers have entered the market offering alternative real-time PCR assays for the detection of TB, all with claims of high sensitivity and specificity at an affordable price and/or in systems where testing can be scaled up alongside GeneXpert. CapitalBio (China) offer a CFDA-approved real-time PCR assay to detect TB from respiratory samples. As with most other commercially available assays, the sample extraction method is not dedicated or integrated and is typically manual. However, the real-time PCR assay can be performed on most models of real-time PCR machines rather than a dedicated instrument. In addition to the real-time PCR assay, CapitalBio also offer microarrays for detection of alleles associated with both MDR-TB or to identify non-tuberculous mycobacteria (NTM). These, too, are CFDA-approved with CE-IVD marking, but not endorsed by WHO. The use of microarrays as more effective tools for the rapid identification of MDR and XDR TB is gaining some interest. As opposed to real-time PCR or line probe methods, microarrays can address greater numbers of alleles associated with drug resistance and therefore may offer superior performance in rapid DST. However, the CapitalBio assays require significant numbers of reagents and ancillary equipment for use. Processing and analysis of slides is also relative complex, and therefore the assays are only appropriate for use at the reference laboratory level. Currently, there are two peer-reviewed, independent published reports on the CapitalBio microarray platform.[27,28]

Seegene (South Korea) has developed a novel method of multiplexing the detection of TB and MDR and XDR resistance alleles via their Dual Priming Oligonucleotide (DPO™) and Tagging Oligonucleotide Cleavage Extension (TOCE™) technologies. These are incorporated in their Anyplex II™ MTB/MDR/XDR real-time PCR assay. The dual priming oligonucleotides are designed to target specific alleles. If present, their by-product is then incorporated into the TOCE™ assay, which can produce a fluorescent amplicon. In this way, multiple alleles can be screened in a single fluorescence channel via high resolution melt curve analysis. For MDR, the assay addresses 25 different targets: 18 for rifampicin resistance (*rpoB* related) and a further seven mutations associated with isoniazid (INH) resistance (*katG* and the *inhA* promoter). For XDR, it addresses seven mutations associated with fluoroquinolone resistance (*gyrA*) and six mutations associated with injectable drugs resistance (*rrs* and the *eis* promoter). Currently, the assay is recommended for use with specific real-time PCR platforms, e.g., the CFX96 from Bio-Rad (USA). The sample preparation component is manual, but Seegene has an instrument that can partly automate extraction

and assay preparation steps. Currently, there are no peer-reviewed, independently-published data on the Seegene NAAT for MTB/MDR/XDR.

The Hain Lifescience (Germany) GenoType® MTBDRplus (v1.0) assay is an LPA that can be used to detect TB and MDR resistance alleles from either culture or smear-positive sputum specimens. Version 1.0 was endorsed by WHO in 2008 on the basis of several published studies (reviewed by Ling DI et al.)[18], and is one of only two technologies listed in Table 1 with WHO endorsement. The assay requires multiplex PCR to create labelled amplicons that are then interrogated on an LPA. There are automated methods available for sample preparation (up to 12), detection (up to 48 tests) and evaluation, and result interpretation. Hain Lifescience has developed a new version, the GenoType® MTBDRplus v2.0, which has not yet been endorsed by WHO. The v2.0 assay was developed to detect TB and MDR from smear-negative specimens in addition to smear-positive and culture samples currently recommended for use with v1.0. Two independent studies with preliminary data show sensitivities greater than 70% when detecting smear-negative TB cases.[29,30] The GenoType® MTBDR*sl* assay was developed to identify second-line drug resistance alleles as a more rapid method for DST.[31] This test is not WHO-endorsed; the Expert Group recommendation was previously described in Section 6.2.[10]

The Toshoh Bioscience (Japan) TRCRapid M.TB has been evaluated in several studies performed in Japan.[13,32,33] This NAAT utilizes transcription-reverse transcription concerted reaction (TRC) targeting ribosomal RNA (rRNA) as the template for amplification. The choice of using rRNA as a target allows the assay to be highly sensitive, as rRNA levels per TB cell are much higher than any genomic DNA target. In addition, the detection of rRNA indicates viable cells rather than dead cells where the rRNA is prone to decay after cell death; this assay therefore may have potential to be used for treatment monitoring in addition to case detection.

The Cepheid (USA) GeneXpert platform and MTB/RIF assay have been described in detail in the previous UNITAID landscape reports and above. It remains the only fully-integrated instrument that can detect MDR-TB and, unlike other technologies, requires only minimal user input when preparing a test sample. In addition, it is endorsed by WHO, with updated guidance on use in extrapulmonary TB and children expected in 2013. Cepheid is currently developing a new drug resistance cartridge to complement the MTB/RIF assay in conjunction with the University of Medicine and Dentistry of New Jersey. They are also investigating the application of new fluorophores to increase the spectral range of the GeneXpert to 10 fluorophores rather than the six currently used. Cepheid has received US $5 million in funding to develop an HIV assay cartridge for the GeneXpert to increase the utility of the device in high TB and HIV burden settings.[34] As noted earlier, Cepheid is expected to release a separate cartridge for HIV viral load - qualitative (whole blood) and quantitative (plasma) in the first half of 2014.[9]

The remaining technologies in Table 1 are minimally- or non-instrumented NAATs aimed at supplanting smear microscopy for TB diagnosis at the peripheral laboratory level by virtue of their simplicity and performance, the stability of associated reagents, and the size/robustness of key equipment. The underlying core technologies have been previously described in detail in the *UNITAID 2012 Tuberculosis Diagnostic Technology Landscape Semi-annual Update* report[35]; therefore, only new and emerging details regarding the current development status of these are included in this report (Appendix 1 provides an overview).[12,35] Of these technologies, Eiken's Loopamp™ MTBC Detection Kit is currently the only one that has undergone evaluation studies and Expert Group Review. As noted earlier, a decision has yet to be made on the suitability of this technology and more evaluations are currently being planned. Since 2011, no peer reviewed publications have been published describing the performance of this assay in its intended target group.[13]

Of this group, only the Genedrive™ (Epistem, UK) can also detect MDR-TB. Based on marketing plans from the company, Epistem—currently in collaboration with Xcelris (India)—is targeting the Indian subcontinent for the release of the Genedrive™ Mycobacterium iD® Test-kit (Figure 3A). For global distribution in other markets, Epistem has reached an agreement with Becton Dickinson (USA) for supply and distribution. Currently, there are no published performance data on this assay.

Figures 3A-D. Small battery-powered real-time PCR systems and associated technology. A: The Epistem Genedrive™ real-time PCR machine and reaction cartridge. B: The MolBio Truprep™ platform to extract TB DNA. C: The Android phone operated TrueNAT™ platform for real-time PCR analysis of TB. D: The TrueNAT™ reaction chip. The white ceramic square acts as the heating block. Both cartridges contain stabilized test reagents.
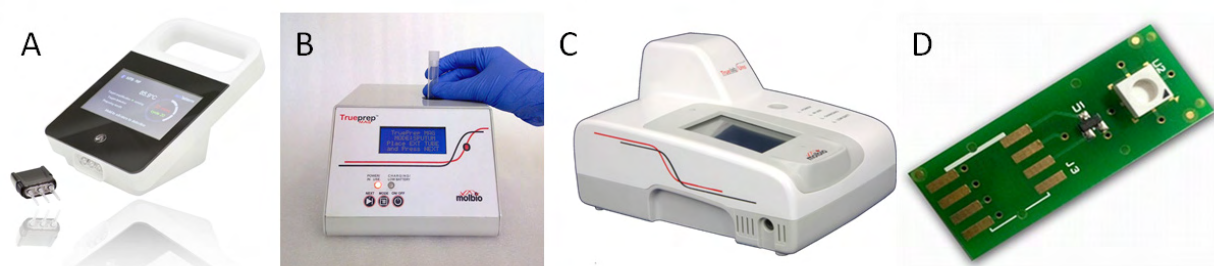
Images used with permission from Epistem and Molbio.

Molbio (India), a joint venture between Tulip Group (Goa, India) and Bigtec Labs (Bangalore, India), have launched the Truelab™ Uno real time microPCR instrument (Figures 3B-3D). The assay utilises a battery powered semi-automatic nucleic acid extraction system for sample preparation. A sample of the extract is transferred to a microPCR chip (TrueNAT™ MTB assay) which is then inserted into the Truelab™ instrument (also battery powered). The controller is an embedded Android phone which gives added utility in terms of data entry, scoring and storing test data and remote access to upload test data and global positioning. Recently a peer reviewed manuscript was published describing the first independent review of the performance of the Molbio TrueNAT™ MTB assay.[36] In this study, the authors demonstrated that specimens could be tested within 1 hour and the performance of the TrueNAT™ assay was good with 91 % sensitivity and 100 % specificity. However, the study size was small and larger studies are required to accurately assess the performance of the TrueNAT™ MTB assay in a great variety of settings and patient groups.

Ustar Biotechnologies has developed the EasyNAT™ TB cross-priming amplification (CPA) assay, and is focused on achieving CFDA approval (expected in 2014). Recent multicenter evaluations performed by the Chinese Centers for Disease Control and Prevention has generated a dataset that is currently undergoing review for CFDA approval of the EasyNAT TB assay.

Finally, a series of four new LPA products has been developed by the Nipro Corp. (Japan): the NTM/MDR-TB, INH, PZA, and FQ strips. Due to limited availability of information on these products, they are excluded from Table 1. All test formats can use either culture or sputum samples. All require nested PCR to amplify labelled target amplicons which are then used to interrogate LPAs. The NTM/MDR-TB strip was designed to identify four *Mycobacterium* species including MTB, and to detect mutations associated with RIF and INH resistance. As their names suggest, the other assays are designed to detect alleles associated with resistance to INH, pyrazinamide (PZA), or fluoroquinolone (FQ). A large multicenter evaluation of the performance of these four tests showed generally good performance of the tests to identify TB, and for DST.[37] Unsurprisingly, the INH-specific assay showed greater sensitivity than the NTM/MDR-TB, which contains fewer targets to assess INH resistance.[37] These tests have not been reviewed for endorsement by WHO. Of final note are technologies that have been discontinued and are no longer commercially available: the INNO-LiPA Rif.TB LPA assay made by Innogenetics NV (Belgium), and the LCx Mycobacterium TB NAAT (based on ligase chain reaction) made by Abbott (USA).[38]

6.4. Technology review of NAAT technologies under early development

Of NAAT technologies under early development, the Alere q is the most notable in that Alere (USA) has received significant funding from BMGF to develop a fully-integrated TB diagnostic test that can run via mains or battery electricity. The funding is up to US $42.2 million and includes a US $21.6 million grant over 2.5 years to develop a TB diagnostic tool based on the Alere q platform. The remaining funding is a low-interest loan for Alere Technologies (Germany) to develop two fully-automated production lines at

their manufacturing plant in Jena, Germany to produce cartridges for this TB assay and an HIV viral load assay. The Alere q can utilize either PCR or isothermal amplification technologies to drive nucleic acid amplification.

Sample preparation, nucleic acid amplification, amplicon detection and result interpretation are fully integrated, thus requiring minimal user input and making this device equally suited for use in POC settings and laboratories. The time to result for the TB assay is anticipated to be less than 30 minutes from specimen collection to result. For TB DNA amplification, the isothermal nicking enzyme amplification reaction (NEAR) assay—developed by Ionian Technologies Inc. (USA, also an Alere subsidiary)—will be used.[12]

The NEAR technology can accurately amplify DNA targets in under 10 minutes. Both the Alere q instrument and the NEAR TB assay chemistry are in late stage development and the recent funding award will be used to co-integrate these components and also to modify the sample preparation component of the cartridge to accommodate a larger specimen volume. It is envisaged that the test cartridge will incorporate a sputum collection cup with liquefaction and bactericidal reagents so that once the sample is collected and the cartridge lid closed, the cassette can then immediately be placed into the Alere q for processing and analysis. The release date for the Alere q is currently unknown, but this project is funded for 2.5 years.

Several other fully-integrated platforms currently employ NAAT to diagnose other pathogens such as *Clostridium difficile* or Group B Streptococci. These could be adapted for the diagnosis of TB in a variety of throughputs. These platforms include the $m2000$ Real Time System (Abbott Laboratories, USA), the BD MAX™ System Technology (Becton Dickinson, USA), the Apollo (Biocartis, Belgium), the Film Array (BioFire Diagnostics Inc. [formerly Idaho Technologies], USA), the Enigma ML (Enigma Diagnostics, UK), the iCubate System (iCubate, Inc., USA), the LIAT™ Analyser (Iquum, USA), and the Verigene® system (Nanosphere, Inc., USA). Of these systems, only iCubate currently offers a multiplexed assay for research use only that includes TB in addition to NTMs and rifampicin, INH, ethambutol and streptomycin in a single cartridge. Both Biocartis and Enigma are currently developing MDR-TB assays for their platform technologies.

Figures 4A-4D. Integrated platforms that are in development. A to C: The Wave80 EOSCAPE technology. Samples are added to a cup and mixed with lysis buffer (A). The cup is then inserted onto the extraction and amplification cartridge (B). This complete unit is then placed into the test reactor to start interrogation (C). D: The Northwestern Global Health Foundation (NWGHF) platform with test cassettes in the right foreground.
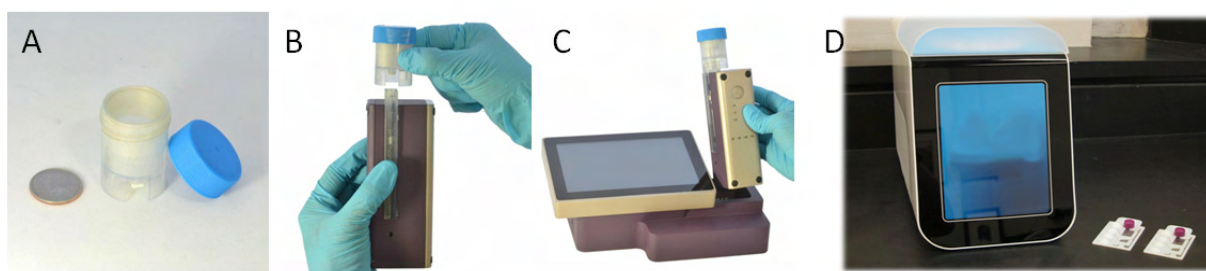
Images used with permission from Wave80 and NWGHF.

Wave80 is developing TB and RIF-FQ assays for their fully-integrated EOSCAPE platform (Figures 4A-C); the RIF-FQ is intended as a reflex test for rifampicin and fluoroquinolone resistance. This technology uses nucleic acid amplification, but the exact amplification technology is not known. Market release is anticipated by the end of 2014.

Other TB diagnostic technologies for use in peripheral settings are being developed by NWGHF (USA), Fluorosentric (USA), NanoBioSys Inc. (South Korea), and Sequella Inc. (USA). The NWGHF technology involves a fully-integrated system using qualitative real-time PCR for detection of TB DNA from sputum;

the only manual step being the processing of sputum prior to adding a sample to the test cartridge (Figure 4D). A rifampicin resistance assay is also currently under development. Result scoring and data collection is automatic with data uploading to a central database via an onboard modem. NWGHF plan to market this product in 2015.

Fluorosentric (USA) is currently developing their dynamic flux amplification (DFA) into the portable tool for use in peripheral laboratories using an optical read out. DFA takes advantage of natural opening and closing of regions of the nucleic acid by targeting those opened regions with specific primers so that only these regions of DNA are amplified by the primers. No release date for this product has been given.

NanoBioSys Inc. is developing semi-automated sample extraction and real-time PCR amplification systems based on microfluidic chips. Sample liquefaction and loading onto the extraction chip is manual. The UltraFast LabChip can accommodate 1-12 samples per chip and automatically process them in the UltraFast LabChip Sample Prep G2 system in only 15 minutes. NanoBioSys also currently markets a TB real time assay for use in the UltraFast LabChip Real-time PCR G2-3 device and claim 6, 10, 18, 48 or 96 samples processed in 30 cycles. The preparation of the reaction mixtures and their placement into the chip is manual. There are no regulatory data on these products and no published peer-reviewed studies on the performance of these tools.

The B-SMART™ technology by Sequella Inc. is a nucleic acid-based system that can measure TB viability and also drug resistance. The product is in early development and utilizes a novel approach to detect viable TB cells via infection with a bacteriophage reporter method.[39] Upon infection and replication in the host TB cell, the phages are engineered to create a novel nucleic acid, the surrogate marker locus (SML). The SML RNA is then amplified via nucleic acid sequence-based amplification, after which amplicons are detected via a lateral flow assay. Detection of the SMLs indicates viable bacteria in the test specimen. Sequella is developing a low-cost, partially-integrated system in which both TB diagnosis and drug resistance can be assessed.

Several companies are developing microarrays to detect TB and resistance to first-line drugs. The principle advantage of these technologies is that they can interrogate a greater number of targets than the LPAs all on a single test chip; therefore, they potentially offer greater sensitivity and specificity to a wider variety of drug resistance markers in a single test. Autogenomics (USA) produce the INFINITI®, an FDA-approved platform, and also an MDR-TB assay that is on the market. Sample preparation and PCR amplification and labelling of target DNA is performed manually, but the remaining steps to process the microarray and interpret data are automatically performed by the INFINITI® platform.

Akonni Biosystems (USA) and Veredus Labs (Singapore) also have products that are commercially available for research use and/or in development. Akonni currently markets tools for MTB DNA extraction that need only a pipettor and also offers the TruArray MDR-TB test kit (a labelling kit and MTB specific microarray), in addition to a complementary suite of instruments, the Akonni TruDiagnosis Systems. Akonni is currently developing a more simplified array system for use outside of the reference laboratory, but details are not currently available. For high-throughput screening of large numbers of specimens, Akonni has a fully-automated platform, TruSentry. The market release date of their simplified array technology is currently unknown.

Figure 5A-B. The VerePLEX™ Biosystem and VereMTB™ Detection Kit. A: The VereMTB™ Detection chip, in which PCR and microarray hybridization is performed. B: The VerePLEX™ Biosystem, including a five-unit processing station for the on-chip PCR and the subsequent hybridization and washing of the chip. On the right is the reader that interrogates amplicon binding on the array.

Images used with permission from Veredus labs.

Veredus Labs is currently developing the VerePLEX™ Biosystem and VereMTB™ Detection Kit, whereby target amplification and array hybridization are performed in a single-array chip within a dedicated instrument (Figure 5). The sample preparation is manual but PCR amplification and labelling and the following downstream steps are all performed on-chip within the VerePLEX™ Biosystem. This platform and assay is available for research use only as of 2012.

6.5. Technology-related information gaps hindering market entry

As reviewed in the previous sections, there is now considerable industry interest in TB diagnostics, with over 50 companies working on TB diagnostics.[40] However, test developers have several information needs. In a recent informal survey of over 25 test developers, the following critical frequently-asked questions were identified:[40,41]

- What is the global burden of TB (including latent TB, TB/HIV and MDR/XDR-TB) and what is the current and future TB treatment landscape?
- What is the current testing landscape for TB (including latent TB and DST), and what diagnostics are in the pipeline? What is the level of access to current TB diagnostics?
- What is the market size and potential for new TB diagnostics, and what are the market dynamics around TB diagnostics?
- What are the unmet diagnostic needs and target product profiles (TPPs) of greatest relevance?
- Where and how can test developers and companies get funding and technical assistance, and secure the specimens/strains necessary for test development and QC?
- What kind of validation is required for a new TB diagnostic in order to enter the market and where can companies get support for such validation?
- What are the regulatory requirements for TB diagnostics, both in-country and globally?
- Are global policy endorsements required? If so, what kind of evidence is necessary for global policy endorsements and scale-up?
- How do countries procure TB diagnostics? How autonomous is their decision making? How much is decision making influenced/guided by WHO and/or donors?
- Once a product has been validated, registered, and put on the market, and once policy endorsements are obtained, what are the challenges for uptake and scale-up?

Beyond these high-level questions, test developers have nuanced questions (Appendix 2). For example, what is the likely trajectory of the TB epidemic and future patient demographics, over the next 5-10 years? How is the treatment landscape likely to evolve over the next 5-10 years? What is the market potential and barriers for new tests, after accounting for the roll-out of Xpert® MTB/RIF? What needs do technologies like Xpert® MTB/RIF meet? How much of the market will they address? What problems remain?

While some of these questions were addressed in previous market analyses and needs assessments,[42-44] updated analyses are necessary to support product development in today's rapidly evolving landscape (see Section 2 on market considerations). Test developers are particularly interested in identifying the most important attributes on which to focus development efforts—e.g., cost, sensitivity/specificity, infrastructure requirements, time to result, throughput, sputum versus other samples, manual versus automated, POC versus centralized lab testing, integrated or reflex drug resistance test, etc. To help advance the field, a new website resource has been created (www.tbfaqs.org), and resources that address the major FAQs have been posted (Figure 6).[40]

Figure 6. Website resource on FAQs by test developers (www.tbfaqs.org)

Recently, based on a TB Diagnostics Research Forum meeting held in October 2012, a framework for action has been published (Figure 7) on the need for alignment of new TB drug regimens with methods for DST.[45] With the recent FDA approval of bedaquiline, and the impending introduction of new TB drug regimens, there is an urgent need to improve existing DST methods and introduce newer assays that can handle a wider range of TB drugs.

Figure 7. Proposed framework to achieve successful implementation of new TB regimens and DST methods

Short term
- Identify all mutations in *Mycobacterium tuberculosis* that occur reasonably frequently and that result in resistance to existing and new drugs; priority should be placed on obtaining resistance information from clinical samples that are accompanied by treatment outcome data
- Develop a collection of sequenced sensitive and resistant strains that can be used to assess new DST assays
- Use modelling to define which strategies for deployment of DST will have the greatest population-level effect and be most cost effective; various strategies would include different DST assays that vary in their speed, sensitivity and specificity, cost, and technical specifications and different DST algorithms, used in the context of various baseline resistance levels
- Undertake surveillance of moxifloxacin resistance in new patients with tuberculosis and of pyrazinamide resistance in new and previously treated patients, and patients with and without multidrug-resistant tuberculosis
- Do operational research to assess and optimise systems for sputum transport and reporting results (including prompt initiation of treatment in response)
- Develop clear target product profiles to guide diagnostics developers about the necessary product specifications and likely market demand
- Do analyses of the tuberculosis diagnostics market size and potential to inform investment decisions by test developers

Medium term
- Use existing diagnostics platforms to develop, field test, and commercialise DST assays—particularly for fluoroquinolones and pyrazinamide—that can be implemented at the subdistrict level
- Monitor for clinical resistance generated during the roll-out of new tuberculosis drugs (ie, new chemical entities) and identify the molecular basis for such resistance
- Refine models of long-term impact based on early surveillance data during roll-out of novel regimens
- Develop DST assays for new tuberculosis drugs and use them to do ongoing surveillance
- Develop and strengthen systems for using next-generation sequencing for tuberculosis drug surveillance

Long term
- Develop new diagnostic platforms that are rapid, inexpensive, and can be implemented at the subdistrict level
- Develop a universal regimen for tuberculosis that has at least three novel chemical entities and that therefore minimises the need for DST while treating all forms of tuberculosis

Source: Wells W et al. Lancet Infect Dis. 2013; reproduced with permission.[45]

6.6. Conclusions on technology considerations for TB NAATs

A review of the current TB NAAT landscape shows that a variety of options are either commercially available or in late-stage development. These products are intended for use in a variety of settings, from the reference laboratory to the peripheral laboratory. The tests are designed for detection of TB, first and/or second-line drug resistance or for TB diagnosis and drug resistance combined. The reference laboratory-based tests offer higher throughput of testing or improved screening of samples for drug resistance markers but are typically more expensive and, due to their complexity, require dedicated infrastructure and staff. Other technologies are simplified to permit scaled testing outside of a reference laboratory but still need skilled staff and mains electricity to power equipment. Some NAATs are either fully or partially integrated in terms of sample preparation, amplification and detection. These tools are also designed to use either mains electricity or battery power. By limiting the need for skilled user input and reliance on electrical power, these tests are intended to serve laboratories where even the Xpert® MTB/RIF cannot be deployed due to inconsistent power supply. Like the Xpert technology, these tools are intended to replace smear microscopy as the primary diagnostic method. These tests and equipment are also being designed to challenge the current price points of the GeneXpert platform and Xpert® MTB/RIF assay. However, GeneXpert still remains the leading technology in this area and is the last product endorsed by WHO in 2010.

A variety of newer NAATs are expected to offer an alternative to Xpert® MTB/RIF in the coming years.[12] Several products offer greater batched processing, but require more user input (e.g., Loopamp [Eiken] or TrueNAT [Ustar]). Both Epistem and Molbio have partially-integrated platforms that may offer good performance and are battery powered. Looking further out in the development continuum, the Alere Inc., NWGHF, and Wave80 platforms may offer similar performance to the Epistem and MolBio systems but, as fully-integrated systems, are expected to offer faster time-to-result than the Xpert at a similar or lower cost.

Until recently, the cost and complexity of microarray technology had greatly limited its utility as a tool for TB diagnosis and screening for drug resistance. Less complex, automated or partially integrated microarray systems may offer a rapid and more informed assessment of drug resistance than other integrated diagnostic platforms that detect resistance to rifampicin. Growing concern about MDR and XDR-TB has created a need for high-performance tools that can offer much faster results than conventional culture-based methods. In particular, the Akonni and Veredus Labs seek to offer faster results with simplified, dedicated equipment that integrates several steps for accurate and actionable data.

Despite these advances, however, the impact of new NAATs on improved TB case detection or DST screening over the next two to three years is expected to be limited. Commercialized technologies and those in late-stage development hold promise in expanding the potential for TB diagnosis via NAATs. However, of the emerging NAAT platforms for TB diagnosis, only the Eiken/FIND manual NAAT product (LAMP) has been reviewed by the WHO Expert Group and was not endorsed. Efforts are underway to generate better evidence for the use of this tool in low-resource settings (e.g., microscopy centres), and it is anticipated that pooled evaluation data from 10 independent study sites (meeting the key recommendations of the Expert Group) will be presented for further review by Q2 in 2014.

Two other products, from Molbio and Epistem, have focused their efforts mostly on markets in Asia, specifically India. The Molbio product is already available in India, while the Epistem product is awaiting regulatory approval from the Drug Controller General of India (DCGI). Published data on these assays are very limited and this is a barrier for policy endorsements. Ustar Biotechnologies is focusing market entry efforts on China, and currently their EasyNAT TB assay is pending CFDA approval (expected in 2014). For these tests to effectively challenge GeneXpert in other markets, product evaluation on a larger scale may be needed to identify how they can most benefit national TB programs and to develop the necessary evidence base for WHO to endorse their use.

Other new, fully-integrated technologies (e.g., the Alere q, Wave80 and NWGHF products) are anticipated for market release in 2014-2016 or later. However, for WHO endorsement and entry into many donor-funded programs, a clear path for generating the required evidence base is urgently needed if new products are to be endorsed in the next 3-4 years. Few organizations other than FIND are active in this important area.

Therefore, an improved and more focused technology evaluation pipeline is urgently needed to engage key stakeholders, including evaluators, country programs, developers, and donors.

In terms of ease of use, the recent NAATs are not fully integrated—with sample preparation being manually performed—and this may be prove to be challenging for implementation in peripheral microscopy centres in resource-limited countries.[12,35] While the cost per test is lower or similar to the Cepheid MTB/RIF assay, there will be additional costs to be met by TB programs for training, implementation and maintenance of quality management programs (i.e., quality assurance [QA] and quality control [QC]). This area is currently not being adequately addressed and, indeed, even with Xpert® MTB/RIF, there are still no established guidelines for quality management in the current test algorithms. Several other new NAAT being developed by Alere, NWGHF and Wave80 that also integrate the sample preparation component will also require similar guidelines for quality management.

Many newer NAAT developers are either small companies or academic groups without established large-scale production facilities or distribution networks. If technologies are shown to be effective, further investments or partnerships will be necessary to meet demand. For example, partnerships formed by Bigtec Labs with Tulip Group, and by Epistem with BD, give them access to large and established global distribution networks.

With the increased need to more rapidly perform accurate DST in MDR-TB endemic regions, there is an increasing number of products that include at least rifampicin resistance screening; however, there is a concern that most of these are not endorsed and, in addition, that many are reference laboratory based. Some of the newer NAATs targeting peripheral laboratory use incorporate limited drug resistance testing in integrated formats or via follow up tests. While culture-based DST is still necessary to confirm drug susceptibility, the evolution of microarrays to address greater numbers of resistance alleles may expedite improved detection of MDR-TB. Currently, these tests are reference laboratory-based, but both Akonni and Veredus are looking to develop test formats that enable DST outside of the reference laboratory. However, ease of use, throughput and the cost per test will need to be more carefully established to identify if they can add value to increased detection of MDR-TB and, ultimately, improve treatment of MDR-TB cases.

Of the many NAATs described in this document, the Alere q holds promise based on a broad range of factors, though this is tempered by the key risk that the TB diagnostic tool is undergoing development of its cartridge component, a significant engineering challenge. Alere Inc. is a leading diagnostics manufacturer with global distribution of a large product portfolio of diagnostic assays for infectious diseases and is experienced in supplying developing world markets. The recent BMGF investment in developing the Alere q has reduced risk associated with integrating the core technologies into this product, which are already at very late stage development, and the funding amount should ensure that this work can be performed in a relatively short period of time (2.5 years). In addition, the low-interest loans to build production lines demonstrate that Alere is already considering ensuring adequate supply shortly after their product(s) reach the market. This is further strengthened by the impending release of the Alere q viral load assay using the same instrument. Alere also has significant experience working in developing country markets given the recent introduction of the Alere PIMA CD4 instrument and test.

In summary, there is a growing portfolio of TB NAAT assays that are close to market or in late-stage development. However, none is expected to be endorsed by WHO in the next year and few are anticipated to have the necessary evidence base for endorsement after that. To increase TB patients' access to improved diagnostics, significant concerted efforts and funding are required to identify products that are most needed and likely to have the greatest impact. This should be followed by expedited evaluation efforts to generate the evidence base for both endorsement and scale-up. In parallel, diagnostic test manufacturers must make sufficient investments to complete test evaluation and regulatory approvals. They must also ensure production facilities can meet anticipated needs and understand how their specific technology is positioned to meet the needs of diagnostic market. Additional needs related to successful scale-up of new technologies, detailed in the previous UNITAID landscape reports, include: global policy recommendations; decision-making processes, engagement and commitment at country-level; fit with user needs; regulation; and laboratory capacity.

7. Market landscape

7.1. Market analyses

With the rapid expansion of the TB diagnostics pipeline, roll-out of the Xpert® MTB/RIF technology, and increasing investments, there is considerable industry interest in TB diagnostics.[12,40,41] In 2013, more than 50 diagnostic companies and test developers are actively engaged in TB.[40,41] A recent survey (see www.tbfaqs.org) showed that to inform their business case, developers require data on the current market size for TB diagnostics, both globally and in high-burden countries.[40,41]

Among the various information gaps identified by surveying test developers (Appendix 2)[40,41] the following questions specifically address market size and dynamics:

- What is the current market size for TB diagnostics, both globally and in high-burden countries?
- What is the market potential for new tests? What is the expected market growth rate?
- How is the market segmented by low, middle vs. high income countries? How is the market segmented by where the test might be utilized (i.e., reference lab, microscopy centre, basic healthcare facility)? Is there a different market segment based on patient risk factors?
- How is the market served currently?
- What are the key market barriers for uptake (i.e., what are the market access challenges)? What will drive uptake?
- How likely is that most high-burden countries will scale-up Xpert® MTB/RIF? What needs do technologies like the GeneXpert meet? How much of the market will they address? What problems remain? Is the potential remaining market only there if access is increased (i.e., currently no testing is being done)?

Updated market analyses are necessary to answer these questions. One published comprehensive global assessment of the TB diagnostics market has been published to date (FIND and WHO Special Programme for Research and Training in Tropical Diseases [TDR], 2006).[46] The TB diagnostics landscape has greatly changed since the publication of this market analysis.[12,47]

Efforts are underway to quantify the current TB diagnostics market, accounting for the on-going roll-out of Xpert® MTB/RIF and other changes in the landscape. This effort involves BMGF, McGill International TB Centre, UNITAID, FIND, the Stop TB Partnership's New Diagnostics Working Group and country partners and national treatment programmes. The scope of the proposed project is to conduct a rapid assessment of the *served* available market for TB diagnostics (i.e., current algorithms, regulatory and policy landscape, testing volumes/sales, total dollar value expenditure on diagnostics, and market segmentation) in four high-burden countries: India, China, Brazil, and South Africa. This market analysis will cover the 2012 to 2013 period, providing a snapshot of the current market in these emerging economies. This analysis, expected to be completed by early 2014, will provide in-depth information for each of the four countries on TB epidemiology, laboratory, and policy landscape, numbers of cases and people tested, test volumes and dollar value of the market in the country, segmented by type of test, and public vs. private sectors.

7.2. Market approaches to improve access to WHO-endorsed tools in the private sector

In many high-burden countries, the private healthcare sector is a major provider of health care.[48] In India, Bangladesh, Cambodia and Pakistan, for example, 70 to 80% of first contact care happens in the private sector, which in these countries represents a heterogeneous mix of qualified and unqualified providers, modern and alternative health systems, and facilities that range from for-profit to charitable institutions. Quality of care, therefore, is highly variable.[49-51]

On the one hand, the private health sector is often seen as part of the problem—diagnostic and treatment practices are known to be suboptimal, as demonstrated in studies from countries such as Pakistan and India.[52-54] Studies have shown considerable delays in TB diagnosis, and patients often move from one provider to another, and between private and public sectors, before they are finally diagnosed and put on

TB treatment.[55-57] And while they do this, they continue to transmit the infection to those in their communities. By the time patients are diagnosed, many have advanced cavitary disease. Poor TB patients also seek private care where the costs of care can be quite high, pushing poor families further into poverty. Thus, poorly managed TB is a major driver of the epidemic, and a critical risk factor for mortality and drug-resistance.[58]

On the other hand, given their dominant role in TB care, engagement with private providers is critical for achievement of TB control targets.[59] In particular, since private providers are often the first point of contact, their involvement is critical for early and accurate diagnosis. Unfortunately, because of unregulated markets and perverse incentives, diagnostic practices in the private sector can be suboptimal. In India, for example, until 2012, inaccurate TB serological (antibody) tests were the most dominant TB test in the private sector.[53]

Thus, a key challenge in TB control is significant engagement with the private sector—for example, replacing suboptimal tests with WHO-endorsed, validated tools at affordable prices, and ensuring that all TB cases are appropriately managed. This will require innovative business models and delivery approaches, and such models are being piloted in countries like India, Pakistan, Bangladesh and Indonesia.[48,60] Two examples are discussed below.

7.3. The IPAQT initiative in India

In the public sector, sputum microscopy is the most dominant TB test in India. However, for several reasons, including poor regulation and financial incentives, blood is the most popular sample for TB testing in the private sector.[52] In June 2012, following the negative policy recommendation by WHO, the Indian government banned the use, import, manufacture or sale of all TB serological tests for TB (Figure 8). To address the concern that serological antibody-based tests will be replaced by blood-based interferon-gamma release assays (IGRAs) (e.g., QuantiFERON-TB Gold), the Indian RNTCP has explicitly addressed use of IGRAs in their advertising campaign (Figure 8).

Figure 8. Advertisement, published in leading Indian newspapers, on the Indian governmental ban on serological antibody tests

This ban, however, raised a new challenge and an opportunity: how can validated, WHO-endorsed, sputum-based TB tests replace the inappropriate blood tests in the private sector? Quality tests like Xpert® MTB/RIF, LPA, and liquid culture were very expensive in the private sector. For example, the Xpert test cost the patient as much as Rs. 3000 (US $55) or more in private laboratories, a substantially higher price compared to the banned ELISA tests (which cost about Rs. 1000 [US $20] for two antibodies). This is because WHO-endorsed tests are available at FIND-negotiated low prices only to the public and non-profit sectors in high-burden countries. In addition, import duties, financial incentives, and laboratory and distributor margins further inflate test costs, making them virtually unaffordable to the average private-sector patient. This had posed a big challenge for replacing the banned serological TB tests with WHO-endorsed tests in India.

To overcome these challenges, and taking advantage of the opportunity created by the serology ban, a new initiative was recently launched in India, to improve the affordability of WHO-endorsed TB tests in the private market. Initiative for Promoting Affordable, Quality TB tests (IPAQT www.ipaqt.org) is a coalition of accredited private labs in India, supported by industry groups and non-profit groups (e.g., Clinton Health Access Initiative), that has made three WHO-approved tests (i.e., Xpert® MTB/RIF, Genotype® MTBDRplus, and MGIT) available at affordable prices to patients in the private sector.[61,62]

To drive a sustainable change in the private TB diagnostics market, it is critical to align the commercial interests of the various players in the value chain in the highly fragmented private market. An effective means of ensuring a synergistic intervention was to organize a group of laboratories interested in adoption

of better tests into a partnership that would be eligible for lower input pricing in exchange for agreeing to abide by certain guiding principles which include, among others, limiting their margins per test to ensure affordable price to patients and case notification (Figure 9). In exchange for offering lower prices, suppliers (manufacturers and distributors) would, in turn, receive greater and more predictable volumes from the large but currently untapped private market for new TB tests, thus creating a "win-win-win" situation where laboratories, suppliers, and patients all benefit.

Member labs in IPAQT have access to lower, FIND-negotiated prices for quality tests in exchange for their commitment to pass on the benefits to patients and adhere to the guiding principles. Specifically, the Initiative aims to:

1. Facilitate the delivery of WHO-endorsed tests to the TB patient at affordable prices;

2. Promote the use of WHO-endorsed TB tests by building awareness of these new, validated/endorsed tests among health providers, laboratories and patients;

3. Discourage the use of tests that are inaccurate or not recommended by WHO and the RNTCP;

4. Encourage notification of all TB cases to the RNTCP;

5. Improve QA by working with accredited labs and implementing an external QA program.

Figure 9. Guiding principles of Initiative for Promoting Affordable, Quality TB tests (IPAQT www.ipaqt.org)
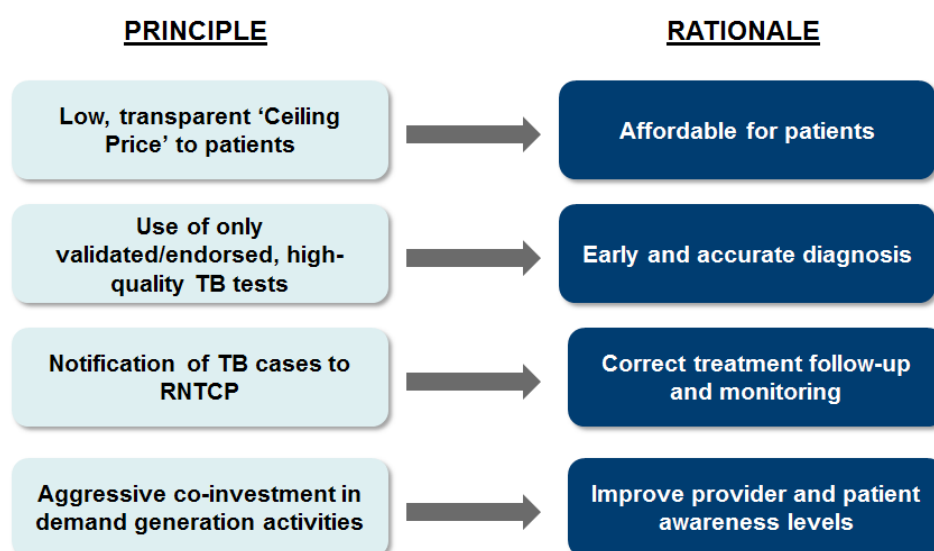
Because this model operates on a high-volume, low-margin, mass-market model (as compared to a premium pricing model), the cost of Xpert® MTB/RIF is now reduced to Rs 1700 [US $32] (maximum price labs can charge patients). The LPA (Genotype® MTBDRplus) is now available at Rs 1600 [US $30]. These prices are approximately 50% less than the private market prices before IPAQT was launched. Laboratories in IPAQT will soon offer other WHO-endorsed tests (e.g., MGIT) at transparently advertised prices. TB cases diagnosed will be notified to the RNTCP for linkages to free TB drugs, where necessary.

It is expected that despite the lower per-test margin, aggressive investment by the labs in demand generation would result in higher volumes and higher absolute profits and drive widespread uptake of the validated/endorsed tests to achieve the targeted volumes and therefore achieve financial returns and health impact.

The IPAQT initiative has a pan-India presence—with more than 36 labs (which adds up to over 3000 franchisee labs and 10000 + collection centres around the country) committed to providing these tests at affordable prices. The number of labs is expected to increase significantly in the months ahead.

7.2.2. Social franchising model in Pakistan, Bangladesh and Indonesia

Recently, Khan and colleagues implemented a novel package of public-private mix approaches in one intervention area of Karachi, and compared case notification rates with a control area.[60] Interventions included a communications campaign to increase demand for tuberculosis diagnosis and treatment services, involvement of laypersons as TB screeners in private practitioner clinics and hospitals, and mobile phone-based incentives—all combined with referrals to a private hospital that offered free tuberculosis care. The authors found a substantial increase in case notifications in the intervention area vis-à-vis the control area.[60] Building on this experience, efforts are now underway to roll out Xpert® MTB/RIF and a package of interventions using social enterprise models in Bangladesh, Indonesia, and Pakistan. Unlike social franchising, which depends on donor funding to provide services, social enterprises seek to generate revenue to sustain services.[63] However, unlike traditional businesses, profits from social enterprises are reinvested, improving care and reducing patient costs rather than paying dividends.[63] The TB REACH-funded projects are exploring many other innovative business models for new TB diagnostics, and future editions of the UNITAID landscape report will cover the lessons from these ongoing experiments.

8. Market shortcomings

The development and roll-out of Xpert® MTB/RIF has undoubtedly had a positive influence on the TB diagnostics landscape. However, challenges remain. The high cost of this technology, dependence on a single-source supplier, cartridge shortage and supply chain issues, exclusion of the private sector in high-burden countries from negotiated pricing agreements, and difficulties in implementing this test in lower tiers of the healthcare delivery system (i.e., primary care centres and peripheral microscopy labs) are critical concerns. Also, it is unclear if programs are implementing Xpert® MTB/RIF as a POC testing program to ensure same-day initiation of TB treatment. Implementation of this technology in centralized, reference laboratories for DST purposes will probably have limited impact on TB incidence, especially in settings where patient delays are substantial.

There is a need for improved NAATs that are more affordable and more robust and decentralizable than Xpert® MTB/RIF. While next-generation molecular tests have emerged since Xpert® MTB/RIF, none of them has undergone rigorous field trials at the intended use setting (i.e., peripheral microscopy labs), and all of them have substantial challenges with sample processing and DNA extraction in peripheral labs. Furthermore, there appears to be no clear pathway for these technologies to be rapidly evaluated for policy review and scale-up. This bottleneck needs to be urgently addressed and resolved. It remains to be seen if recent investments such as the BMGF grant to Alere will produce a NAAT technology that can be used in primary care centres and microscopy labs in the next 3 to 4 years.

The *UNITAID 2012 Tuberculosis Diagnostic Technology Landscape Semi-annual Update* report noted other potential barriers to adoption and scale-up of POC technologies.[35] In addition, the *UNITAID Strategy 2013-2016* detailed shortcomings related specifically to markets for TB diagnostics.

In summary, market shortcomings and the reasons for these include:

Availability: There is no true POC TB diagnostic test: GeneXpert still requires basic laboratory infrastructure. ***Reasons:*** Unclear potential market and lack of clarity on available market share after GeneXpert scale-up reduce developers' willingness to invest in research. Significant technical challenges in developing a true POC product.

Acceptability/Adaptability: Current diagnostics are not adapted for specific patient groups or decentralized healthcare settings. For example: limited DST ability; no ability to perform multiple different tests (multi-platform functionality); not suited for children (the tests require sputum which is hard to collect

from children); not suited for populations with low levels of mycobacteria in sputum (children, HIV co-infected patients, cases of extrapulmonary disease). *Reasons:* Technical difficulty in developing technologies to address specimen collection and other challenges presented by specific patient groups.

Affordability: New technologies are expensive: the GeneXpert machine costs $17,500 (4-module), and each cartridge costs about $10 to preferred buyers, or considerably more in the private sector (retail cost: $60). *Reasons:* Monopolistic supplier. High complexity of incorporating multiple reagents into a robust cartridge.

Quality: No information on quality of diagnostics to guide procurement. Continued use of inappropriate tests, particularly in the private sector. *Reasons:* Limited global quality assurance processes for TB diagnostics; current reliance on ad hoc recommendations from WHO STAG-TB committee. Limited in-country regulation of laboratories.

Delivery: Supply constraints affecting delivery of GeneXpert cartridges. *Reasons:* Monopolistic market with limited production capacity. No alternative suppliers for purchasers to use.

Barriers to adoption of novel innovative technologies hinder uptake. *Reasons:* Novel product types require extensive training and integration into diagnostic and clinical algorithms.

9. Potential opportunities for market intervention

As noted in the *UNITAID Strategy 2013-2016,* potential interventions may include efforts to:

- **Accelerate market entry for innovative POC TB diagnostics, including any with comprehensive DST capability and ability to use specimens other than sputum.** Indeed, the need for a biomarker-based, simple, low-cost, instrument-free rapid test remains a key priority. Such a test could potentially be implemented at points of first-contact in the community and help triage persons who require confirmatory testing. Although biomarker discovery is an active area, no test under development is close to the market. Companies and test developers have an opportunity to address this key gap in the pipeline. Ongoing initiatives such as updated market analyses and development of target product profiles should facilitate greater engagement of test developers in meeting this priority need.

Based on broader market scoping by UNITAID, additional potential interventions may include efforts to:

- **Support global efforts to develop quality assurance policies and systems for TB diagnostics**; and
- **Facilitate development of open platforms or generic competition, and facilitate development of TB diagnostics for use in underserved patient groups**, including extrapulmonary TB, children, and people living with HIV.

As noted in the *UNITAID Call for Letters of Intent (29 May—22 September 2013)*, UNITAID has an interest in innovative market approaches that improve access to TB commodities, and recognizes that many patients seek care from programmes funded by country governments or in the private sector. New business models are being tested to increase access to WHO-endorsed TB tests in high-burden countries (e.g., India, Pakistan, Bangladesh) with a significant private sector focus, as described above. These initiatives will inform wider scale-up of such delivery models and could influence future iterations of global and national policies.

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APPENDIX 1

Comparison of key test characteristics, performance, and projected costs of the GeneXpert® MTB/RIF diagnostic platform and emerging fast-following technologies (Adapted from *2012 Tuberculosis Diagnostic Technology Landscape, Semi-annual Update*; UNITAID)

	Xpert® MTB / RIF (Cepheid)	TrueLab™ MTB Detection (Molbio)	Genedrive™ MTB iD (Epistem)	Loopamp® TB Detection (Eiken)	NATeasy™ TB (Ustar)
Diagnostic capabilities	MTB diagnosis & Rif resistance	MTB diagnosis	MTB diagnosis & Rif resistance	MTB diagnosis	MTB diagnosis
Amplification	PCR	PCR	PCR	LAMP	CPA
Detection	Real time, fluorescence	Real time, fluorescence	End point analysis, melt curve, fluorescence	Real time with turbidity or endpoint via fluorescence	End point, immunochromato-graphic strip
Degree of automation/ integration	Fully integrated sample preparation, amplification & detection	Semi-automated sample preparation, then automated amplification/ detection	Manual sample preparation, then automated amplification/ detection	Manual sample preparation. Strategies for fully automated amplification & detection or by manual method	Manual sample preparation, amplification & detection
IAC	Yes	Yes	Yes	No	Yes
Electronic data transmission	Yes†	Yes	No	No	No
Disposables/ cost	Liquefying reagent, MTB/RIF cartridge. Retail cost: US $60, discount cost: <US $10	DNA extraction kits, PCR chip. Cost: US $10-12 ‡~	MTB iD® Test-kit: paper based sample preparation. Test cartridge cost: US $10-17 ‡	DNA Extraction Kit and MTB Complex Detection Reagent kit. Cost: N/A	DNA purification kit, CPA reagent tubes, XCP Nucleic Acid Detection Device. Cost: US $6 per test
Endorsed by WHO	Yes	No §	No §	No §	No §
CE IVD mark	Yes	No	Yes	Yes	Yes
Evaluations	>18%	3	2	5	4
Dedicated Instrument-ation/cost	GeneXpert/ US $17,500 (4 module)	Truelab™ Mag and UNO (two instruments)/ < US $ 7,000 ¶	Genedrive®/ < US $4,000	LF-160 or LA-500/ price NA	US $6 per test
Additional instruments required	None	None	None	Alternatively: PCR machine, vortexer, UV Lamp	Water bath/heating block/PCR machine vortexer, centrifuge
Electricity	Uninterrupted line power	Rechargeable Battery	Rechargeable Battery	Uninterrupted line power	Uninterrupted line power
Temperature control	Operating temperature <30 °C	2-30 °C	N/A	2-30 °C	Refrigerated reagent storage #
Technical skills required ††	Low	Low-Medium	Low-Medium	Medium	Medium
Time to result	<2 hrs	<1 hr	<45 minutes	<1 hr	<2 hrs

	Xpert® MTB / RIF (Cepheid)	**TrueLab™ MTB Detection (Molbio)**	**Genedrive™ MTB iD (Epistem)**	**Loopamp® TB Detection (Eiken)**	**NATeasy™ TB (Ustar)**
Throughput	16-20 tests per 8 hr work shift for 4-module instrument	12 tests per 8 hr work shift	Not yet determined	Not yet determined	Not yet determined
Sample handling	1 sample per module, random access	Single sample per instrument	Single sample per instrument	Single sample, or batch processing with potential for random access via 8 test sets¥	Single sample or batch processing
Clinical sensitivity ‡‡	99.8% SSM+/C+ 72.5% SSM-/C+	99.12% SSM+/C+ 75.86% SSM−/C+	No published data	98.5% SSM+/C+ 55.6 SSM-/C+	96.9% SSM+/C+ 87.5% SSM-/C+
Clinical specificity ‡‡	99.2% SSM-/C-	100% SSM-/C-	No published data	96.2% SSM-/C-	98.8% SSM-/C-
Intended entry market	Global	India ##	Global ¶¶	EEA, India	China, Indonesia

† The data generated from the GeneXpert®can be uploaded to a web-based server if connected to the internet.

‡ Tentative cost per test, reflects nonsubsidized pricing unlike the volume-generated pricing associated with the Xpert® MTB/RIF assay.

§ Devices and assays are undergoing evaluation and demonstration at multiple test sites.

~ This cost includes both the Truelab™ UNO and Truelab™ micro PCR system.

¶ The price includes both the extraction system and PCR device.

The Ustar reagents are thermostable for up to 2 weeks, permitting some transport without cold chain.

†† The technical skills required are described as low (1–3 days training of a non-expert user with seventh grade level education or equivalent) or medium (4–5 days training of a user with higher skill level).

‡‡ Performance is based on limited published data.

¥ The LA-500 instrument can operate with 4 independent set of reaction tubes, measuring turbidity.

§§ Based on using a standard sample preparation approach, not the sample preparation method envisioned in the future test devices.

% Based on the meta-analysis of Xpert® MTB/RIF studies by Chang et al.

Although targeting the Indian market, Tulip Diagnostics currently has sales markets in over 57 countries.

¶¶ Epistem have a collaborative agreement with Xcelris Labs (India) to market the test in India. A recent agreement with Becton Dickinson (USA) is intended to cover the remaining global market except the USA.

CPA: Cross-priming amplification; IAC: Internal amplification control; LAMP: Loop-mediated amplification; MTB: Mycobacterium tuberculosis; NA: Not available; NAAT: Nucleic acid amplification technique; NALF: Nucleic acid lateral flow; Rif: Rifampicin; SSM+/C+: Positive by sputum smear microscopy and culture; SSM-/C+: Negative by sputum smear microscopy, positive by culture; SSM-/C-: Negative by sputum smear microscopy and culture; UV: ultra violet.

APPENDIX 2

TB diagnostics: top 10 FAQs by test developers

Based on input from 25 + companies and individuals, these are the most critical questions of relevance to test developers and companies wanting to develop TB diagnostic technologies.

Source: Pai M. *Int J TB and Lung Dis* 2013 and www.tbfaqs.org

1.   TB BURDEN AND TREATMENT LANDSCAPE

What is the global burden of TB (including latent TB, TB/HIV and MDR/XDR-TB) and what is the current and future TB treatment landscape?

   a. What is the current burden and predictions for future, disease distribution (highest burden countries), current and future patient demographics, and trends over the next 5-10 years?

   b. What is the treatment landscape today and for the next 5-10 years? What is the level of access to current TB treatment?

   c. What TB drugs are currently important for drug susceptibility testing (DST), and which drugs will need to be considered for DST in the near future?

2.   CURRENT DIAGNOSTICS LANDSCAPE AND PIPELINE

What is the current testing landscape for TB (including latent TB and DST), and what diagnostics are in the pipeline? What is the level of access to current TB diagnostics?

   a. What TB diagnostic tests are currently on the market, and what products are likely to enter the market in the near future?

   b. Which tests are currently included in policy recommendations and widely used? What are the currently used diagnostic algorithms in high-burden countries? Who develops the algorithms and what is the process for changing a diagnostic algorithm in response to a new diagnostic technology entering the market?

   c. What is the current level of access to available TB diagnostics in high-burden countries?

3.   MARKET SIZE, POTENTIAL AND DYNAMICS

What is the market size and potential for new TB diagnostics, and what are the market dynamics around TB diagnostics?

   a. What is the current market size for TB diagnostics, both globally and in high-burden countries? What is the market potential for new tests? What is the expected market growth rate?

   b. How is the market segmented by low, middle vs. high income countries? How is the market segmented by where the test might be utilized (i.e., reference lab, microscopy centre, basic healthcare facility)? Is there a different market segment based on patient risk factors?

   c. How is the market served currently? What are the key market barriers for uptake (i.e., what are the market access challenges)? What will drive uptake?

d. How likely is that most high-burden countries will scale-up Xpert® MTB/RIF? e. What needs do technologies like the GeneXpert meet? How much of the market will they address? What problems remain? Is the potential remaining market only there if access is increased (i.e., currently no testing is being done)?

e. Are market access barriers lower for second or third, rather than the first product in its class?

f. What is the risk for new products that have to compete against entrenched competitors?

4. TARGET PRODUCT PROFILES

What are the unmet diagnostic needs and TPPs of greatest relevance?

a. Which attributes* within the TPP are the most important to focus on? What are the top 4-5 features that are needed in a TB diagnostic test for developing countries?

*Attributes include target cost, sensitivity/specificity (which is more important and what is the minimum acceptable level?), infrastructure requirements (e.g., power, temperature control), time to result, through-put, sputum versus other samples, manual versus automated, requirements for reporting of test results, point-of-care versus centralized lab testing, integrated or reflex drug resistance test, which drugs to include in DST, TB only test versus multiplexed platform, other key assays (i.e., HIV, CT/NG) that need to be available on the same platform, shelf-life requirements, instrument/test connectivity requirements, importance of subgroups such as HIV-infected and children, etc.

b. At what price/cost can a new TB diagnostics be sold (depending on volume)? What is the current and projected pricing environment over the next 5-10 years?

c. What are the differences in the market opportunities for a screening test and separately for DST? How is the price/cost affected if the new test is a screening (broadly used) test versus an "add-on" or reflex test?

d. How critical is it to include DST in the test? Which drugs are critical for DST now, and in the future? Is it advantageous to have a platform that can detect a large number of mutations? What is the cost-benefit ratio of having these additional elements in the test?

5. PRODUCT DEVELOPMENT SUPPORT

Where and how can test developers and companies get funding, technical assistance and secure necessary specimens/strains for test development and quality control?

a. How do successful/profitable, as well as start-up companies, gain funding to support TB diagnostic development?

b. Which are the key funding/donor agencies (e.g., NIH, BMGF, USAID, DFID, Wellcome) and what are their funding priorities in TB product development?

c. If donors support product development, what are their expectations in terms of pricing, global access, IP, etc.?

d. Which are the product development partnerships (e.g., FIND, PATH, IDRI) that can provide support with TB dx development and what are their criteria/conditions for providing support?

e. Where can test developers access well-characterized specimens (including non-infectious artificial sputum), strains, sequences for drug-resistance mutations and biosafety level 3 facilities?

6. PRODUCT VALIDATION SUPPORT

What kind of validation is required for a new TB diagnostic in order to enter the market and where can companies get support for such validation?

a. How many validation studies will be required to introduce a new TB diagnostic? Are test accuracy studies adequate, or clinical impact studies required? How much geographical diversity is needed for the clinical trials and validation?

b. What validation studies were required for, and conducted by Cepheid to bring their TB test to market? How much did it cost and who paid for it?

c. Who can provide clinical trials and validation support to companies?

d. What will it cost to conduct clinical validation studies? Will donors and funding agencies pay for product validation?

e. Which academic institutions and laboratories are capable of TB test validation and field trials?

f. Which are the product development partnerships (e.g., FIND, PATH, CHAI, IDRI) or agencies (e.g., CDRC) that can provide support with validation and what are their criteria/conditions for providing support?

7. REGULATION

What are the regulatory requirements for TB diagnostics, both in-country and globally?

a. What is required for the registration of new diagnostics in the major, high TB burden countries?

b. Will multiple regulatory approvals be necessary? What will it cost? Is there a pathway to get simultaneous approvals from multiple regulatory agencies?

c. How critical is FDA approval for global markets and what will it take to get FDA approval?

d. What are the advantages and disadvantages of getting CE mark versus WHO prequalification versus FDA approval and which of these is needed for major markets?

e. How strong is the intellectual property in the TB diagnostics area, and specifically within major, target countries?

8. POLICY

Are global policy endorsements required? If so, what kind of evidence is necessary for global policy endorsements and scale-up?

   a. What global policy endorsements or approvals are critical for success (e.g., WHO, CDC, FDA, others)? What level of interaction and evidence is required for these endorsements?

   b. Is WHO endorsement/policy the most important factor for accessing global markets? If not, what is? Historically, what is the timeline for these endorsements/policies? What will it cost to collect evidence for such approvals and policies? How does WHO decide on which technologies to consider for policy review?

   c. Is there a WHO prequalification process for TB diagnostics, and if so, how long will the process take? What is the difference between WHO policy and WHO prequalification for TB?

   d. What kind of evidence is required at the country level to get policy endorsements and registration? Is WHO endorsement or prequalification alone sufficient for country-level policy adoption and registration? Are country evaluations still required?

9. PROCUREMENT AND MARKET ACCESS

How do countries procure TB diagnostics? How autonomous is their decision making? How much is it influenced/guided by WHO and/or donors?

   a. Who are the major buyers in developing countries (e.g., Ministries of Health, laboratories, National TB Programmes, international donors)? What is the reimbursement environment for diagnostics?

   b. What are the most important MOH concerns, and how does the MOH procure, direct procurement, and make decisions on vendors (e.g., tenders)?

   c. How important are 'consumers' in the various developing markets?

   d. What are the market access challenges and options for addressing them? Specifically, is procurement linked to regulatory approval? Will each country require an independent study of a TB diagnostic, or is a single study sufficient?

   e. What are the logistics and distribution challenges? How fragmented is the distribution pathway? What will be necessary to set up a distribution and supply chain? Who pays for distribution, the manufacturer or the purchaser? Are these issues separate for public and private sector purchasing and distribution, or is there a single national or centralized process in countries?

   f. Are there regulations such as taxes/import duties in country that affect business models?

   g. What will be required by developers/suppliers to provide sales and after-sales support, as well as service and maintenance in a reliable manner?

   h. Will countries use a mix of different products using the same technology (i.e., different NAAT tests from different manufacturers)?

10. SCALE-UP

Once a product has been validated, registered and put on the market, and once policy endorsements are obtained, what are the challenges for uptake and scale-up in high-burden countries?

   a. Which validated tests have been successfully scaled-up, what were reasons for the success, and how long did it take to reach scale?

   b. How do country level policy makers make decisions on technologies to scale-up?

   c. If some tests have not been scaled-up even after policy endorsements, why? What are the biggest barriers and how can they be overcome? Are the barriers in BRICS countries very different from other high-burden countries?

   d. When and how do donors fund/subsidize and support roll-out of TB diagnostics?

1. Who are the major players in funding scale-up (e.g., UNITAID/The Global Fund to Fight AIDS, Tuberculosis and Malaria/BMGF/PEPFAR/USAID), and their historical role and funding mandate/priorities?

2. Since donors are already supporting the roll-out of GeneXpert, will they consider other technologies for buy-down and/or scale-up?

3. Is WHO endorsement mandatory for donor support for scale-up?

4. What are the long-term prospects after donor funding ends?

5. Are there markets that are not dependent on donor funding? What is the willingness/ability to pay for new diagnostics in donor-dependent versus non donor-dependent countries?