UNITAID

2012

TUBERCULOSIS
Diagnostic Technology Landscape

SEMI-ANNUAL UPDATE

DECEMBER 2012

UNITAID Secretariat
World Health Organization
Avenue Appia 20
CH-1211 Geneva 27
Switzerland
T +41 22 791 55 03
F +41 22 791 48 90
unitaid@who.int
www.unitaid.eu

UNITAID is hosted and administered by the World Health Organization

TABLE OF CONTENTS

List of Acronyms

AIDS	Acquired immune deficiency syndrome
CDC	Centers for Disease Controls and Prevention (USA)
CPA	Cross-priming amplification
DNA	Deoxyribonucleic acid
DST	Drug susceptibility testing
EPTB	Extrapulmonary tuberculosis
EQA	External quality assurance
FIND	Foundation for Innovative New Diagnostics
GHTF	Global Harmonization Task Force
GLI	Global Laboratory Initiative
GPRS	General packet radio service
GPS	Global positioning system
GSM	Global system for mobile communications
HIV	Human immunodeficiency virus
IAC	Internal amplification control
IPC	Internal process control
LAMP	Loop-mediated amplification
LCD	Liquid crystal display
MDR	Multi-drug resistant
MTB	*Mycobacterium tuberculosis*
MTBC	*Mycobacterium tuberculosis* complex
NAAT	Nucleic acid amplification test
NALF	Nucleic acid lateral flow
NHLS	National Health Laboratory Service
NTM	Non-tuberculosis mycobacteria
NTP(s)	National tuberculosis programs(s)
PATH	Program for Appropriate Technology in Health
PCR	Polymerase chain reaction
PEPFAR	U.S. President's Emergency Plan for AIDS Relief
POC	Point of care
RIF	Rifampicin
RNTCP	Revised National Tuberculosis Control Programme (India)
TAG	Treatment Action Group
TB	Tuberculosis
TDR	Special Programme for Research and Training in Tropical Diseases (WHO)
TPP(s)	Target product profile(s)
TST(s)	Tuberculin skin test(s)
USAID	United States Agency for International Development
USD	United States dollar
WHO	World Health Organization
XDR	Extensively drug resistant

Overview

The *Tuberculosis Diagnostic Technology Landscape* is published annually and is prepared as part of a broad and on-going effort to understand the technology landscape for tuberculosis (TB) diagnostics. The first edition of the landscape report was published in July 2012. It is available at:

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

This document is a semi-annual update, focused mainly on nucleic acid amplification test (NAAT) technologies, specifically roll-out of the Xpert® *mycobacterium tuberculosis* (MTB)/rifampicin (RIF) resistance test, and a review of fast-follower NAATs that are on the market or will be on the market by early 2013. This report also provides an update on the ongoing work to assess the market size for TB diagnostics and develop target product profiles (TPPs) for new TB diagnostics. Challenges for point-of-care (POC) testing and market dynamics and barriers for roll-out of new TB diagnostics are also reviewed. The material in this landscape is current through December 2012.

Methods

The *Tuberculosis Diagnostic Technology Landscape: Semi-Annual Update 2012* was compiled by Madhukar Pai (McGill University, Montreal) and David Boyle (Program for Appropriate Technology in Health [PATH], Seattle) with support from UNITAID. The material in this landscape 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 or agencies. In particular, materials from the following three published articles by the authors were adapted, with permission from the authors and copyright holders:

1. Niemz A, Boyle DS. Nucleic acid testing for tuberculosis at the point-of-care in high-burden countries. Expert Rev Mol Diagn. 2012 Sep;12(7):687-701.
2. Pai NP, Vadnais C, Denkinger C, Engel N, Pai M. Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. PLoS Med. 2012 Sep;9(9):e1001306.
3. Pai M, Palamountain KM. New tuberculosis technologies: challenges for retooling and scale-up. Int J Tuberc Lung Dis. 2012 Oct;16(10):1281-90.

Acknowledgements and conflicts of interest

Madhukar Pai has no commercial/financial conflicts. He has received grant funding for TB diagnostics research from Grand Challenges Canada and the Bill & Melinda Gates Foundation. 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.

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

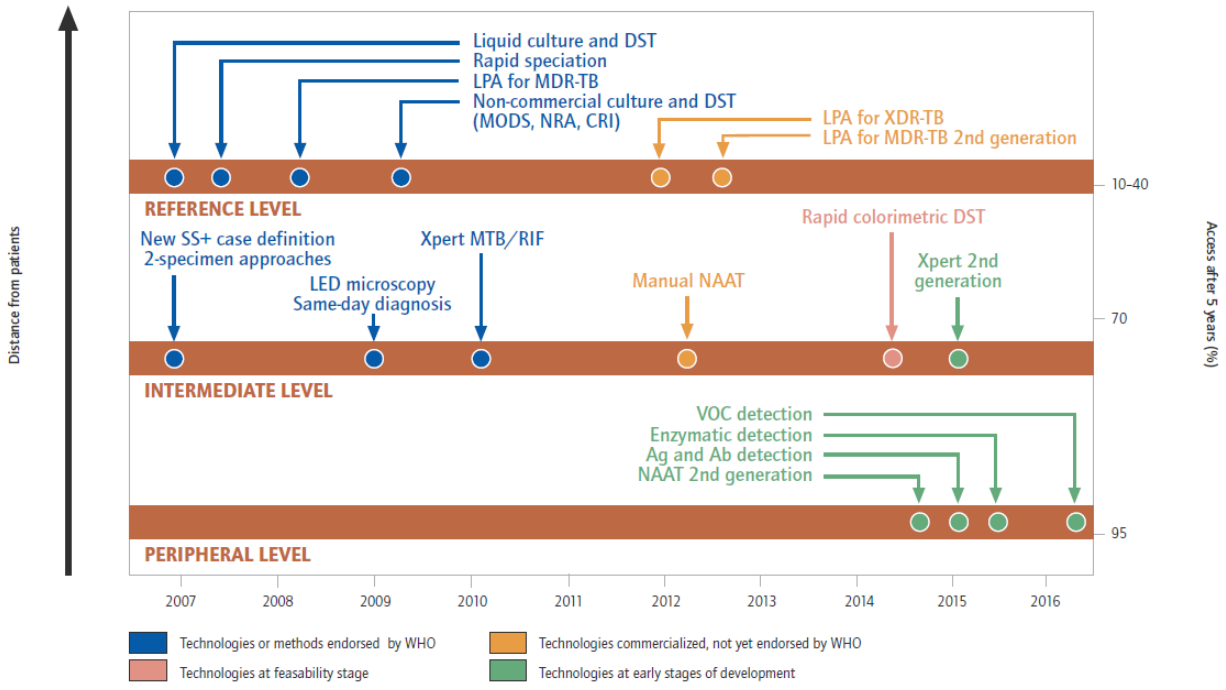
Section 1.
Update on Molecular TB Tests

2012 TB diagnostics pipeline; update on roll-out of Xpert MTB/RIF

Findings from the 2012 World Health Organization (WHO) Global Tuberculosis Report show that TB continues to be a major public health threat, with an estimated 8.7 million new cases in 2011, and an estimated 1.4 million deaths from TB.[1] Early case detection and rapid treatment remain the most important TB control strategy, and accelerating uptake of new TB diagnostic technologies is critical for ensuring early diagnosis and reduced TB transmission.

The 2012 TB diagnostics pipeline (Figure 1) shows a number of technologies that have been endorsed or reviewed by WHO, and a pipeline of technologies under development.[1] Many of the technologies under development are intended for use at the most peripheral level of the health care system.

Figure 1. 2012 TB diagnostics pipeline



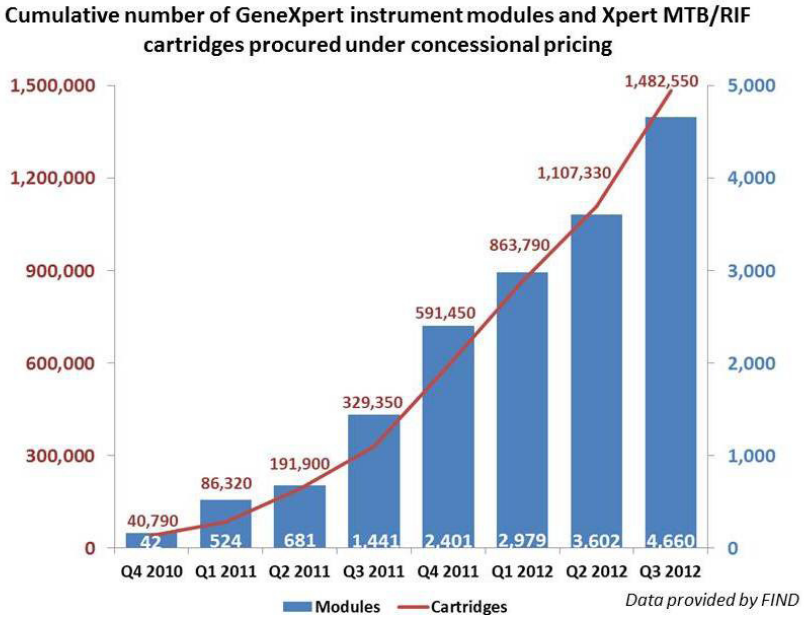
Abbreviations: **DST** Drug susceptibility test; **NAAT** Nucleic acid amplification test; **LTBI** Latent TB infection; **Ag** Antigen; **Ab** Antibody; **MODS** Microscopic observation drug-susceptibility; **NRA** Nitrate reductase assay; **CRI** Colorimetric redox indicator assay; **LED** Light-emitting diode; **LPA** Line probe assay; **VOC** Volatile organic compound.

Source: WHO Global Tuberculosis Report, 2012[1]

The Xpert® MTB/RIF technology (Cepheid Inc., Sunnyvale, CA; http://www.cepheid.com) was endorsed by WHO in 2010.[2] In 2011, WHO published rapid implementation guidelines on this technology to address operational and technical considerations. Several published studies have confirmed the high accuracy of this test and its superior performance vis-à-vis the conventional sputum smear microscopy (reviewed elsewhere[3-5]). A recent publication by Weyer and colleagues describes the policy process that led to WHO endorsement of Xpert® MTB/RIF, reviews findings from subsequent research studies, and provides a comprehensive overview of operational issues surrounding scale-up of this test.[6]

According to WHO, as of 30 September 2012, a total of 898 GeneXpert instruments and 1,482,550 Xpert® MTB/RIF cartridges have been procured worldwide in the public sector in 73 of the 145 countries eligible for concessional pricing (Figure 2).[7] Over half of all the cartridges have been procured for use in South Africa alone. Updated quarterly sales figures are publicly available via the WHO website for monitoring the roll-out of Xpert® MTB/RIF.[7]

Figure 2. GeneXpert instruments and Xpert® MTB/RIF cartridge sales



Note: data as of 30 September, 2012

Source: WHO & FIND, Geneva[7]

In June 2012, UNITAID, the Bill and Melinda Gates Foundation, 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 $9.98 per cartridge (from the original price of $16.86). This purchase price is applicable to over 145 purchasers in low- and middle-income countries. As part of the agreement, UNITAID is also supporting scale-up through an accelerated roll-out of the test in high-burden countries via the WHO Stop TB Department and the Stop TB Partnership Secretariat. The three-year project will support the implementation of over 200 GeneXpert instruments and 1.4 million Xpert cartridges in 21 countries, starting in 2013.

In November 2012, the Stop TB Partnership announced US $27 million in new funding for partners implementing innovative projects aimed at improving TB case finding, via the TB REACH initiative. This project will support a third wave of 37 new projects and the continuation of 13 projects from the previous wave of funding. TB REACH will supply more than half a million test Xpert® MTB/RIF cartridges in this round of funding.

In December 2012, PEPFAR announced an additional $11 million to provide up to 150 GeneXpert instruments and 450,000 test cartridges in 14 high-burden countries across sub-Saharan Africa and in Burma. This program will be implemented by USAID and US Centers for Disease Control and Prevention (CDC), and accelerate access to Xpert® MTB/RIF in countries with a high prevalence of TB/HIV co-infection.

Given these developments, the TB diagnostics landscape is highly dynamic. Box 1 summarizes the key events and initiatives that will shape the landscape in 2013.

Box 1. Initiatives that will shape the molecular TB diagnostics landscape in 2013

- Expansion of the ongoing scale-up of Xpert® MTB/RIF by the South African National TB Control Programme.
- Buy-down of the Xpert® MTB/RIF price to $9.98 per cartridge by UNITAID, BMGF, USAID and PEPFAR.
- Implementation of over 200 GeneXpert instruments and 1.4 million Xpert® MTB/RIF cartridges in 21 countries via the TBXpert project (2013-2015).
- TB REACH grants to improve case finding in high burden settings, including social enterprise models for Xpert® MTB/RIF use by the private sector in Pakistan, Bangladesh and Indonesia.
- PEPFAR funded implementation of 150 GeneXpert instruments and 450,000 test cartridges in 14 high-burden countries across sub-Saharan Africa and in Burma.
- Policy and roll-out decisions on Xpert® MTB/RIF by countries that are currently completing feasibility and operational studies (e.g. Brazil, Indonesia and India).
- Emergence of fast-follower NAATs, with likely uptake in the private sector in emerging economies.

Update on WHO review of newer NAAT technologies

The previous *Tuberculosis Diagnostic Technology Landscape* (July, 2012) highlighted a variety of emerging NAATs for the diagnosis of pulmonary MTB and the rapid identification of drug resistance.[8] Since then, the performance of two commercial NAATs (by Eiken Chemical Co. Ltd., [Japan] and Hain Lifescience [Germany]) has been reviewed by WHO Expert Groups and key findings from the Expert Group recommendations were included in the 2012 WHO Global Tuberculosis Report.[1]

The Eiken NAAT, the Loopamp™ MTB complex (MTBC) Detection Kit, is a manual assay using the loop-mediated amplification (LAMP)[9] platform to detect TB DNA in sputum specimens.[10] The WHO Expert Group reviewing the Loopamp assay concluded that there was insufficient evidence to proceed with the development of policy guidance.[1]

The Hain Lifescience NAAT, the Genotype MTBDR*sl* (*Mycobacterium tuberculosis* drug resistance second line), is designed for the rapid molecular detection of the most common alleles associated with resistance to second line drugs.[11] The WHO Expert Group found that while the Genotype MTBDR*sl* test's specificity for detecting resistance to fluoroquinolones and second-line injectables was high, its sensitivity was suboptimal.[1] Therefore, while the test has the potential to be used as a rule-in test for extensively drug resistant (XDR) TB (where capacity to use line probe assays is available), the WHO Expert Group concluded that it cannot be used as a replacement test for conventional phenotypic drug susceptibility testing (DST).[1] The Expert Group also noted that there is incomplete cross-resistance between the second-line injectable drugs, and that the assay does not allow for specific resistance to individual second-line injectable drugs to be determined. Detailed conclusions from both WHO Expert Group meetings are expected to be published soon.

Technology review of fast-follower NAAT technologies

Recently a suite of new fast-follower technologies has emerged for the NAAT-based diagnosis of MTB. All include specimen preparation, DNA amplification, and product detection in real time or by endpoint analysis. The key characteristics of these technologies in terms of technology, consumables user steps, performance, and other important characteristics are shown in Tables 1 and 2 (presented later in this section). As Cepheid's GeneXpert technology is the only stand-alone NAAT platform currently recommended by the WHO for TB diagnosis, this technology is described first and other fast-follower developers are listed in alphabetical order thereafter.

Cepheid Xpert® MTB/RIF Assay

The underlying technology and performance of the Cepheid Xpert® MTB/RIF assay have been previously described in detail.[12-15] The development work was a collaborative effort between Cepheid Inc. (California, USA), University of Medicine and Dentistry of New Jersey (New Jersey, USA) and the Foundation for Innovative New Diagnostics (FIND, Geneva, Switzerland). Briefly, the Xpert® MTB/RIF assay is a test that uses a self-contained, fully integrated and automated platform with its operation requiring minimal technical expertise. The test processes allow sample processing, real time polymerase chain reaction (PCR) analysis, and result determination in less than 2 hours. User input is limited and requires only that the user first liquefies and inactivates the sputum sample (with a buffer provided, a 15 minute process) and the transfer of 2 mL of this to the test cartridge which is then sealed and inserted into the test device for processing (Figure 3).

Figure 3. The Xpert® MTB/RIF test cartridge and the GeneXpert® IV instrument by Cepheid

Source: reproduced with permission from M. Pai

The GeneXpert® platform is modular with a variety of capacities in 1, 4, 8, 16 and 48 test modules, each of which are random access. The cartridge uses a mesofluidic design to transport liquid material through a variety of processes to generate a test result. The MTB cells are first captured on a filter, then washed and lysed to release the genomic DNA. The lysed solution is transferred to reconstitute the lyophilized PCR reagents whereby TB DNA is amplified by nested real-time PCR for optimal synthesis of the target amplicon. The assay specifically targets a 192-base pair (bp) region of *rpoB*, which is highly conserved for MTB.[16] MTB amplicon detection is achieved via a set of five differently-labeled molecular beacon probes which each hybridize to discrete target regions and together tile an 81-bp region within the amplified DNA.[15] The simultaneous detection of at least two fluorescent signals derived from the beacons indicates an MTB positive result. In addition the Xpert® MTB/RIF assay includes an internal control where *bacillus globii* spores are processed together with the sample confirming the functionality of all processes in the test cartridge and the GeneXpert® instrument respectively.

In addition to MTB diagnosis, the development of the *rpoB* assay target was driven by the understanding that many of the mutations that confer rifampicin resistance are located within the 81-bp region.[16,17] Judicious selection and design of the 5 molecular beacon sequences allows detection in real time of the most prevalent mutations associated with rifampicin resistance. A rifampicin resistance result is indicated by any molecular beacons which take a markedly longer time to produce a fluorescent signal (≥3.5 cycle thresholds) or is not detected when compared to others during the amplification of a positive sample. While the Xpert® MTB/RIF assay can only infer rifampicin resistance, mono-resistance to rifampicin is rare and >90% of isolates also exhibit resistance to isoniazid. Therefore, the detection of rifampicin resistance can be used as a surrogate indicator for multidrug-resistant TB (MDR-TB). This feature has great utility in regions with high burdens of MDR-TB to more rapidly identify cases of MDR-TB.

The basic performance and operational characteristics of this technology are compared with the fast followers in Tables 1 and 2.

Table 1. Comparison of primary components and test characteristics associated with specimen processing/DNA extraction protocols and equipment required for NAAT-based diagnostic platforms

	Cepheid	Eiken	Epistem	Molbio	Ustar
Assay	Xpert® MTB/RIF	Loopamp™ PURE DNA	Genedrive®	Trueprep™ MAG	EasyNAT™
Specimen type	Liquefied sputum	Raw or sediment from liquefied sputum	Liquefied sputum	Liquefied sputum	Liquefied sputum
Automated	Yes	No	No	Semi	No
Sample volume	2 mLs	60 µL	N/A	1 mL	1 mL
Integrated sample preparation	Yes	No	No	No	No
Supplementary kits required	No	No	N/A	Yes (2)	No
Steps*	2	14	N/A	8	7
Dedicated Accessories	No	Yes	N/A	Yes	Yes
Proprietary equipment	Yes	Yes†	No	Yes	No
Pipettors	No	Yes‡	Yes	Yes‡	Yes
Generic equipment	No	Yes†	N/A	No	Yes
>2 Consumables¥	No	Yes	Yes	Yes	Yes
Processing Time	~45 minutes	~15 minutes**	~10 minutes	~25 minutes	~60 minutes**

* Number of steps taken from the instructions for use with each test.

† The Loopamp PURE DNA kit is designed for use with either dedicated hardware from Eiken or a generic heating device.

‡ Dedicated fixed volume pipettors are offered with these kits but are not critical.

¥ This includes pipettor tips and microcentrifuge tubes.

** Sample preparation times derived from the instructions for use with each test.

Eiken Loopamp™ MTBC Detection Kit

The Eiken Loopamp™ MTBC Detection Kit was co-developed by Eiken Chemical Co., Tokyo, Japan (http://loopamp.eiken.co.jp/) and the FIND, Geneva, Switzerland using LAMP, Eiken's proprietary amplification method, to amplify MTB DNA. The test targets *gyrB* and *IS*6110. This product is now commercially available, though not WHO endorsed, and requires the combined use of an extraction kit (Loopamp™ PURE DNA Extraction Kit) and test reagents (Loopamp™ MTBC Detection Kit), plus ancillary equipment. The specimen type is either raw sputum or NALC-NaOH-treated sputum sediment. Both kits are designed for storage at 2 – 30 °C.

For the extraction of MTB DNA from sputum, a relatively simple sample preparation process using three sequentially interlocking tubes has been developed. This reduces the need for consumables and opportunities for contamination of specimens (Figure 4A). Specimens can be processed either singly or as a batch. The Loopamp™ PURE DNA Extraction Kit requires a heater to incubate the lysis reaction. An aliquot of specimen is added to the heating cap and subsequently lysed via exposure to NaOH and heating at 90 °C for 5 minutes. After brief cooling, the lysis cap is manually interlocked with an adsorbent tube and the contents of each component mixed by shaking (Figure 4A). This step removes confounding substances from the lysed sample in order to improve amplification. The final step of this process is the addition of the injection cap to the base of the adsorbent tube. This permits the controlled release of lysed material from the adsorbent tube directly into the reaction tube (Figures 4A and 4B).

Figure 4A. Steps in Loopamp™ PURE DNA extraction method and subsequent staged processes to extract DNA from sputum sample prior to LAMP detection for MTB.

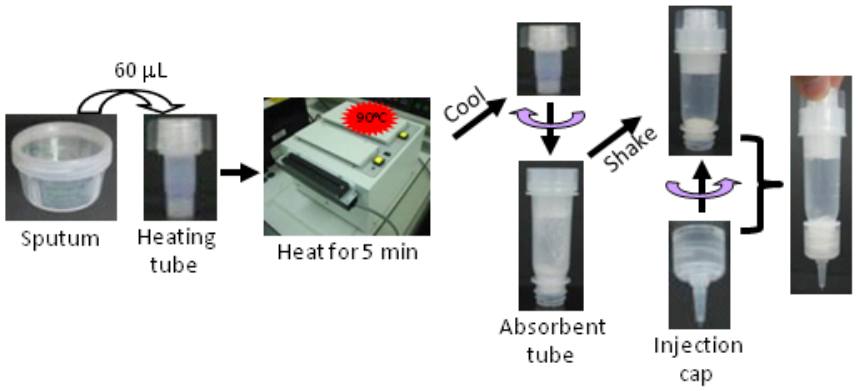
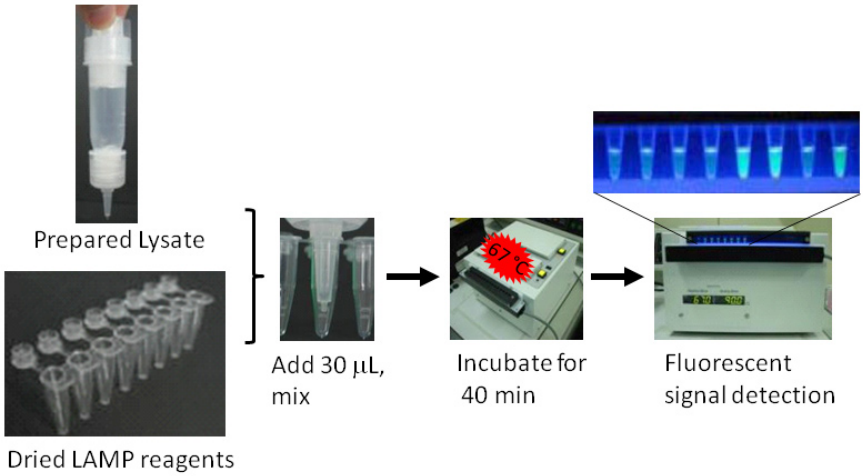


Figure 4B. Setup of LAMP TB assays with subsequent incubation and determination of test result via fluorescence.



Source: reproduced with the permission of FIND and Eiken Chemical Co.

For the MTB DNA amplification reaction, the Loopamp™ assay has added utility in that it can use either a dedicated platform made by Eiken (the LF-160 or LA-500) or generic heating equipment. The test reaction tube is marked with two lines to indicate an acceptable fill volume, which informs the user to apply the correct volume of lysed material (~30 µL). No other liquids are added. The Loopamp™ MTBC reagents are supplied as lyophilized pellets in the cap of each reaction tube which are then immediately sealed after the tube is filled. The tubes are then fully mixed by inverting several times to reconstitute the LAMP reagents in the sample.

The reactions are incubated at 67 °C for 40 minutes, followed by heat inactivation of the enzyme. The reactions are analyzed in real time via turbidity or at end point using fluorescence. The biosynthesis of DNA releases pyrophosphate that reacts with magnesium ions in the reaction mixture to create magnesium pyrophosphate, an insoluble white salt that can be detected spectrophotometrically or visually.[18] The Loopamp Realtime Turbidimeter (LA-500), manufactured by Eiken, can incubate and read four reaction strips (32 reactions) independently. Operation requires mains electricity and a computer.

For fluorescent detection, the Loopamp assay incorporates a dye molecule, Calcein, which fluoresces under UV light if its quencher (manganese) is displaced by pyrophosphate produced during DNA amplification.[19] One protocol uses the LF-160 reactor (Eiken) to incubate the reactions. After incubation, the test result of each sample is determined via the Fluorescence Visual Check Unit where the sample tubes are irradiated by UV (Figure 4B). The user must wear eye protection to do this. If a generic heater is used, the samples are visually inspected after incubation using a handheld UV lamp. The result is based on green light emitted from the positive control

tube and no fluorescence in the negative (Figure 4B). After these have been confirmed as correct then the test samples are scored as positive (green light emitted) or negative (colorless). Although an initial report on performance was reviewed by the Expert Review Group for the WHO, the only published performance data currently available on the Eiken Loopamp is by Mitarai et al.[10] (Table 2).

Epistem Genedrive™ Mycobacterium iD® Test-kit

Epistem Ltd. UK (http://www.epistem.co.uk/) has developed the Genedrive® real-time PCR instrument (Figure 5) for use in low-resource settings to detect a variety of infectious diseases including MTB. The Genedrive™ is a lightweight, portable bench-top real-time PCR instrument capable of single-test processing with sample preparation, assay incubation, and test result in under 45 minutes. The Genedrive™ can be powered via mains electricity or via an internal rechargeable battery. Epistem has developed the Mycobacterium tuberculosis iD® Test-kit, an assay enabling MTBC detection and rifampicin resistance screening using the Genedrive® instrument. Genedrive™ technology uses innovative solutions to extract DNA, resulting in an integrated, real-time PCR system with low power requirements for precise thermal cycling and simplified precision optics included in a disposable cassette. The performance and precise technical details of this technology and the Mycobacterium iD® Test-kit have not yet been fully described in a peer-reviewed journal, but based on details from the manufacturer it is described as a fully-integrated, cartridge-based, and easy-to-use NAAT system for MTB and rifampicin resistance diagnosis from either sputum or urine. No information is available on the thermal stability of its reagents, but it should be noted that test reagents are lyophilized presumably for long-term storage at ambient temperature.

Figure 5. Genedrive™ instrument and test cassette

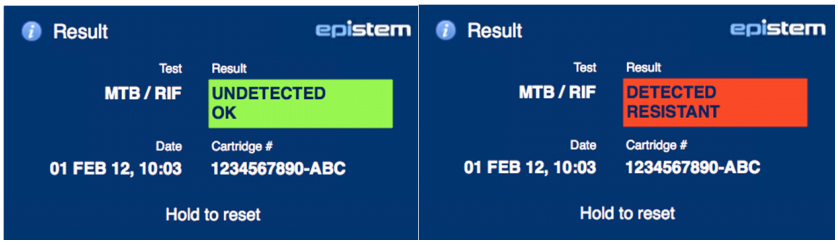
Note: The test cassette (containing lyophilized PCR reagents in each tube) is loaded with sample extracts and inserted into the Genedrive™ (porting visible at the front of the Genedrive™) prior to amplification.

Source: reproduced with the permission of Epistem, UK.

The precise details of all necessary components to perform MTB testing have not been released. Processing from sputum includes liquefaction, lysis, and release of DNA. The assay uses a novel paper-based filtration device to remove or dilute inhibitory compounds prior to PCR. Epistem state that this process takes only 10 minutes. The test cassette contains three reaction tubes made of high-quality optical material in which the test reactions are performed. These are used for MTB detection, rifampicin resistance screening, and an internal process control (IPC), respectively. Data entry is via touch screen.

The lyophilized reagents in each test bed are rehydrated by the addition of 20 µL of DNA extract to each. Innovative design of the thermocycling component permits a test time of 30 minutes. Test results are monitored in real time and post-amplification melt curve analysis of each reaction permits the confirmation of the MTB test and the IPC results, in addition to the identification of mutant alleles in *rpoB* that can indicate rifampicin resistant MTB. The test data are interpreted by software and the test result is displayed on the Genedrive screen (Figure 6). Preliminary and unpublished performance data provided by the manufacturer claims a limit of detection of 30 colony-forming units/mL using spiked sputum, but no peer-reviewed performance data are available to date. The key performance characteristics of this technology in comparison to the GeneXpert® and the other fast-follower technologies described in this report are listed in Table 2.

Figure 6. Genedrive™ screen images of test negative and positive results displayed after MTB analysis.



Note: ease of result interpretation by the user is enhanced by colorimetric differences between a negative (green) and positive test result (red). In addition to MTB identification, rifampicin resistance is also indicated.

Source: reproduced with the permission of Epistem, UK.

Molbio Diagnostics Truelab™ TB Assay

Molbio Diagnostics Private Ltd. (http://molbiodiagnostics.com) is a joint venture between Bigtec Labs (Bangalore, India), and the Tulip Group (also Bangalore, India), a leading manufacturer of rapid diagnostic tests. The Molbio technology utilizes PCR amplification with fluorescence detection in real time. The core amplification technology utilizes a novel test chip that acts as the amplification platform to amplify the target DNA. A host instrument operated using an embedded Android cell phone controls the necessary operating software to perform and determine the test result with the added benefits of data storage, wireless transmission of test data, communications, and GPS capability. All reagents and instruments are designed to be stable at 2-30 °C in 10-80 % humidity and all hardware can operate from battery or mains power.

Figure 7. Materials and equipment required to prepare DNA for the Molbio TB assay.

Note: From left to right: All core reagents for liquefaction and DNA purification from sputum are supplied in the sputum kit. The Accessories kit with ancillary tubes for specimen collection, processing and collection of the DNA eluate. The fixed-volume pipettors for the transfer of liquid materials and reagents during sample preparation. The Trueprep MAG instrument with liquid crystal display (LCD) screen user interface, and extraction tube holder on top of the device to facilitate sample processing.

Source: reproduced with permission of Molbio Diagnostics Pvt. Ltd. India

The Truelab™ micro PCR system for the diagnosis of MTB is a semi-automated process requiring dedicated instruments, the Trueprep™ MAG sample preparation device and the Truelab™ Uno Real Time micro PCR Analyzer (Figure 7). The key materials for sample preparation are shown in Figure 7. The primary characteristics of sample preparation are listed in Table 1.

For the extraction of MTB DNA, several proprietary components are essential, including the sputum kit, accessories pack, and Trueprep™ MAG sample prep device (Figure 7). Specimens are processed individually. The DNA extraction process uses solid phase extraction with silica coated magnetic beads to purify the MTB DNA. Sputum is liquefied and a 1 mL aliquot is used for processing. The sample is mixed with lysis buffer in the extraction tube, which is then inserted into the MAG device (Figure 7). Cell lysis is achieved via chaotropic salts and heating for 5 minutes at 85°C and the sputum processing regimen is selected from a menu of test protocols by the user. The Trueprep sample preparation component is semi-automated, with the user sequentially adding or aspirating liquids as described in the kit procedure. While processing a sample, an audible alarm from the Trueprep™ MAG device alerts the user for each stage requiring user input. The DNA is eluted in a final volume of 100 µL, manually transferred to an elution tube prior to storage and/or subsequent analysis via real time PCR in the Real Time micro PCR Analyzer.

The subsequent analysis for MTB is via real time PCR in a novel chip-based assay, the Truenat™ MTB system (Figure 8). The individual chips are automatically processed in the Truelab™ Uno Real Time micro PCR Analyzer (Figure 8). The MTB assay target is *IS*6110. The micro PCR chips use a 6 µL volume of the DNA eluate, which is pipetted into the reaction chamber. Key design features of the device include the use of a chip-based ceramic temperature block for thermal cycling rather than a dedicated heating block, the prevention of reuse, and reaction evaporation during thermal cycling.

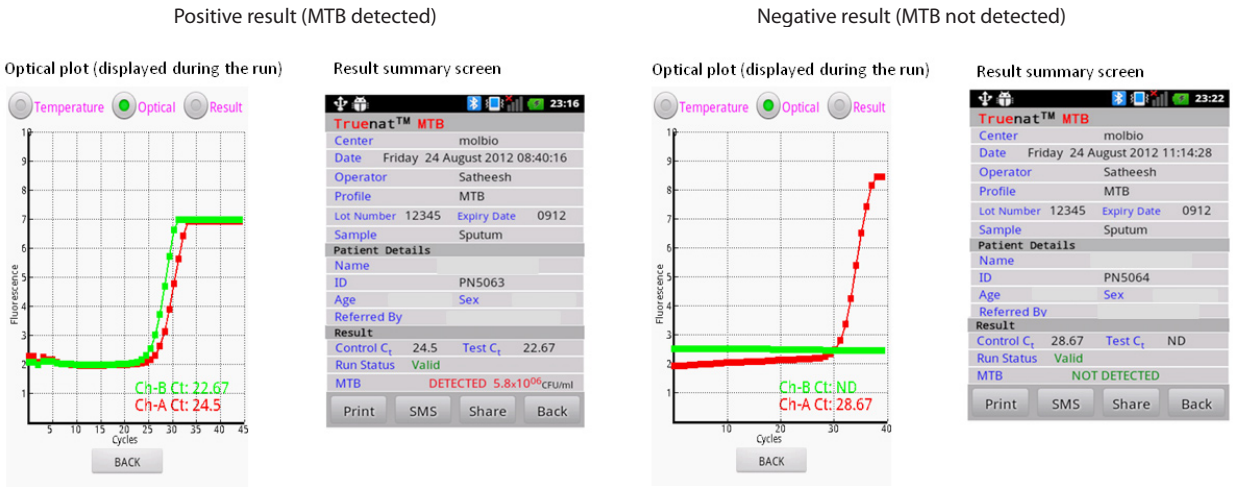
Figure 8. Truenat™ micro PCR chip (left) and Truelab™ Uno Real Time micro PCR Analyzer (right)

Note: All key PCR reagents are contained in the square white reaction well on the chip. The micro PCR chip is placed in the chip tray of the Truelab™ Uno PCR analyzer (right). Eluted DNA is added via pipettor into the reaction well and chip tray is inserted into the device for analysis via real time PCR.

Source: reproduced with permission from Molbio Diagnostics Pvt. Ltd. India

The Truelab™ Uno micro PCR Analyzer simultaneously reads fluorescence via two wavelengths in real time to detect MTB DNA amplification in one wavelength and the IPC in the other. The IPC qualifies the DNA extraction process and the performance of the PCR reagents and equipment. The time to final result is 35 minutes. The software controlling the operation of the chip uses Android-based software and the user interface for operation and data input is via the Android unit touch screen (Figure 9). The test data are displayed during PCR and results are generated after completion of PCR (Figure 9).

Figure 9. Screenshots of test result formats as displayed on Truelab™ Uno Real Time micro PCR Analyzer



Source: reproduced with permission from Molbio Diagnostics Pvt. Ltd. India

The use of an Android-based device in the platform gives added utility in terms of wireless operation and communication with the ability to locate via global positioning system (GPS). The Wi-Fi application allows the importing of data and other communication. The device can store up to 1,000 test results and a report can be

produced via a wireless thermal printer. The manufacturers claim that the time taken for a single test is one hour (from the specimen extraction to a PCR result). Further data relating to the current status of the Molbio technology in terms of field evaluations, regulatory certification, and initial markets for product release is listed in Table 2. Currently there are no peer-reviewed data describing the performance of the Molbio assay.

Ustar Biotechnologies EasyNAT™ TB Isothermal Amplification Diagnostic Kit

Ustar Biotechnologies, Hangzhou, China (http://www.bioustar.com) have developed the EasyNAT™ TB assay, a manually operated kit for the isothermal detection of MTB DNA via amplification using cross priming amplification (CPA).[20,21] The assay is designed for manual processing of individual or batched tests, and includes materials for sample preparation (box 2, Figure 10), DNA amplification (box 2), and result detection (box 1, Figure 10). Box 1 may be stored at 2-30 °C, while the recommended storage temperature for Box 2 is -20 °C. The reagents in Box 2 are stable at ambient temperatures under 30 °C for 2 weeks but extended storage is recommended at -20 °C. Specimens recommended for use with the EasyNAT™ TB assay include sputum, cerebrospinal, pleural, peritoneal, pericardial, ascites, and synovial fluid. This product is now commercially available, though not WHO endorsed.

Figure 10. Ustar EasyNAT TB kit.

Note: Larger box contains CPA amplicon detection cassettes. Examples of the amplification cassettes shown opened (lower left) and packaged (center left). Smaller box contains DNA extraction buffer, other buffers and the MTB CPA reagents packaged in foil pouches (center right).
Source: reproduced with permission from Ustar Biotechnologies, Hangzhou, China

The test uses generic equipment and several key consumables to perform sample preparation, sample amplification and amplicon detection (Table 1). Test equipment includes a heat block, vortexer, and micro centrifuge. Disposable consumables are also required. Specimens can be individually or batch processed with this method. The DNA preparation procedure processes 1 mL of liquefied sputum with MTB cells pelleted via centrifugation with subsequent washing steps. MTB cells are then lysed in a buffer with heating at 95 °C, after which the sample is cooled and centrifuged. The supernate is used for subsequent testing.

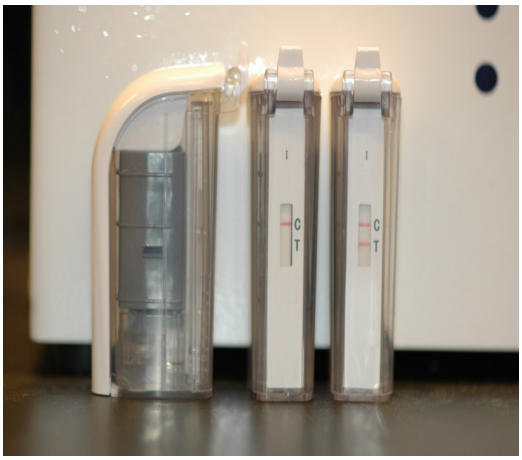
The MTB CPA reaction is prepared by resuspension of the glassified reagent pellet with rehydration buffer followed by addition of 4 µL of the prepared DNA extract. Prior to incubation, 20 µL of sterile mineral oil is layered on top of each reaction mixture to prevent evaporation during incubation. Reactions are incubated for 1 hour at 65 °C. If MTB DNA is present, hapten labeled CPA amplicons are produced.[21]

To identify the test result after incubation, Ustar have developed and patented an enclosed LFS-based detection tool (Figures 10 and 11). This is a rapid and simple mode of detection with no dedicated equipment required. The unopened reaction tube is placed into a cassette which is inserted into a housing whose lid is then closed. Upon closure, a flow buffer and the reaction tube contents are simultaneously released to allow both to mix and flow across the LFS. The test cassette has two test stripes to indicate the test result and the IPC (Figure 11).

The assay utilizes an IPC that is present in the rehydration buffer and assures the performance of the CPA reagents and the operation of the detection cassette. The test results are read in under 30 minutes after cassette activation. In a TB negative reaction, only the control stripe (C) will appear. If both the test stripe (T) and the C stripe develop, the specimen is positive for MTB (Figure 11). If the strip remains blank, the test is invalid and must be repeated. The current use of rapid diagnostic tests (RDTs) in many developing countries for diseases such as HIV and malaria may be an advantage in training users as they are familiar with the interpretation of these tests.

Key details describing the EasyNAT™ TB assay in relation to the other fast followers described in this report are listed in Tables 1 and 2. Currently, there are limited clinical data on test accuracy (Table 2).[20]

Figure 11. Ustar encased CPA amplicon detection cassette.



Note: Side view of an activated cassette shown at left. Unopened reaction tube is placed into a grey cartridge that is placed into the cassette and forced down into the cassette upon closure of white handle. Negative test (center) is depicted by formation of visible single red band at control (C) stripe. Positive reaction (right) depicted by visible control stripe and appearance of second lower band (T). This is CPA amplicon derived from MTB DNA and indicates a MTB-positive test.

Source: reproduced with permission.

Table 2. Comparison of key test characteristics, performance, and projected costs of the GeneXpert® MTB/RIF diagnostic platform and emerging fast-following technologies

	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, immunochromatographic 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: $60, discount cost: <$10	DNA extraction kits, PCR chip. Cost: $10-12 ‡	MTB iD® Test-kit: paper based sample preparation. Test cartridge cost: $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: $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 Instrumentation /cost	GeneXpert/ $17,500 (4 module)	Truelab™ Mag and UNO (two instruments)/ < $ 6,000 ¶	Genedrive®/ < $4,000	LF-160 or LA-500/ price NA	None
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[f]	Low	Low-Medium	Low-Medium	Medium	Medium
Time to result	<2hrs	<1 hr	<45 minutes	<1 hr	<2 hrs

Throughput	16-20 tests per 8hr work shift for 4-module instrument	12 tests per 8hr 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+	No published data	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-	No published data	No published data	96.2% SSM-/C-	98.8% SSM-/C-
Intended entry market	Global	India ##	India&	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.
Truelab 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.[10,20]
¥ 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.[3]
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.
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.
Source: adapted from Neimz and Boyle[14], reproduced with permission from Expert Reviews Ltd, UK

It is important to note that data on Xpert MTB/RIF in the above table are derived from a large number of published studies, while very limited or no published evidence is available for the fast-follower technologies reviewed. Also, data provided reflect initial or early versions of the fast-follower technologies. Ongoing improvements made by the test developers (e.g. automated sample preparation) will be reviewed in future editions of the UNITAID landscape reports.

Conclusions on technology considerations for fast-follower NAATs

The emergence of the fast-follower NAATs is a welcome development that can enhance the affordability of NAATs in high burden settings and allow for more decentralized deployment. There are currently very limited data on performance of all fast followers, a prerequisite to better understand the potential application of these tools for TB diagnosis in low-resource settings. However, peer-reviewed publications are expected within the next 12 months. All of the technologies have varying requirements for the significant user input they require as compared to the Xpert MTB/RIF assay, and so additional training will be necessary if these tools are to be implemented, especially in peripheral microscopy centers. The manual tests developed by Eiken and Ustar offer higher rates of throughput, a benefit in areas with very high demand for testing. However, both require greater user input and extended sample preparation, which introduce a risk of contamination. To mitigate the risk of amplicon contamination, both have included sealed endpoint detection methods.

With the current global impetus to move highly sensitive NAATs to decentralized settings, it is imperative that their performance is not compromised by manufacturing, transport, storage, the environment, or the user. If test equipment is portable then quality assurance (QA) of performance must be conducted before testing begins to demonstrate appropriate functionality. Furthermore, the performance of minimally-supervised users must be monitored via routine proficiency testing. With manual assays designed for batched sample processing, adequate training and subsequent assessment of skills is critical for the technology to be effective. While all of the technologies include QC components to validate test data, there is a large gap in terms of ensuring adequate performance of equipment and users via uniform standards. Currently, there is no standardized external quality assurance (EQA) devoted to the Xpert® MTB/RIF or the four fast-follower NAATs described in Table 1.

To support EQA and the subsequent proficiency testing necessary to ensure appropriate use, a variety of EQA panels have been developed, including those by Global Laboratory Initiative (GLI, WHO), National Health Laboratory Service (NHLS, South Africa), Vircell (Spain), CDC (USA) and Maine Molecular Quality Controls Inc. (USA). The GLI TB EQA panel is currently being used for EQA of the Xpert® MTB/RIF but a longer-term solution is required as GLI may be unable to continue this indefinitely. The CDC panel is currently used with the PEPFAR-funded roll out of the Xpert® MTB/RIF. The NHLS have determined that EQA panel for Xpert® MTB/RIF assay will require the following elements: (i) testing material must contain whole *M. tuberculosis*; (ii) transportation of EQA material must be safe; (iii) testing procedures must be compatible with the current Xpert® MTB/RIF testing protocol; (iv) health care workers who do not have laboratory skills must be able to perform the EQA testing in non-laboratory settings; and (v) the EQA program must be cost-effective and sustainable. Such a program – using whole, inactivated *M. tuberculosis* spotted onto filter paper – has been developed and piloted in South Africa as part of the NHLS rollout of Xpert® MTB/RIF to ensure controlled assessment of equipment performance prior to starting specimen testing.[22] The same group (in collaboration with the CDC, FIND, and GLI) has recently performed a multi-center, observational study to evaluate the five available EQA panels with the Xpert® MTB/RIF. Published data are currently not available but are expected soon.

Based on information supplied by the manufacturers, the Epistem and Molbio technologies have sample preparation methods that – unlike the Xpert® MTB/RIF assay – involve varying degrees of manual user input, which add cost and create a risk of inadequate sample preparation or test contamination. However, unlike the Xpert® MTB/RIF assay, their smaller footprint, independence from mains power, and dedicated computer may permit their roll-out into smaller, less-controlled environments, including rural microscopy laboratories. All tools use dedicated equipment to differing degrees, but a primary advantage of the Epistem and Molbio tools is that user interpretation of the test result is performed *in silico* and so user misinterpretation of test results is avoided.

The Loopamp (Eiken) assay has made inroads developing a simple tool for sample preparation with an easy-to-interpret endpoint analysis method via potentially basic instrumentation. The Genedrive (Epistem) assay is the only fast follower that can indicate rifampicin resistance in addition to MTB diagnosis in the presence of an IPC to confirm test results. The Android-based Molbio system is particularly interesting in that it addresses the need for the transmission of results to the provider, patient and TB control program without internet connectivity, a key factor for monitoring test data from remote sites in relative real time. Both devices are battery powered. Ustar have also created a tool that is inherently simple when compared to conventional NAAT diagnostic methods and have addressed end-point detection without compromising test site contamination using a test format currently familiar to many health care workers in low-resource settings.

In determining the appropriate use of these technologies, there are therefore a variety of outstanding clarifications to be addressed in terms of intended use, the need for dedicated hardware and consumables, ease of use, potential supply chain issues, associated costs (training and proficiency testing/quality control), the ability of the manufacturers to meet anticipated future production demands, and necessary steps for timely WHO endorsement. To determine the suitability of these technologies for use by end-users, the following questions must be addressed:

a. How will these tests fit with current TB diagnostic algorithms, and can they be successfully implemented in peripheral microscopy laboratories in high burden countries?

b. Will sample preparation/processing methods allow for truly decentralized implementation, at least at the microscopy center level?

c. What is the acceptable trade-off between higher throughput and lower cost on one hand and more manual involvement on the other as compared to partially integrated assays with higher cost per test but reduced needs for user input?

d. How can appropriate quality control procedures for test integrity be developed for and maintained in peripheral facilities with minimal oversight from National Tuberculosis Programs (NTPs)?

e. What is the tolerance of test hardware to excessive heat, humidity, and dust?

f. Will the fast-follower NAATs be more affordable and cost-effective compared to the Xpert® MTB/RIF assay given the recent price reduction of the GeneXpert® technology?

g. Will these NAATs receive sufficient donor or investor support to undergo validation and demonstration studies that are required for WHO review and endorsement?

h. What is the regulatory and policy pathway for these technologies for country-level adoption and scale-up?

Section 2.
Market Analyses, Unmet Needs, and Target Product Profiles

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.[14] In 2012, more than 40 diagnostic companies and test developers are actively engaged in TB.[23] 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.[23]

The following questions are most important to test developers:

- What is the market potential and what are the market 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?

Market analyses are necessary to (i) support new product development by convincing industries and investors that investments in new TB tools are needed, (ii) inform TPPs that can guide product development and scale-up, and (iii) guide donor/funder decisions. 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).[24] Findings from this analysis showed that over US$ 1 billion was spent worldwide on TB diagnostics annually. One-third (US$ 326 million) of this was spent, and 73% of test requests were generated, outside of North America, Europe, Japan, Australia and New Zealand. In the high-income countries noted above, latent TB testing (i.e. tuberculin skin tests [TSTs]) dominated, while active TB testing (sputum smears and chest x-rays) dominated in the rest of the world.[24]

The TB diagnostics landscape has greatly changed since the publication of the FIND/TDR market analysis in 2006.[14,25] Several tests have undergone WHO review and endorsement, and new technologies have emerged – many of which are replacing or supplementing older and less expensive technologies. For example, interferon-gamma release assays (IGRAs) are increasingly used in low-burden countries for latent TB testing, either as replacements for the tuberculin test or as add-on tests.[26] In high-burden countries, tests like liquid cultures, line probe assays and Xpert® MTB/RIF are gaining increasing acceptance and uptake.[1] Furthermore, high-burden countries, especially emerging economies, are showing a substantially increased interest in new technologies for TB.[27] India, China, and South Africa account for more than 40% of the global TB burden and are therefore major markets for TB testing. With rapid economic growth, these countries are also making major investments in TB control.

South Africa has already made major investments in their nation-wide roll-out of the Xpert® technology.[28,29] The incremental capital cost of implementing Xpert® technology in South Africa between 2011 and 2016 has been

estimated to be USD 22 million.[28] The incremental recurrent cost over this time period has been estimated at USD 287 to 316 million, depending on whether scale-up is gradual or accelerated.[28]

A recent market size assessment conducted in India showed that about $222 million is spent on TB diagnosis in India every year, with the private sector accounting for over 60% of this expenditure (Table 3).[30]

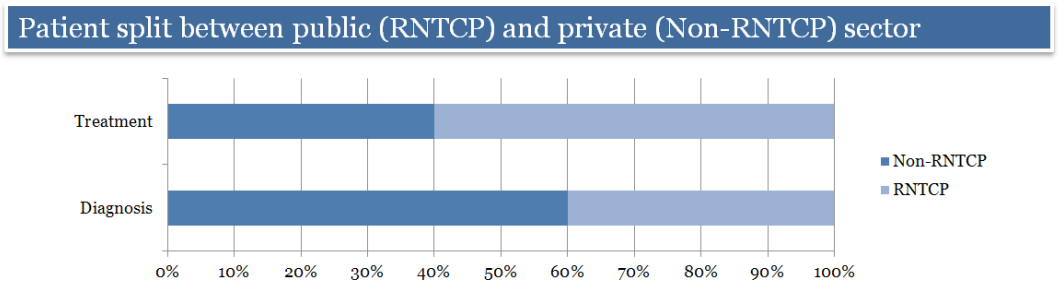
Table 3. Annual volume of TB tests performed in India and their market value[30]

TB testing market segmentation	Volume	Market value ($)
Revised National TB Control Programme (RNTCP) (dominated by direct sputum smears)	15,121,044	38,025,943
Non-RNTCP (dominated by serological tests)	10,988,841	138,570,955
Common tests (X-rays and TSTs)	15,274,290	45,649,624
Total tests	**41,384,175**	**222,246,522**

Note: This analysis was conducted before the Government of India ban on TB serological tests in June 2012.

In India, the private sector accounts for 60% of total TB testing (Figure 12), and there are major differences between the public and private sectors with respect to the tests used. In the public sector (i.e. RNTCP), sputum smears are the most widely used tests, with nearly 7.5 million persons with suspected TB undergoing smear microscopy every year.[31] In contrast, private sector testing is dominated by chest x-rays and serological antibody detection tests.[32]

Figure 12. Indian private sector accounts for large volume of TB diagnostic testing market[30]



Results from this market size analysis in India are being updated to account for changes in the private market based on the Indian Government's June 2012 ban on import, sale, and use of TB serological tests.

In summary, although the exact size of the global TB diagnostics market in 2012 is not precisely quantified, it is very likely to be higher than $1 billion/year because of the abovementioned trends. Efforts are underway to quantify the current TB diagnostics market, accounting for the ongoing roll-out of Xpert® MTB/RIF and other changes, such as the Government of India ban on TB serological tests. This information will be included in future updates of the UNITAID Landscape reports. Existing, publicly available market analyses have been compiled at: http://tbevidence.org/resource-center/market-analyses/

Unmet needs and gaps

While much progress has been made with development and roll-out of new TB diagnostics, many critical gaps and needs remain. Table 4 (adapted from Peter J et al.[5]) summarizes the most important unmet needs.

Table 4. Critical unmet diagnostic needs and gaps in the TB diagnostics pipeline[5]

Unmet diagnostic need or gap in the diagnostic pipeline	Rationale for need and/or research question(s)
Development of a simple, affordable, instrument-free POC test for active TB using sputum samples	• High burden countries, severely limited resources • Poor laboratory infrastructure and technical skills • Patients have difficulty accessing health-services and default prior to diagnosis
Development of rapid, non-sputum based POC test for the diagnosis of extrapulmonary TB (EPTB) and childhood TB	• EPTB and children most often unable to produce sputum • Biological samples such as urine readily available • Certain forms of EPTB (e.g. TB meningitis) carry very high mortality and rapid diagnosis would save lives
Clinical and public health impact evaluations of Xpert MTB/RIF at different health care levels; operational research and cost-effectiveness evaluations of MTB/RIF; optimal positioning of MTB/RIF in diagnostic algorithms	• Rapid WHO endorsement and plan for global implementation • Benefits of a 2-hour test result may be lost if not used at point of treatment and rapidly available to patients • Operational performance and actual cost efficacy unknown
Development of a rapid 'rule-out' or triage test, especially for TB-HIV co-infection in high burden settings	• TB can be atypical clinically in HIV co-infection but progresses rapidly with high mortality rate • High TB drug-related morbidity in HIV-infected patients • Other pathogens can mimic TB presentation and cause mortality if untreated
Development of simple-to-perform, improved rapid molecular DST assays for first- and second-line drug resistance	• Growing epidemic of multidrug resistant (MDR) and XDR TB • All phenotypic DST methods require at least 10-14 days to provide results
Predictive biomarker(s) to identify latently infected people likely to progress to active TB and who will benefit most from preventive therapy	• Interferon-gamma release assays (IGRAs) and TSTs have suboptimal predictive value for progression to active TB • Isoniazid preventative therapy needs to be targeted to those who will benefit most

Detailed TPPs are necessary for all the above product-specific needs in order to guide investments and engage industries and donors in meeting the unmet needs.

Target product profiles for new TB diagnostics

Currently, significant effort and funding is being invested in new TB diagnostics development, especially POC tests and platforms. However, little work has gone into the development of TPPs. Test developers are particularly interested in learning about the most important unmet diagnostic needs and TPPs of greatest relevance to the TB community.[23] In particular, to focus product development efforts, they seek information on 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 and temperature control), time to result, throughput, sputum versus other samples, manual versus automated, requirements for reporting of test results, POC 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 that need to be available

on the same platform, instrument/test connectivity requirements, and importance of subgroups (e.g. HIV/TB co-infection and children).

At present, although there is widespread agreement that a simple POC test is urgently needed, there is lack of consensus on which TPP attributes will have the biggest impact on reducing the incidence of TB in disease-endemic countries.[25,33-35] There is also awareness that a single TPP may not be able to fulfill the needs of patients, providers and lab workers across a multitude of settings and uses (triage, diagnosis, monitoring, and resistance detection).

Biologically, TB is a spectrum, ranging from latent infection to active disease that can manifest in a variety of forms (pulmonary and extrapulmonary), and drug-resistant disease. Even latent TB infection is considered a spectrum, extending from sterilizing immunity to active infection that requires preventive therapy.[36] Furthermore, POC testing can cover a spectrum of technologies[37] (simplest [without instruments] to more sophisticated), patients (children versus adults; pulmonary versus extra-pulmonary; persons with suspected TB versus asymptomatic contacts, HIV co-infection or not), purpose (rule-in versus rule-out tests; triage/referral versus confirmatory diagnosis), users (lay persons; community health workers; nurses; doctors; to highly trained lab professionals), settings (in-home testing; testing in the community; testing in the clinic; to testing in laboratories and hospitals), specimens (sputum versus non-sputum), and approach (decentralized versus centralized testing). This suggests that a variety of TPPs are necessary to meet clinical and practical needs.

Since existing TB tests have well-known limitations, tests to replace them must have the right combination of novel characteristics (e.g. smear replacement, serology replacement). For example, some technologies are more expensive (e.g. Xpert® MTB/RIF) and more affordable molecular tests are deemed necessary, suggesting that new TPPs will include low cost and affordability as a key requirement. Also, in some settings with high rates of drug resistance, the need for universal DST at the time of TB diagnosis will dictate adjusted TPPs.

The impending introduction of new TB drugs will certainly mandate an expanded array of DST capable technologies. In October 2012, a partnership between the FIND and the Global Alliance for TB Development (TB Alliance) was announced. This partnership will seek to introduce new and better drug regimens by developing tests that can detect resistance to fundamental TB drug components.

There are currently two POC TPPs that are publicly available (compiled at http://tbevidence.org/resource-center/target-product-profiles):

- A simple, instrument-free ("dipstick"- or "pregnancy test"-type) test for active TB that can be performed on finger-stick or non-sputum samples at the most remote health care level (e.g. rural health posts or mobile clinics), that can deliver results within minutes, and can be used by community health workers and nurses.[33] [**Appendix Table 1**]
- An affordable, cartridge-based NAAT for active TB that can be performed on sputum samples in microscopy centers or health centers with attached peripheral laboratories, which can deliver results within an hour and can be used by a trained lab technician or health care worker.[38] [**Appendix Table 2**]

Examples of efforts already underway to refine and develop detailed TPPs of greatest relevance and impact use the following approaches[39]:

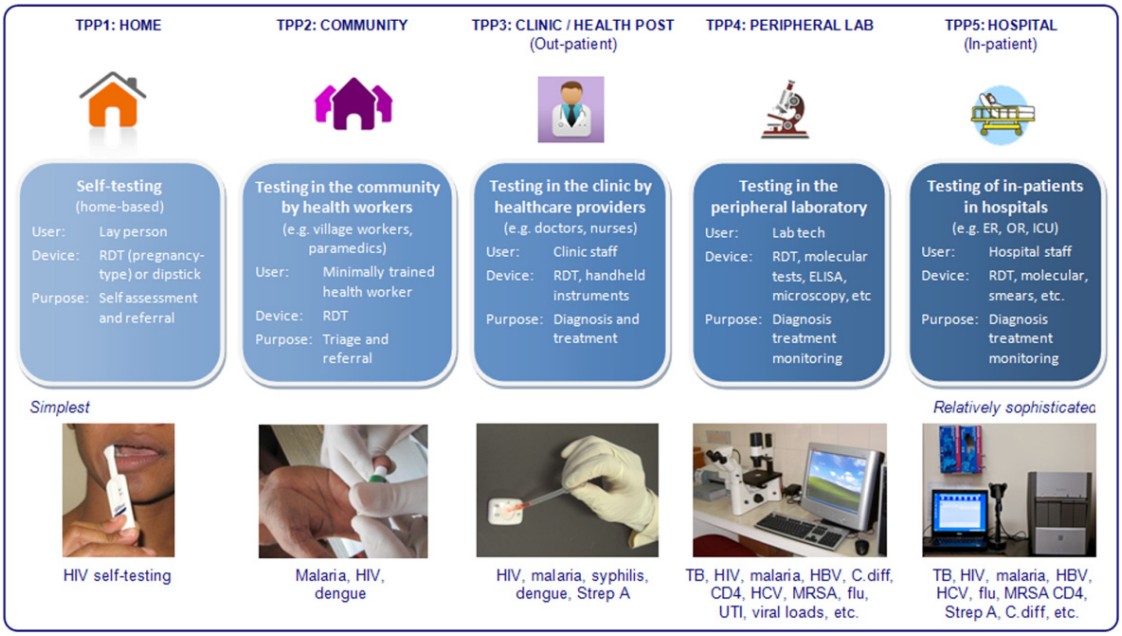
1. Patient, clinical and user assessments to identify tests that meet perceived needs.
2. Replacement of existing tests (e.g. smears, serology, and TSTs) in current diagnostic algorithms.
3. Case studies of successfully scaled-up tests and pragmatic trial results, incorporating their features into new test TPPs.
4. Market analyses to evaluate gaps in the market and potential for new tests to capture unaddressed markets by expanding coverage and use.
5. Mathematical modeling to explore the likely impact of various TPPs on reducing TB incidence.
6. Qualitative and quantitative research to better understand patient health-seeking and provider behaviors in the community and elsewhere to design technologies where early diagnosis is likely to succeed.

7. Operational research to map out where individuals in the population seek health care, where health care services are available, what resources (including lab capacity) exist at each level of health care, what fraction of patients with suspected TB access each level of health care (patient volumes), where TB treatment services are available, and where technology deployment is likely to capture the largest fraction of patients with TB early in the infectious period.
8. Implementation science to understand the most important barriers to POC testing to use such data to design TPPs that can overcome delivery obstacles and health system limitations.

Diversity of POC TPPs and barriers for POC testing in resource-limited settings

It is widely believed that POC tests should be equipment free, simple, inexpensive RDTs (that is, those that meet the "ASSURED" criteria: affordable, sensitive, specific, user friendly, rapid and robust, equipment-free, and delivered) that are always be performed outside of laboratories and hospitals by non-laboratorians. A recently published framework revisited this paradigm and proposed that POC testing should be viewed as a spectrum of technologies (simplest to more sophisticated), users (lay persons to highly trained), and settings (homes, communities, clinics, peripheral laboratories, and hospitals) [Figure 13].[37] A deeper appreciation of this diversity in TPPs, and likely barriers in each setting, might help test developers and public health managers identify the most impactful product and delivery model.

Figure 13. Diversity of target product profiles, users, and settings within the spectrum of POC testing



Source: Pai NP et al. *PLoS Med* 2012[37]

Technology as such does not define a POC test. Rather, it is the successful use at the POC that defines a diagnostic process as POC testing. Thus, the focus must be on POC testing programs, rather than POC technologies. Regardless of which technology is used, where, and by whom, the most critical elements of POC testing are rapid turn-around and communication of results to guide clinical decisions and completion of testing and follow-up action in the same clinical encounter (or at least on the same day).

While many health care systems routinely implement sputum smear microscopy, this test is rarely implemented as a POC program which allows for same-day diagnosis and treatment for TB. For example, the average time from smear-based diagnosis to initiation of TB treatment is 8 days in India, even though microscopy is done in decentralized microscopy centers.[40] Likewise, while the Xpert® MTB/RIF test can be used in clinic settings to allow for same-day treatment decisions[41], health care systems generally have not implemented the test in such

decentralized settings.[42] In fact, the vast majority of published studies on Xpert® MTB/RIF have deployed the technology in reference/centralized laboratories. Thus, if Xpert® MTB/RIF is not deployed in a decentralized way, it will not reach its full potential to reduce TB transmission and incidence.[43]

Systems for rapid reporting of test results to care providers, and a mechanism to link test results to appropriate counseling and treatment, are as important as the technology itself. If systems for reporting the results and follow-up care are not in place, then POC testing is unlikely to have an impact on clinical or public health outcomes. Also, POC testing programs require viable business models to ensure that they are sustainable in the real world and will actually get used. This means POC testing must fit within real-world workflow patterns and economic/incentive structures.

The mere availability of rapid or simple tests does not automatically ensure their adoption or scale-up. A range of barriers prevent the successful use of POC testing, including economic, regulatory, and policy-related barriers, as well as user/provider perceptions and cultural barriers (Table 5).[37]

Table 5. Potential barriers to adoption and scale-up of POC technologies[37]

Barrier for POC testing	Example
Economic	It may be more expensive to place test instruments at the POC, as compared to laboratories. Some POC tests may be priced at a level that is unaffordable in many countries. Private care providers may receive incentives from laboratories for each test that they order; this means they can earn more by sending their patients to labs rather than do any POC testing.
Policy-related	Existing guidelines and policy documents may not provide clear recommendations on how to include POC tests in existing algorithms. Lack of a strong evidence-base for POC tests can result in weak evidence and uncertain policy recommendations.
Regulatory	Poor regulation of diagnostics may result in easy availability of suboptimal and poor-quality rapid tests on the market; this makes it challenging to scale up validated POC tests.
Laboratory capacity	Some POC tests may require peripheral labs with sufficient capacity to run them (e.g., NAATs). Poor laboratory capacity poses a barrier for scale-up of such technologies.
Infrastructure	Clinics and primary care centers often lack infrastructure such as constant power supply, refrigerators, storage space, waste disposal units, phlebotomy supplies, and temperature control; this makes it hard to implement some types of POC tests.
Quality control and quality assurance	Even simple POC tests require quality assurance and training before they can be performed. Primary care providers may not have the expertise or training to do them with quality assurance.
Work-flow balance	Staff shortages and high workload may reduce uptake of POC testing. Health care providers are overburdened with a high volume of patients, and work-flow and time constraints do not permit easy use of POC tests.
Training	Unqualified and informal care providers may lack the knowledge and training needed to implement even simple RDTs. Erroneous results then erode the health system's faith in POC testing. Lack of continuous, ongoing proficiency testing can result in diminishing performance of POC testing programs.
Supply chain	Supply chain deficiencies can lead to suboptimal or poor quality POC tests, which, in turn, may discredit POC testing.
Infection risk	Health providers may be unwilling to do tests that may expose health care workers to the risk of infection.
Administrative/ operational	It is not easy for health providers to seek reimbursement from insurance providers and third-party payers when POC tests are used in community or home settings.

Technical/medical	Doctors and front-line care providers in some settings may prefer clinical diagnosis and empiric treatment over diagnostic certainty. Widespread empiric treatment of common diseases reduces the felt need for any testing, POC or otherwise.
Awareness	Health workers and care providers may not be aware of the various tests that are now available for POC use. Thus, they may still refer their patients to laboratories for testing.
Health system-related	Laboratory professionals in hospitals and larger health care facilities are sometimes opposed to any testing performed outside of lab settings. Some fear this will impact their own business; some also worry about relinquishing control over testing.
Fit with user needs	Available rapid tests are often single-disease focused when primary care providers are more worried about syndromes of unknown etiology (e.g., febrile illness, chronic cough). So, available tests may not always meet user needs.
Cultural/societal	Perceived lack of confidentiality and stigma may reduce acceptance of POC testing in the community (e.g., HIV rapid tests).

As Xpert® MTB/RIF and fast-follower NAATs are increasingly implemented, it is important for TB control programs to maximize the impact of POC technologies while ensuring that the deployment strategy addresses diagnostic and treatment delays (Box 2). Implementation research and operational modeling may help identify the most efficient and optimal placement strategy for new POC technologies.[41,44-46]

Box 2. Critical issues and questions for maximizing the impact of Xpert MTB/RIF and newer POC NAATs

- If Xpert® MTB/RIF is implemented in centralized/reference laboratories, will it have an impact on reducing diagnostic and treatment delays?
- If Xpert® MTB/RIF is mostly used for drug-resistance screening or for smear-negative TB, will it have an impact on TB transmission and incidence?
- Will the implementation of Xpert® MTB/RIF and newer NAATs in a passive case detection approach reduce patient delays in seeking care? What is the role of these technologies in intensified and active case finding?
- Can NAATs be successfully implemented at the point-of-care to enable same-day TB diagnosis and treatment (i.e. a "test and treat" approach)? Will health care systems invest in such POC testing programs?
- If informal and private sector health providers are important providers of first contact care in many settings, what is the best strategy for deploying new diagnostics at the first point of contact?

The availability of new tools does not mean they will be adopted, used correctly, scaled-up, or have public health impact.[27,47,48] Experience to date with new diagnostics suggests that many NTPs in high burden countries struggle to adopt and scale up new tools, even when these are backed by evidence and global policy recommendations.[47,49,50] As reviewed recently[27], there are several common barriers to effective national adoption and scale-up of new technologies: global policy recommendations that do not provide sufficient information for scale-up; complex decision-making processes and weak political commitment at country-level; limited engagement of and support to NTP managers; high cost of tools and poor fit with user needs; unregulated markets and inadequate business models; limited capacity for laboratory strengthening and implementation research; and insufficient advocacy and donor support.[27] Overcoming these barriers will require enhanced country-level advocacy, resources, technical assistance and political commitment.

Appendix Table 1.
Proposed minimum set of specifications for the design of any new POC diagnostic test for TB[33]

Test Specification	Minimum Required Value
Medical decision	Treatment initiation
Sensitivity – Adults *(for pulmonary TB only; regardless of HIV status)*	Pulmonary TB: - 95% for smear positive, culture positive - (60-)80%* for smear negative, culture positive *[Detection of EP-TB being a preferred but not minimal requirement]*
Sensitivity – Children *(including EP-TB; regardless of HIV status)*	- 80% compared to culture of any specimen and - 60% of probable TB (noting problem of lack of a gold standard)
Specificity – adults	95% compared to culture
Specificity – children	- 95% compared to culture - 90% for culture-negative probable TB (noting problem of lack of a gold standard)
Time to results	3 hours max. (patient must receive results the same day) *[Desirable would be <15min]*
Throughput	20 tests/day, minimum, by 1 lab staff
Specimen type	Adults: urine, oral, breath, venous blood, sputum [Desired: NON sputum-based sample type and use of finger prick instead of venous blood] Children: urine, oral, capillary blood (finger/heel prick)
Sample preparation	- 3 steps max. - Safe: biosafety level 1 - Ability to use approximate volumes (ie, no need for precise pipetting) - Preparation that is not highly time sensitive
Number of samples	One sample per test
Readout	- Easy-to-read, unambiguous, simple “yes”, “no”, or “invalid” answer - Readable for at least 1 hour
Waste disposal	- Simple burning or sharps disposal; no glass component - Environmentally acceptable disposal
Controls	- Positive control included in test kit - Quality control simpler and easier than with SSM
Reagents	- All reagents in self-contained kit - Kit contains sample collection device and water (if needed)
Storage/stability	- Shelf life of 24 months, including reagents - Stable at 30°C, and at higher temperatures for shorter time periods (to be defined) - Stable in high humidity environments

Instrumentation	- If instrument needed, no maintenance required - Instrument works in tropical conditions - Acceptable replacement cost - Fits in backpack - Shock resistant
Power requirement	Can work on battery
Training	- 1 day max. training time - Can be performed by any health worker
Cost	<US$10 per test after scale-up

*Consensus could not be reached on a definite minimum value. The group could not reach consensus for three test specifications: Sensitivity in smear-negative adults: 60% vs 80%; diagnosis of EP-TB in adults as a minimal requirement; rejection of use of sputum as a sample. For EP-TB diagnosis in adults, the interim decision was to define this specification as highly desirable but not a minimal requirement. Similarly, for exclusion of sputum as an acceptable sample, the interim decision was to define this as highly desirable but not a minimal requirement.

Source: This simple, instrument-free, dipstick POC TPP was developed by Médecins Sans Frontières, Treatment Action Group (TAG) and other partners.[33] First, survey interviews with field practitioners were conducted in order to identify the top-priority medical needs in resource-limited settings concerning new TB diagnostics. Second, an expert meeting convening field practitioners, laboratory experts, diagnostic test developers and researchers was held with the objective of defining the minimal test specifications for a new TB POC test. Available at: http://www.msfaccess.org/content/towards-lab-free-tuberculosis-diagnosis

Appendix Table 2.
Optimal and minimal product characteristics for proposed affordable, cartridge-based NAAT[38]

Characteristic	Optimal	Minimal
Cost of consumables (all) FOB	< 4 USD	< 8 USD
Diagnostic specificity	> 99 %	>97%
Reagent Kit stability	24 months at 40°C, 70% humidity, incl. transport stress (48h at 50°C)	12 months at 30°C, 70% humidity, incl. transport stress (48h at 50°C)
Sample preparation and Assay processing (total steps)	Integrated	Minimal sample processing; no more than 3 – 5 steps (requiring operator intervention)
Thermal Tolerance of Platform/ Assay	Operation between 15°C and 40°C	Operation between 15°C and 35°C
Time to Market	≤ 24 Months	≤ 36 Months
Time-to-result	< 1 hour	<2 hours
Additional equipment required	None	Minimal (e.g. Heat block)
Analytic sensitivity	< 10^2 cfu/ml	< 10^3 cfu/ml
Analytic specificity	No cross reactivity with other organisms including non-tuberculous mycobacteria (NTM)	No cross reactivity with other organisms including NTM
Biosafety	No need for biosafety cabinet, and direct disposal of consumable	No need for biosafety cabinet, and autoclaved consumable
Controls	Internal full-process positive control and negative controls	External controls
Cost of instrumentation	< 5,000 USD	< 10,000 USD
Diagnostic sensitivity	> 98% smear-positive and 80% smear-negative patients	95% of smear-positive and 65% smear-negative patients
Drug resistance screening	Detection of rifampicin, isoniazid, and fluoroquinolone resistance testing via a separate cartridge with additional consumable cost (reflex testing)	Rifampicin drug resistance testing via a separate cartridge with additional consumable cost (reflex testing)
Electronics and data analysis	Integrated	Separate computer required
Instrumentation	Single device	Sample prep + amp/detection
Power	None, optional battery or solar operation	110-220V AC current; DC power with rechargeable battery lasting up to 8 hours of testing
Quantitation	Semi-quantitative	Qualitative
Reagent integration	All reagents in consumable	< 4 external reagents
Result capturing & documentation | Data display	Electronic and printed, wireless transmission capable s).	Electronic

Sample type	Sputum	Sputum
Throughput	> 48 Samples per day, asynchronously	12 tests per 8-hour day
Training & education needs	<1/2 day, health care worker	<1 day, trained laboratory technician
Goal of Test	Diagnosis of active pulmonary TB in adults and children for the purpose of treatment initiation; diagnosis of MDR TB by diagnosis of drug resistance to rifampicin, isoniazid and fluoroquinolone	Diagnosis of active pulmonary TB in adults for the purpose of treatment initiation
Reference Test	Liquid culture	Liquid culture
Equipment	Small, portable or hand-held device	Small, table-top device; portable device optional
Additional 3rd party consumables	None	None, except for sample collection
Cold Chain	None required at any point in supply chain or storage	None required at any point in supply chain or storage
Clean Water Requirements	None	None
Duration of valid sample (time from taking sample to insertion into device)	2 hours without refrigeration	If running samples sequentially, 5 minutes; if batching without random access, 2 hours
Waste/disposal requirements	Disposal by incineration of infectious disease materials; simple trash for other materials	Incineration of infectious disease materials; recyclable or compostable plastics and consumables for other materials
Service/Maintenance	No annually scheduled preventive maintenance required; device has capability to send an alert or to be detected remotely when it is not functioning properly. Mean time to failure of at least 12 months; 18 months preferred	No on-site service and maintenance required; broken devices can be swapped with replacement device; device has capability to send an alert or to be detected remotely when it is not functioning properly. Software updates can be pushed out remotely over Global System for Mobile Communications (GSM) and data networks. Mean time to failure of 18 months
Calibration	None required	Minimal user calibration required
Test/platform size/footprint/ Portability	Small, portable device (<2 kg) or handheld analyzer	Small, table-top analyzer or portable device (<5 kg)
Regulatory requirements	Manufactured pursuant to GMP, ISO 13485:2003 certified and authorized for use by a regulatory authority that is a member of the Global Harmonization Task Force (GHTF); registered for in vitro diagnostic use.	Manufactured pursuant to GMP, ISO 13485:2003 certified and authorized for use by a regulatory authority that is a member of the GHTF; registered for in vitro diagnostic use.

User Interface	Simple test menu, integrated LCD screen; simple key pad or touch screen.	Simple test menu; integrated LCD screen; simple key pad or touch screen
Data Export (for External Quality Assurance)	Full data export over mobile phone network (data transmission can automatically select between General packet radio service (GPRS) or more advanced networks and GSM, based on available coverage). GPRS should be able to utilize the internet File Transfer Protocol to transmit data. Data transfer should be initiated every 6 to 12 hours automatically by the analyzer. Data can be exported in a format compatible with HL7 standards, where appropriate. Instrument tracks and transmits QA data over time (e.g. identify shifts or trends).	Full data export over mobile phone network

Source: This TPP for an affordable, cartridge-based NAAT for active TB that can be performed on sputum samples in microscopy centers or health centers was developed by the Bill & Melinda Gates Foundation, with input from partners.[38] This TPP that was used for a request for applications, released in September 2012, from Chinese companies to support the creation of a validated, low-cost, nucleic-acid assay for clinical TB detection on platforms capable of operation in rudimentary laboratories in low-resource settings. Available at: http://gongyi.qq.com/a/20121031/000023.htm

References

1. World Health Organization. Global tuberculosis control: WHO report 2012. Geneva: WHO; 2012. Report No.: 978 92 4 156450 2.
2. World Health Organization. Roadmap for rolling out Xpert MTB/RIF for rapid diagnosis of TB and MDR-TB. In. Geneva: World Health Organization; 2010.
3. Chang K, Lu W, Wang J, et al. Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: a meta-analysis. JInfect 2012;64:580-8.
4. Lawn SD, Nicol MP. Xpert(R) MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. FutureMicrobiol 2011;6:1067-82.
5. Peter J, van zyl-Smit R, Denkinger C, Pai M. Diagnosis of TB: state of the art. Eur Respir Monogr 2012;58:125-44.
6. Weyer K, Mirzayev F, Migliori GB, et al. Rapid molecular TB diagnosis: evidence, policy-making and global implementation of Xpert®MTB/RIF. Eur Resp Journal 2012 [published ahead of print].
7. WHO monitoring of Xpert MTB/RIF roll-out. 2012. (Accessed 22 November, 2012, at http://www.who.int/tb/laboratory/mtbrifrollout/en/index.html.)
8. UNITAID. TUBERCULOSIS: Diagnostic Technology Landscape. Geneva: WHO; 2012.
9. Notomi T, Okayama H, Masubuchi H, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 2000;28:E63.
10. Mitarai S, Okumura M, Toyota E, et al. Evaluation of a simple loop-mediated isothermal amplification test kit for the diagnosis of tuberculosis. IntJ TubercLung Dis 2011;15:1211-7.
11. Hillemann D, Rusch-Gerdes S, Richter E. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of Mycobacterium tuberculosis strains and clinical specimens. J Clin Microbiol 2009;47:1767-72.
12. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. The New England journal of medicine 2010;363:1005-15.
13. Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 2011;377:1495-505.
14. Niemz A, Boyle DS. Nucleic acid testing for TB at the point-of-care in high burden countries. ExpRevMolDiagn 2012;12:687-701.
15. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. JClinMicrobiol 2010;48:229-37.
16. Hunt JM, Roberts GD, Stockman L, Felmlee TA, Persing DH. Detection of a genetic locus encoding resistance to rifampin in mycobacterial cultures and in clinical specimens. DiagnMicrobiolInfectDis 1994;18:219-27.
17. Whelen AC, Felmlee TA, Hunt JM, et al. Direct genotypic detection of Mycobacterium tuberculosis rifampin resistance in clinical specimens by using single-tube heminested PCR. JClinMicrobiol 1995;33:556-61.
18. Mori Y, Nagamine K, Tomita N, Notomi T. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. BiochemBiophysResCommun 2001;289:150-4.
19. Tomita N, Mori Y, Kanda H, Notomi T. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. NatProtoc 2008;3:877-82.
20. Fang R, Li X, Hu L, et al. Cross-priming amplification for rapid detection of Mycobacterium tuberculosis in sputum specimens. J ClinMicrobiol 2009;47:845-7.
21. Xu G, Hu L, Zhong H, et al. Cross priming amplification: mechanism and optimization for isothermal DNA amplification. SciRep 2012;2:246.
22. Scott LE, Gous N, Cunningham BE, et al. Dried culture spots for Xpert MTB/RIF external quality assessment: results of a phase 1 pilot study in South Africa. J Clin Microbiol 2011;49:4356-60.

23. Pai M. TB diagnostics: Top 10 FAQs by test developers. In: Stop TB Partnership's New Diagnostics Working Group Annual Meeting,; 2012; Kuala Lumpur, Malaysia: Stop TB Partnership. URL: http://www.stoptb.org/wg/new_diagnostics/assets/documents/M.Pai_Top%2010%20FAQs.pdf; 2012.

24. Special Programme for Research & Training in Tropical Diseases (TDR) and Foundation for Innovative New Diagnostics (FIND). Diagnostics for tuberculosis. Global demand and market potential. Geneva: World Health Organization; 2006.

25. Pai NP, Pai M. Point-of-care diagnostics for HIV and tuberculosis: landscape, pipeline, and unmet needs. DiscovMed 2012;13:35-45.

26. Denkinger CM, Dheda K, Pai M. Guidelines on interferon-gamma release assays for tuberculosis infection: concordance, discordance or confusion? Clin Microbiol Infect 2011;17:806-14.

27. Pai M, Palamountain KM. New tuberculosis technologies: challenges for retooling and scale-up [State of the art series. New tools. Number 4 in the series]. Int J Tuberc Lung Dis 2012;16:1281-90.

28. Meyer-Rath G, Schnippel K, Long L, et al. The impact and cost of scaling up GeneXpert® MTB/RIF in South Africa. PLoS One 2012;7:e36966.

29. Schnippel K, Meyer-Rath G, Long L, et al. Scaling up Xpert MTB/RIF technology: the costs of laboratory- vs. clinic-based roll-out in South Africa. Trop Med Int Health 2012;17:1142-51.

30. TB Diagnostics in India: Market Analysis and Potential 2012. (Accessed 31 August, 2012, at http://tbevidence.org/wp-content/uploads/2012/07/TB_Diagnostics_Market_Analysis__July16_2012_FINAL.pdf.)

31. Central TB Division. TB India 2011. Revised National Tuberculosis Control Programme. Annual Status Report. New Delhi, India: Directorate General of Health Services, Ministry of Health and Family Welfare; 2011.

32. Jarosawlski S, Pai M. Why are inaccurate tuberculosis serological tests widely used in the Indian private healthcare sector? A root-cause analysis. J Epidemiol Global Health 2012;2:39-50.

33. Batz H-G, Cooke GS, Reid SD. Towards lab-free tuberculosis diagnosis. In: Treatment Action Group, Stop TB Partnership, Imperial College London; MÉDECINS SANS FRONTIÈRES; 2011.

34. Weyer K, Carai S, Nunn P. Viewpoint TB Diagnostics: What Does the World Really Need? J Infect Dis 2011;204 Suppl 4:S1196-202.

35. Lienhardt C, Espinal M, Pai M, Maher D, Raviglione MC. What Research Is Needed to Stop TB? Introducing the TB Research Movement. PLoS Med 2011;8:e1001135.

36. Barry CE, 3rd, Boshoff HI, Dartois V, et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat Rev Microbiol 2009.

37. Pai NP, Vadnais C, Denkinger C, Engel N, Pai M. Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. PLoS Med 2012;9:e1001306.

38. Bill and Melinda Gates Foundation. Simple and Affordable Molecular Testing for Tuberculosis. Request for Letters of Intent (LOI). In: http://gongyiqqcom/zt2012/Diagnostics/: BMGF; 2012.

39. Denkinger C, Pai M. TPPs for TB Diagnostics: one size will not fit all. URL: http://tbevidence.org/wp-content/uploads/2012/09/Merieux-TPP-Talk-Final.pdf. In: Moving Forward in the Diagnosis of Infectious Diseases in Resource Limited Countries 5th Forum Edition Fondation Merieux; 2012; Annecy, France: Fondation Merieux; 2012.

40. Paul D, Busireddy A, Nagaraja SB, et al. Factors associated with delays in treatment initiation after tuberculosis diagnosis in two districts of India. PLoS One 2012;7:e39040.

41. Clouse K, Page-Shipp L, Dansey H, et al. Implementation of Xpert MTB/RIF for routine point-of-care diagnosis of tuberculosis at the primary care level. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde 2012;102:805-7.

42. Lawn SD, Kerkhoff AD, Wood R. Location of Xpert(R) MTB/RIF in centralised laboratories in South Africa undermines potential impact. Int J Tuberc Lung Dis 2012;16:701; author reply 2.

43. Menzies N, Cohen T, Lin HH, Murray M, Salamon J. Population Health Impact and Cost-Effectiveness of Tuberculosis Diagnosis with Xpert MTB/RIF: A Dynamic Simulation and Economic Evaluation. PLoS Med 2012;9:e1001347.

44. Langley I, Doulla B, Lin HH, Millington K, Squire B. Modelling the impacts of new diagnostic tools for tuberculosis in developing countries to enhance policy decisions. Health care management science 2012;15:239-53.

45. Squire SB, Ramsay AR, van den Hof S, et al. Making innovations accessible to the poor through implementation research. Int J Tuberc Lung Dis 2011;15:862-70.

46. Davis JL, Dowdy DW, den Boon S, Walter ND, Katamba A, Cattamanchi A. Test and treat: a new standard for smear-positive tuberculosis. J Acquir Immune Defic Syndr 2012;61:e6-8.

47. Ramsay A, Steingart KR, Cunningham J, Pai M. Translating tuberculosis research into global policies: the example of an international collaboration on diagnostics. Int J Tuberc Lung Dis 2011;15:1283-93.

48. Ramsay A, Steingart KR, Pai M. Assessing the impact of new diagnostics on tuberculosis control. Int J Tuberc Lung Dis 2010;14:1506-7.

49. van Kampen SC, Ramsay AR, Anthony RM, Klatser PR. Retooling national TB control programmes (NTPs) with new diagnostics: the NTP perspective. PLoS One 2010;5:e11649.

50. Ramsay A, Cuevas LE, Mundy CJ, et al. New policies, new technologies: modelling the potential for improved smear microscopy services in Malawi. PLoS ONE 2009;4:e7760.