



# Rapid molecular TB diagnosis: evidence, policy making and global implementation of Xpert MTB/RIF

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**ABSTRACT** If tuberculosis (TB) is to be eliminated as a global health problem in the foreseeable future, improved detection of patients, earlier diagnosis and timely identification of rifampicin resistance will be critical. New diagnostics released in recent years have improved this perspective but they require investments in laboratory infrastructure, biosafety and staff specialisation beyond the means of many resource-constrained settings where most patients live. Xpert MTB/RIF, a new assay employing automated nucleic acid amplification to detect *Mycobacterium tuberculosis*, as well as mutations that confer rifampicin resistance, holds the promise to largely overcome these operational challenges. In this article we position Xpert MTB/RIF in today's TB diagnostic landscape and describe its additional potential as an adjunct to surveillance and surveys, taking into account considerations of pricing and ethics. In what could serve as a model for the future formulation of new policy on diagnostics, we trace the unique process by which the World Health Organization consulted international expertise and systematically assessed published evidence and freshly emerging experience from the field ahead of its endorsement of the Xpert MTB/RIF technology in 2010, summarise subsequent research findings and guidance on who to test and how, and provide perspectives on scaling up the new technology.



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## Introduction

With 8.7 million incident cases of tuberculosis (TB) and 1.4 million deaths estimated in 2011 [1], TB is a leading cause of morbidity and mortality worldwide. However, public health services globally reported only 5.8 million (66%) of the estimated TB cases in 2011. Moreover, less than 5% of notified TB cases were tested for drug resistance [1], which is often diagnosed after prolonged diagnostic delays [2–4]. Of the 310 000 notified new and re-treatment cases with pulmonary TB estimated to have multidrug-resistant (MDR)-TB in 2011, just under 60 000 (19%) were reported to the World Health Organization (WHO) [1].

The main reasons for these gaps are inadequate diagnostic capacity and an over reliance on chest radiography and/or sputum smear microscopy as diagnostic tools. Patients with HIV-associated TB, those with sputum smear-negative and/or extrapulmonary disease, and drug-resistant TB patients are particularly affected by the failure of microscopy as a primary diagnostic tool. The “classical” diagnosis of HIV-associated and drug-resistant TB is complex, expensive, slow and technically demanding, relying on conventional culture and drug susceptibility testing (DST). The long delay (up to several weeks) required to obtain results has devastating consequences for patients who go undiagnosed (and therefore untreated or inappropriately treated), or are diagnosed too late [5].

Detecting more cases, detecting them early and rapidly identifying drug resistance are essential for improving individual patient health and avoiding transmission in the community. This requires universal access and early detection using contemporary tools and innovative strategies [1, 4, 5].

The past decade has seen unprecedented growth in the TB diagnostic pipeline and accelerated efforts to establish the necessary laboratory infrastructure [5]. Nevertheless, although recommended by WHO, the latest generation liquid culture diagnostics and molecular line probe assays for rapid detection of MDR-TB have not yet solved the diagnostic dilemma in most resource-limited settings, largely due to the need for expensive laboratory infrastructure, extensive biosafety precautions and specialised staff [5]. A new rapid test that overcomes many of the current operational difficulties was recommended for use by WHO in December 2010: the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is an automated, real-time nucleic acid amplification technology run on the multi-disease platform GeneXpert (Cepheid). The Xpert MTB/RIF assay represents a paradigm shift in the diagnosis of TB and drug-resistant TB by simultaneously detecting *Mycobacterium tuberculosis* and rifampicin resistance-conferring mutations in a closed system suitable for use outside conventional laboratory settings in less than 2 h, directly from sputum samples [6, 7].

## Objectives

This article has three primary objectives. The first is to describe the dynamic process followed by WHO in policy development for TB diagnostics, using the example of Xpert MTB/RIF assay as a pathfinder. The second is to summarise subsequent evidence on the use of Xpert MTB/RIF, clarify common misconceptions about the technology, and provide perspectives on the role of the assay in improved case detection and care delivery. The third is to summarise the relevance of the technology for TB prevalence surveys and drug resistance surveillance, its impact on case and treatment outcome definitions, and discuss issues around affordability, sustainability, ethics and research priorities.

## Methods

Existing policy and guidance documents on Xpert MTB/RIF are summarised to illustrate the WHO policy formulation process for new TB diagnostics. Outcomes are presented from a Global Consultation organised by WHO immediately prior to endorsement of the assay. Experiences shared by early implementers of the assay during two subsequent WHO global meetings are also summarised.

For additional evidence on the Xpert MTB/RIF assay since WHO endorsement, active scanning of the emerging literature was performed. PubMed and EMBASE results were searched to find articles dealing with the Xpert MTB/RIF test. Combinations of the following search terms were used: “tuberculosis”, “multidrug-resistant tuberculosis”, “extensively drug-resistant tuberculosis”, “Xpert MTB/RIF” and “rapid diagnosis”. Although the search was not restricted to publications in English, articles not reporting an English summary were excluded.

Citations were independently screened by four investigators (K. Weyer, W. van Gemert, G.B. Migliori and R. Centis) by examining titles, abstracts and full articles to identify relevant studies, which are stored in the WHO database and regularly updated (last update September 21, 2012). Unpublished sources of data (multicentre laboratory validation and demonstration studies coordinated by FIND (Foundation for Innovative New Diagnostics; Geneva, Switzerland) and unpublished data from investigator-driven, single-centre studies) shared with WHO at the time of policy development were also included. Although this perspective article includes all available evidence on Xpert MTB/RIF, the formal criteria for a systematic review were not followed.

**Brief overview of the Xpert MTB/RIF technology**

The GeneXpert platform, launched in 2004, simplifies real-time PCR-based molecular testing by integrating and automating the key processes of sample preparation, amplification and detection. Core components of the system include the instrument, a personal computer, a barcode scanner and the software (fig. 1), together with disease-specific, single-use, disposable cartridges containing lyophilised reagents, buffers and washes. Target detection and characterisation is performed in real time using a six-colour laser detection device.

The Xpert MTB/RIF cartridge for the simultaneous detection of TB and rifampicin resistance was developed within 4 years, following a unique collaboration between academia and industry, brokered by FIND and financially supported by the US National Institutes of Health (Bethesda, MD, USA) and the Bill and Melinda Gates Foundation (Seattle, WA, USA) [8–13]. This collaboration serves as a blueprint for TB diagnostics development, consisting of clear end-user product specifications, adequate research funding, collaboration among academic partners on the core components of the technology, pooling of research resources, controlled clinical validation trials, large-scale field evaluations under well-designed operational research protocols, and a flexible response by industry engaging early on with FIND in negotiations on cost and preferential pricing.

The end-product was a fully automated, closed (and therefore safe) real-time PCR system, requiring basic not specialised laboratory infrastructure, operator skills or biosafety precautions [8–13]. The Xpert MTB/RIF assay employs five unique nucleic acid hybridisation probes (molecular beacons), each labelled with a coloured fluorophore responding to a specific target sequence within the *rpoB* gene of *M. tuberculosis*. More than 95% of mutations associated with rifampicin resistance occur in an 81-base pair core region of the *rpoB* (a bacterial RNA polymerase) gene and together these five molecular beacons encompass the entire core region. The generation of all five fluorescent colours during PCR amplification indicates the presence of rifampicin-susceptible *M. tuberculosis*, while any mutation in the core region prevents the binding of the respective molecular beacon, resulting in the absence of colour and indicating rifampicin resistance (fig. 2) [8–13].

**Evidence-based policy development**

In 2008, WHO adopted the international GRADE process (Grades of Recommendations Assessment, Development and Evaluation) for evidence synthesis and evaluation [14]. GRADE currently underpins all WHO recommendations and guidelines [15]. Recently refined for the evaluation of diagnostics [16], GRADE provides a systematic assessment of the quality of evidence underlying policy formulation, as well

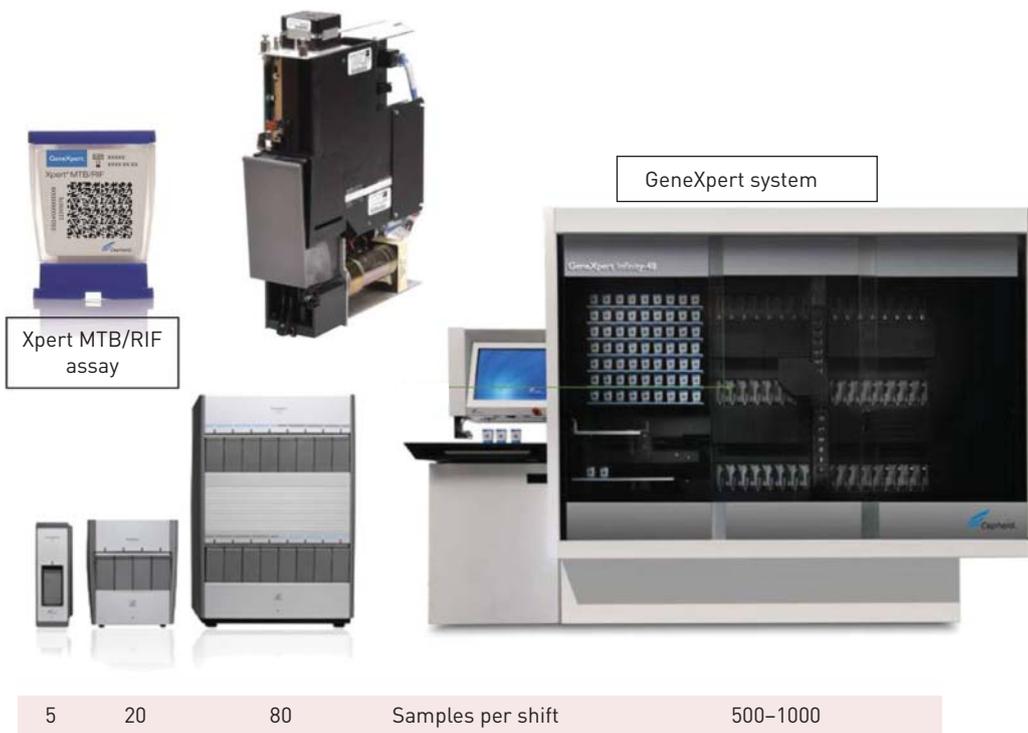


FIGURE 1 The GeneXpert system (personal communication; Foundation for Innovative New Diagnostics, Geneva, Switzerland).

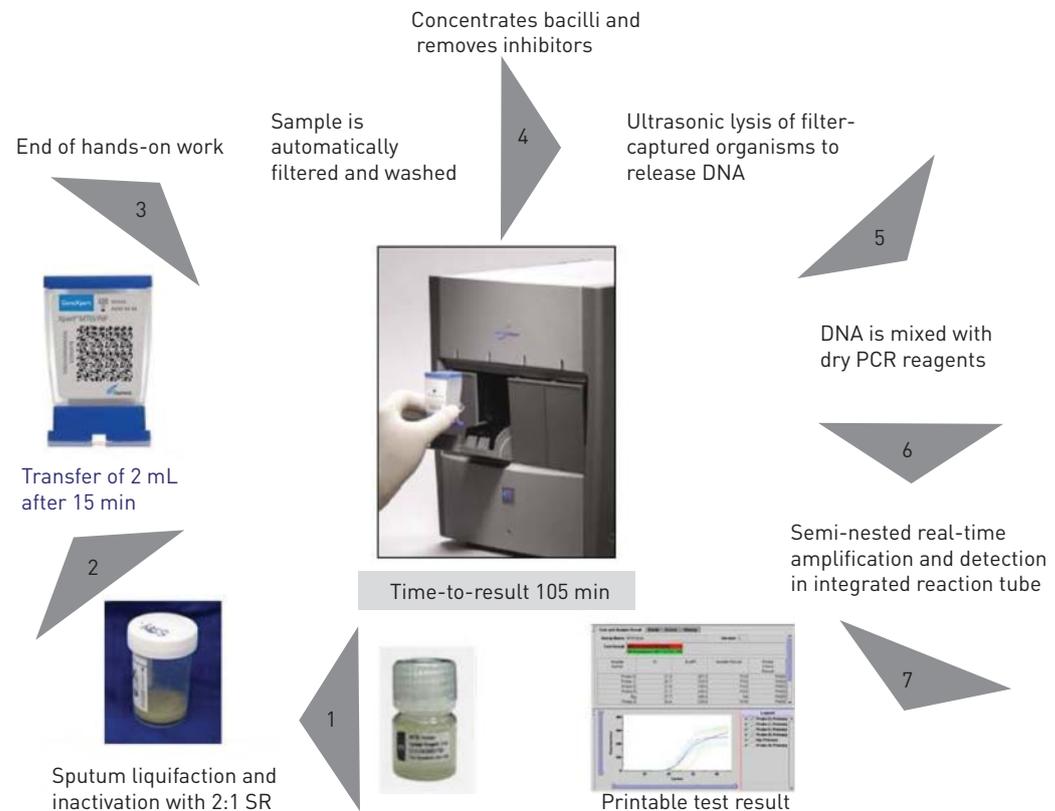


FIGURE 2 The step-by-step Xpert MTB/RIF assay process. SR: sterilising reagent. Reproduced from [6] with permission from the publisher.

as the strength of policy recommendations, aiming to achieve a balance between test performance, risks and benefits, and patient/public health impact [14, 16]. The process is overseen by the WHO Guidelines Review Committee, which was specifically established for this purpose [15].

Figure 3 illustrates the structured approach to policy development on new TB diagnostics established in 2008 by the WHO Stop TB Department, while table 1 outlines the body of evidence required by WHO to proceed with policy formulation on TB diagnostics.

### Dynamic policy development on Xpert MTB/RIF

In early September 2010, an Expert Group convened by WHO assessed the available data on Xpert MTB/RIF, including information from six published papers [8–13], two large multicentre laboratory validation and demonstration studies coordinated by FIND [18, 19], results from cost-effectiveness analyses [17] and unpublished data from 12 investigator-driven, single-centre studies (most of which were subsequently published) shared with WHO under nondisclosure agreements. The GRADE evaluation assessed assay performance, the feasibility and anticipated impact of programmatic implementation, cost-effectiveness, and issues to be addressed in future research.

Recommendations from the Expert Group were subsequently endorsed by STAG-TB (Strategic and Technical Advisory Group for Tuberculosis) in late September 2010, and WHO was advised to proceed immediately with policy guidance, develop a global strategy for rapid update, convene a Global Consultation on implementation considerations, and assist countries with technical support and planning [20].

WHO convened a Global Consultation in early December 2010, which was attended by approximately 120 institutional and country representatives. An agreement was reached on interim diagnostic algorithms and the positioning of Xpert MTB/RIF in defined risk groups (MDR-TB and HIV-associated TB) at different levels of health services. Consensus agreements were incorporated into a subsequent WHO Rapid Implementation document [7] supported by an Xpert MTB/RIF checklist [21] providing practical suggestions for systematic roll-out of the assay to optimise use and benefits of the technology while addressing key operational research aspects in more longitudinal efforts.



FIGURE 3 The World Health Organization (WHO) policy development process for tuberculosis (TB) diagnostics. GRADE: Grades of Recommendations Assessment, Development and Evaluation; QUADAS: Quality Assessment of Diagnostic Accuracy Studies; STAG-TB: Strategic and Technical Advisory Group for Tuberculosis.

In April 2011, WHO convened a meeting with early implementers of the Xpert MTB/RIF assay to refine the proposed diagnostic algorithms, develop a core set of variables to determine the impact of introducing the technology on laboratory workload, and clarify operational and logistical issues. A second WHO meeting of early implementers followed in April 2012 to share experiences during the introduction of the assay under routine TB control programme conditions.

Under its mandate to coordinate the global roll-out of Xpert MTB/RIF, WHO established a dedicated website and electronic data collection tool (<http://who.int/tb/laboratory/mtbrifrollout/en/index.html>), tracking country implementation and partner plans for scale-up in 145 countries eligible for preferential pricing of the assay, as well as to collect information from post-marketing surveillance of operational problems to guide scale-up of the technology under programmatic conditions.

Formal WHO policy recommendations on the use of Xpert MTB/RIF issued on December 8, 2010 arose from a solid GRADE assessment of the available evidence (table 2) [6].

**Analytical studies**

The Xpert MTB/RIF assay has analytic sensitivity of five genome copies of purified DNA, and 131 CFU·mL<sup>-1</sup> of *M. tuberculosis* spiked into sputum. No cross-reactivity with non-tuberculous mycobacteria (NTM) was detected. TB and resistance to rifampicin were correctly detected when NTM DNA or mixed susceptible and resistant strains were present. The sample reagent, added in a 2:1 ratio to sputum, killed >6 log<sub>10</sub> CFU·mL<sup>-1</sup> of *M. tuberculosis* with 15 min of exposure and rendered >97% of sputum smear-positive samples negative by Lowenstein–Jensen culture. No infectious aerosols were detected following the Xpert MTB/RIF inoculation procedure and sample testing [8–13].

**TABLE 1** Evidence required by the World Health Organization (WHO) to enable tuberculosis (TB) diagnostic policy development and policy update

**Phase 1: research and development**

Typically consists of upstream research and development to define and validate a prototype, followed by laboratory validation under international standards that culminate in a design-locked product

WHO interacts with developers if requested to discuss end-user requirements such as biosafety, assay robustness and intended setting of use

**Phase 2: evaluation and demonstration**

The performance of the new diagnostic product should be evaluated in controlled trials at three to five trial sites in high-burden TB and HIV countries, ideally using pre-specified and-user product specifications. These data are often used for product registration with global and/or regulatory authorities such as FDA and/or CE marking.

Product specifications and performance should subsequently be validated in uncontrolled trials under field conditions in five to 10 trial sites in high-burden TB and HIV countries, and include cost-effectiveness studies.

**Phase 3: WHO evidence assessment using GRADE**

For new technologies or new indications for use of technologies already approved by WHO

Submission of dossier with phase 1 and 2 data to WHO

Structured evidence assessment process (described in [figure 3](#))

For fast-follower or generic versions of technologies already approved by WHO

Manufacture of the technology under ISO 13:485 standards; equivalent performance demonstrated in two to three independent supranational TB reference laboratories, to the reference technology already approved by WHO

Structured evidence assessment process (described in [figure 3](#))

WHO is not a regulatory authority and does not recommend technologies for individual country use

**Phase 4: phased uptake and collection of evidence for scale-up**

New diagnostic successfully implemented in routine diagnostic services by early implementers in high-burden TB and HIV countries; systemic assessment of proposed algorithms, laboratory workload, operational constraints, country by cost-effectiveness. Lessons learnt by early implementers used for country adaptation.

**Phase 5: scale-up and policy refinement**

Scale-up of the new diagnostic, with subsequent data used to inform and refine WHO policy guidance in a dynamic and ongoing process

FDA: Food and Drug Administration; CE: Communauté Européenne (European Community); GRADE: Grades of Recommendations Assessment, Development and Evaluation; ISO: International Organization for Standardization.

*Controlled clinical validation trials*

The performance of Xpert MTB/RIF was tested in 1730 patients suspected to be affected by drug-susceptible or pulmonary MDR-TB from Peru, Azerbaijan, South Africa and India. Among culture-positive patients, a single, direct Xpert MTB/RIF test identified 98.2% (551 out of 561) of sputum smear-positive TB cases and 72.5% (124 out of 171) of those with sputum smear-negative TB. The test was specific in 604 (99.2%) out of 609 patients not affected by TB. A second Xpert MTB/RIF test among patients with sputum smear-negative, culture-positive TB increased sensitivity by 12.6% and a third by 5.1%, to reach 90.2%. When compared to phenotypic DST, the Xpert MTB/RIF assay identified correctly 97.6% (200 out of 205) of patients harbouring rifampicin-resistant strains and 98.1% (504 out of 514) of those with rifampicin-susceptible strains. Sequencing resolved all but two cases in favour of Xpert MTB/RIF [18].

**TABLE 2** World Health Organization (WHO) policy recommendations on Xpert MTB/RIF

**The GRADE process confirmed a solid evidence base to support widespread use of Xpert MTB/RIF for detection of TB and rifampicin resistance and resulted in the following main recommendations:**

Xpert MTB/RIF should be used as the initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB (strong recommendation)

Xpert MTB/RIF may be considered as a follow-on test to microscopy in settings where MDR-TB or HIV is of lesser concern, especially in further testing of smear-negative specimens (conditional recommendation, acknowledging major resource implications)

**Remarks**

These recommendations apply to the use of Xpert MTB/RIF in sputum specimens (including pellets from decontaminated specimens)

Data on the utility of Xpert MTB/RIF in extrapulmonary specimens are still limited

These recommendations support the use of one sputum specimen for diagnostic testing, acknowledging that multiple specimens increase the sensitivity of Xpert MTB/RIF but have major resource implications

These recommendations also apply to children, based on the generalisation of data from adults and acknowledging the limitations of microbiological diagnosis of TB (including MDR-TB) in children

Access to conventional microscopy, culture and DST is still needed for monitoring of therapy, prevalence surveys and/or surveillance and recovering isolates for DST other than rifampicin (including second-line anti-TB drugs)

GRADE: Grades of Recommendations Assessment, Development and Evaluation; MDR: multidrug resistant; DST: drug susceptibility testing. Data from [6].

### Field demonstration studies

6648 individuals were prospectively enrolled in South Africa, Peru, India, Azerbaijan, the Philippines and Uganda, comparing Xpert MTB/RIF with microscopy in decentralised microscopy centres, and with culture and phenotypic DST in centralised laboratories. Xpert MTB/RIF detected 90.3% (933 out of 1033) of the culture-confirmed TB cases, compared with 67.1% (699 out of 1041) using microscopy. In sputum smear-negative, culture-positive TB patients Xpert MTB/RIF test sensitivity was 76.9% (296 out of 385) and specificity was 99.0% (2846 out of 2876). Sensitivity for rifampicin resistance was 94.4% (236 out of 250) and specificity was 98.3% (796 out of 810) [19].

While HIV co-infection substantially decreased the sensitivity of microscopy (to 47%), Xpert MTB/RIF performance was not significantly affected. The median time to detection of TB was 0 days (interquartile range (IQR) 0–1) using Xpert MTB/RIF, compared to 1 day (IQR 0–1) for microscopy, 30 days (IQR 23–43) for solid culture and 16 days (IQR 13–21) for liquid culture. The median time to detection of rifampicin resistance was 20 days (IQR 10–26) for line-probe assay *versus* 106 days (IQR 30–124) for phenotypic DST.

The Xpert MTB/RIF test reduced the median time to treatment for sputum smear-negative TB from 56 days (IQR 39–81) to 5 days (IQR 2–8). The indeterminate rate of Xpert MTB/RIF testing was 2.4% (126 out of 5321 samples) compared to 4.6% (441 out of 9690) for culture.

### Unpublished, single-centre evaluation studies

Results from 12 studies with varying design and study populations reported Xpert MTB/RIF sensitivity in detecting TB ranging from 70% to 100% in culture-positive patients and ~60% in those with smear-negative disease. Specificity ranged from 91% to 100%. Pooled average sensitivity for TB detection was 92.5% and pooled average specificity was 98%. Average rifampicin sensitivity and specificity were ~98% and 99%, respectively.

### Operational and logistical issues

The available evidence confirmed the robustness of the Xpert MTB/RIF assay under varying temperature and humidity conditions, the need for minimal staff training, basic biosafety requirements (as for sputum smear microscopy) and high levels of user satisfaction. Operational challenges included the requirement for an ambient temperature <30°C (necessitating air conditioning in hot climates), and uninterrupted and stable electrical power supply (requiring generators in several sites). Storage space and conditions (<28°C) for cartridges, waste generated (considerably more than for microscopy), and the 12-month shelf-life of cartridges were listed as main operational challenges [6, 7].

### Cost, affordability and cost-effectiveness analyses

Using Xpert MTB/RIF for the diagnosis of smear-negative pulmonary TB was deemed cost-effective compared with existing diagnostic strategies in India, South Africa and Uganda, and within WHO acceptable incremental cost effectiveness ratios [6, 7, 17].

The cost of achieving the diagnostic targets in the Global Plan to Stop TB, 2011–2015 [22] with and without use of Xpert MTB/RIF was appraised for three population groups, *i.e.* TB patients considered at risk of having MDR-TB, people living with HIV with TB signs and symptoms, and all people with TB signs and symptoms. Using the FIND negotiated price at the end of 2010 of US\$16.86 per cartridge, there were four main findings. First, a diagnostic strategy using Xpert MTB/RIF with follow-on DST for rifampicin-positive cases was a lower cost approach for reaching the 2015 targets for diagnosis of MDR-TB, both globally and in all high TB and high MDR-burden countries, compared with reliance on conventional culture and DST only. Secondly, using Xpert MTB/RIF to diagnose TB in people living with HIV in high HIV-prevalence settings was of similar or lower cost, compared with the conventional diagnostic algorithm (based on culture and radiography) recommended by WHO, in most countries. Thirdly, the total cost of using Xpert MTB/RIF to diagnose MDR-TB and TB among people living with HIV was a small fraction (<5%) of total spending on TB control in 2010. Finally, the cost of using Xpert MTB/RIF to test all people with TB signs and symptoms was much higher compared with conventional diagnosis based on smear microscopy and radiographs, but in middle-income countries would be relatively affordable compared with total spending on TB care and control.

### Operational research informing policy on Xpert MTB/RIF

Operational research on the Xpert MTB/RIF assay has proliferated subsequent to WHO endorsement. Of particular programmatic relevance are several operational research studies addressing key research questions identified following the WHO Global Consultation. As of July 2012, at least 24 operational research projects in 16 countries were registered, covering multiple implementation aspects [1].

By the end of August 2012, more than 70 peer-reviewed publications, commentaries, viewpoints and editorials had been published [8–13, 17–19, 23–90], including an updated systematic review of 18 studies involving 10 224 specimens, confirming the initial findings [23]. A single Xpert MTB/RIF test detected 90.4% of culture-confirmed pulmonary TB patients (98.7% of smear-positive specimens and 75.0% of smear-negative specimens). Similar accuracy was retained in specimens from HIV-infected patients, showing pooled values of 81.7% sensitivity (95% CI 77.0–85.8%) and 98.0% specificity (95% CI 96.6–98.9%). The accuracy of Xpert MTB/RIF in detecting paediatric TB was 74.3% (95% CI 62.4–84.0%). Accuracy in detecting extrapulmonary TB reached 70.7% sensitivity (95% CI 59.6–80.3%) and 82.6% specificity (95% CI 79.5–85.3%). Accuracy estimates for rifampicin resistance detection were equally similar to the initial data, with pooled sensitivity of 94.1% (95% CI 91.6–96.0%) and pooled specificity of 97.0% (95% CI 96.0–97.7%).

Subsequent studies showed equally consistent results confirming the accuracy of the Xpert MTB/RIF assay in different settings and patient groups, with superior performance over microscopy. Most studies also confirmed the current operational limitations of the GeneXpert system: the sophisticated nature of the device requires care of handling, *i.e.* a stable and uninterrupted electrical supply to avoid interruption of the procedure and subsequent loss of results, an ambient temperature <30°C, security against theft, adequate storage space for the cartridges, and the need for sufficient staff to perform testing [6, 7, 21].

As with any other TB test, the positive predictive value (PPV) of Xpert MTB/RIF testing is adversely affected in low disease-prevalence settings or populations.

#### “Learning by doing”: ongoing technological innovation and field experience

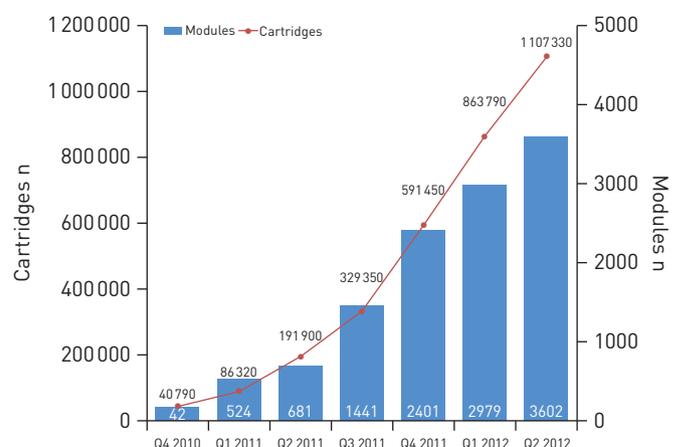
As of June 2012, 67 low- and middle-income countries had implemented the Xpert MTB/RIF assay, with 749 GeneXpert machines, 3602 cartridge modules and 1.1 million cartridges deployed or used (fig. 4).

Initial anxiety about errors and invalid results was reported during implementation of Xpert MTB/RIF in selected countries [24, 65, 70]. Recurring errors at particular sites were linked to improper procedures in specimen collection and/or preparation of samples, and faulty modules and cartridges. Since the evaluation and demonstration studies published in 2011, refinements to the reagents and software of the Xpert MTB/RIF assay have been made to decrease the frequency of false-positive rifampicin resistant results. Specifically, most of the false-positive results for rifampicin resistance were associated with a single probe; this has been redesigned to give more robust performance, with false-positive results for rifampicin resistance now rarely reported. The introduction of the latest generation Xpert MTB/RIF cartridge in December 2011 significantly reduced the number of signal loss (5011) errors, which had been the most commonly reported error [84].

The experience of the South African National Health Laboratory Service (NHLS) with over 300 000 tests found a decreasing error rate in the presence of adequate training and troubleshooting, with a current overall error rate of 2.2% (W. Stevens, NHLS, Johannesburg, South Africa; personal communication), which is very similar to unreadable microscopy results and much lower than acceptable culture contamination rates.

Changes have been made to minimise packaging, thus reducing waste and shipping costs [84]. Real-time stability studies are being performed by FIND and Cepheid to increase the current cartridge shelf-life of 12 months to 24 months. Stability data for 18-month shelf-life determination are expected in May 2013 and for 24 months in November 2013 (M. Perkins, FIND; personal communication). An on-site calibration kit

FIGURE 4 Global uptake of Xpert MTB/RIF up to June 30, 2012. As of June 30, 2012, a total of 749 GeneXpert instruments (comprising 3602 modules) and 1 107 330 Xpert MTB/RIF cartridges had been procured in the public sector in 67 of the 145 countries eligible for concessional pricing. Reproduced from [6] with permission from the publisher.



has been developed which allows users to recalibrate the optical system of the GeneXpert machine, verify the functioning thermal system, and conduct a series of system-level tests to ensure full system functionality within specifications, thus reducing the need for remote calibration of modules [84].

Experiences shared by early implementers showed that treatment of rifampicin-resistant TB cases diagnosed by Xpert MTB/RIF is a major, although controversial, concern [84]. While some argued for cautious roll-out in order to ensure treatment access, others felt that diagnosis in the absence of appropriate treatment allows for increased advocacy for scale-up of treatment and enables patients to make appropriate life decisions and protect the health of their families, while also facilitating interventions for reduced transmission of drug-resistant strains in healthcare facilities.

Early implementers reported high levels of user acceptance and satisfaction [84], describing the technology as fast, easy-to-use, modern, and much less cumbersome than conventional TB diagnostic techniques. They also indicated that the time and resources needed to develop and implement effective diagnostic as well as clinical management algorithms should not be underestimated, and stressed the need for training of doctors and nurses on interpretation of Xpert MTB/RIF results and clinical management of patients [84].

Several early implementers felt that adoption of Xpert MTB/RIF by the large private sector in many high-burden countries would be highly beneficial for increasing patient access to rapid and reliable diagnosis, while replacing poor technologies not endorsed by WHO [84]. Establishment of public-private collaborations was seen as mutually beneficial, allowing private providers to access concessional prices and national TB control programmes to ensure that patients detected in the private sector are duly reported and subsequently registered for appropriate treatment [84].

### Cost, affordability and cost-effectiveness

While the Xpert MTB/RIF assay allows decentralised testing, cost and affordability of the assay are often cited as a barrier to wide-scale implementation. Price negotiations by FIND prior to launching the assay resulted in a significant upfront cost reduction (up to 85%) and preferential pricing of both the GeneXpert instrument and Xpert MTB/RIF cartridges for the public sector in 145 low- and middle-income countries [7]. A further major price reduction in cartridge cost (from US\$16.86 to US\$9.98) was recently achieved following a novel financing agreement between the manufacturer and the Bill and Melinda Gates Foundation, the US Agency for International Development (USAID), the Office of the United States Global AIDS Coordinator (OGAC) and UNITAID [91]. Detailed analyses using the price of US\$9.98 per Xpert MTB/RIF cartridge to assess cost and affordability of diagnostic strategies confirmed and strengthened the findings of the analyses performed for the Global Consultation [85].

Studies of the cost and cost-effectiveness of Xpert MTB/RIF are currently limited to three countries: India, South Africa and Uganda [19, 46, 75–78, 85]. The data show that Xpert MTB/RIF is cost-effective compared with conventional diagnostic strategies, especially when the test is used as recommended by WHO, *i.e.* in persons suspected of MDR and/or HIV-associated TB. Use of the assay has also been found to be cost-effective in pre-antiretroviral treatment (ART) screening and in reducing early mortality during the first 6 months of ART [77, 78]. In South Africa, a diagnostic strategy of combining microscopy and Xpert MTB/RIF was found to have produced the highest accuracy and lowest cost [17].

### Placement of Xpert MTB/RIF in tiered laboratory services

Currently recommended TB diagnostics require different levels of laboratory sophistication due to technical complexity and biosafety concerns. To date, technologies to diagnose drug-resistant TB and smear-negative TB have been suitable for use only at the apex of tiered laboratory services, *i.e.* reference laboratories at central or regional level (table 3). A distinct advantage of Xpert MTB/RIF is its suitability for use at the district and sub-district level and the technology should therefore not be restricted to central/reference laboratory level only [6, 7].

Countries already using high-throughput liquid culture and DST systems or molecular line probe assay (LPA) for rapid diagnosis of rifampicin resistance at central/reference laboratory level should introduce Xpert MTB/RIF at lower health service levels. Selection of sites for placement of Xpert MTB/RIF testing should be guided by: 1) the prevalence of MDR or HIV-associated TB; 2) the current or estimated workload of the facility; 3) availability of adequate infrastructure; 4) availability of staff; and 5) availability and capacity for appropriate treatment [6, 7, 21].

None of the existing TB diagnostic tools are mutually exclusive and implementation (in various combinations in country screening and diagnostic algorithms) is highly setting and resource specific [92]. One size no longer fits all, and expert laboratory input is needed to define the most cost-effective and

TABLE 3 Summary of tuberculosis (TB) diagnostics evaluated by the World Health Organization 2007–2012

**2007: Commercial liquid culture and DST systems**

Automated and manual commercial systems for liquid culture and DST recommended for use at central/reference laboratory level  
 Phased implementation recommended within the context of comprehensive country plans for strengthening TB laboratory capacity  
 Currently regarded as the reference standard for conventional culture and DST and recommended as a stand-alone diagnostic test for TB and drug-resistance detection

**2007: Rapid speciation strip technology**

Rapid chromatographic strip speciation recommended for distinguishing *Mycobacterium tuberculosis* from non-tuberculous mycobacteria  
 Recommended for use in combination with conventional culture and DST systems, at central/reference laboratory level  
 Recommended as a stand-alone speciation test for *Mycobacterium tuberculosis* isolates

**2008: Molecular line probe assay for first-line anti-TB drugs**

Commercial line probe assays recommended for rapid detection of rifampicin alone or in combination with isoniazid resistance detection in smear-positive sputum specimens and *Mycobacterium tuberculosis* isolates grown from culture, for use at central/reference laboratory level  
 Phased implementation recommended within the context of national plans for MDR-TB diagnosis, including development of country-specific screening/diagnostic algorithms  
 Can be used as a stand-alone diagnostic test for rifampicin resistance (but no other resistance) once laboratory proficiency and equivalence with commercial liquid culture systems have been validated  
 Need for conventional culture (for smear-negative sputum specimens and treatment monitoring) as well as phenotypic DST capacity remains

**2010: LED microscopy**

Recommended as immediate replacement for conventional fluorochrome microscopy and as gradual replacement for conventional light Ziehl-Neelsen microscopy  
 Suitable for use at peripheral microscopy, as well as higher laboratory levels

**2010: Selected non-commercial DST methods: MODS, NRA, CRI**

Recommended as interim solutions for rapid rifampicin testing in resource-constrained settings, at central/reference laboratory level  
 Phased implementation under strict laboratory protocols and quality assurance recommended within the context of national plans for MDR-TB diagnosis, including development of country-specific screening/diagnostic algorithms  
 Phased implementation recommended within the context of national plans for MDR-TB diagnosis, including development of country-specific screening/diagnostic algorithms  
 Can be used as stand-alone diagnostic tests for rifampicin resistance (but no other resistance) once laboratory proficiency and equivalence with conventional culture systems have been validated  
 Need for conventional culture (for smear-negative sputum specimens and treatment monitoring) as well as DST capacity remains. MODS and NRA are suitable for direct testing on smear-positive sputum specimens and indirect testing on *Mycobacterium tuberculosis* isolates grown from culture  
 CRI is suitable for indirect testing on *Mycobacterium tuberculosis* isolates only

**2011: Automated real-time nucleic acid amplification technology: Xpert MTB/RIF system**

Recommended as rapid diagnostic test for TB and rifampicin resistance at peripheral microscopy, as well as higher laboratory levels  
 Can be used as stand-alone diagnostic test for TB detection in all settings (including HIV co-infected patients) and for rifampicin resistance in patients at risk of drug-resistant disease  
 Phased implementation and rapid scale-up recommended within the context of national TB and MDR-TB plans, including development of country-specific screening/diagnostic algorithms  
 Need for conventional microscopy and culture remains to monitor treatment and to conduct additional DST

**Evaluated but not yet approved due to lack of adequate evidence**

Sputum concentration and decontamination methods (evaluated 2008)  
 Phage-plaque technology for rapid rifampicin resistance detection (evaluated 2008)  
 Thin-layer agar methods for rapid culture and DST (evaluated 2010)  
 Molecular line probe assays for second-line anti-TB drugs (evaluated 2012)  
 Loop-mediated isothermal amplification test kit for tuberculosis (evaluated 2012)

**Not approved for use**

Commercial serodiagnostic tests for TB diagnosis (evaluated 2011)  
 IFN- $\gamma$  release assays for detection of active TB in all settings (evaluated 2011)  
 IFN- $\gamma$  release assays as replacement for TST to detect latent TB in low- and middle-income (typically high TB and/or HIV burden) settings (evaluated 2011)

DST: drug susceptibility testing; MDR: multidrug resistant; LED: light-emitting diode; MODS: microscopic observation of drug susceptibility; NRA: nitrate reductase assay; CRI: colorimetric redox indicator; IFN: interferon; TST: tuberculin skin test.

efficient algorithms in individual countries, guided by WHO standards and procedures, and within the context of overall, integrated laboratory strengthening activities [92].

**Targeting risk groups for testing**

Maximum benefit from the Xpert MTB/RIF assay is obtained by targeted testing of individuals considered at risk of drug-resistant TB and/or smear-negative TB, such as those co-infected with HIV. Risk groups for drug-resistant TB include all re-treatment categories (*i.e.* failure, relapse and default cases), as well as those

described in WHO guidelines [93, 94] or national policies, including those with HIV infection [95]. These individuals should receive an Xpert MTB/RIF test as a primary diagnostic test, *i.e.* subsequent confirmation of the diagnosis is not required and appropriate treatment should be started on the basis of the Xpert MTB/RIF result.

Published studies have shown significant increases in TB case detection when Xpert MTB/RIF is used as an add-on or replacement test for microscopy, especially in settings with high HIV prevalence [18, 19, 34, 35, 50, 61]. Both diagnostic delay and treatment initiation can be significantly shortened compared to conventional approaches [92], reducing premature death and on-going transmission. The 2012 WHO Global Report [1] also notes the still insufficient screening of HIV patients for TB and the low proportion of patients started on isoniazid preventive therapy.

HIV testing should be routinely offered to all persons suspected of having TB, based on WHO recommendations [95], ideally before investigation with Xpert MTB/RIF. Up to 25% of patients accessing HIV services may have active TB, the vast majority of which would be missed using conventional microscopy as a primary diagnostic tool [96]. The systematic introduction of Xpert MTB/RIF in HIV services would, therefore, make a major contribution to intensified TB case finding efforts and increased uptake of isoniazid preventive therapy.

The distinct advantage of Xpert MTB/RIF in providing a rapid, simultaneous diagnosis of both TB and rifampicin resistance has also given rise to continuing debate and concerns about the implications of positive results in different epidemiological and resource settings [24, 42, 43, 79, 80]. It is therefore important to distinguish the performance characteristics and treatment implications of the assay for: 1) TB detection and; 2) rifampicin resistance detection.

In many settings, the vast majority of persons suspected of having TB will not have risk factors for drug resistance or be HIV-positive. Careful consideration should be given in these circumstances to the resource implications of offering routine Xpert MTB/RIF testing [6, 7] and the low PPV of the assay for detecting rifampicin resistance at a low underlying prevalence (tables 4 and 5). Where resources are limited, national TB control programmes will have to prioritise specific groups for testing, decide whether Xpert MTB/RIF is performed as an initial diagnostic test or as a follow-on test after sputum smear microscopy, and consider the use of chest radiography as a first screening tool.

#### **Predictive values of Xpert MTB/RIF for TB case detection**

In the GRADE framework, diagnostic test accuracy can be interpreted as proxy measures for patient-important outcomes based on the relative importance/impact of false-positive and false-negative results [16]. Poor sensitivity would result in false-negative results with adverse consequences for patient morbidity and mortality and ongoing disease transmission. Poor specificity would result in false-positive results exposing patients to unnecessary treatment while the underlying cause of disease remains undiagnosed.

Test accuracy is also dependent on underlying disease prevalence. Typically, between 10% and 20% of persons with respiratory symptoms may have confirmed TB in high-burden settings. Table 4 presents the predictive values for TB detection using Xpert MTB/RIF (compared to conventional culture) in settings or populations with varying TB prevalence. The negative predictive value (NPV) is over 99% in settings with both low and high prevalence of TB, *i.e.* a negative result reliably excludes TB. Table 4 shows that the vast majority of patients with a negative Xpert MTB/RIF result in such settings will not have TB and very few false-positive results will occur. Even with a low PPV the absolute number of false-positives will usually be very low and the proportion of overall true results (positive and negative combined) far outweigh the proportion of overall false results.

#### **Predictive values of Xpert MTB/RIF for rifampicin resistance detection**

Table 5 presents PPV and NPV for rifampicin resistance detection using Xpert MTB/RIF in settings or populations with varying prevalence of rifampicin resistance. The NPV is over 99% in settings with both low and high prevalence of rifampicin resistance, *i.e.* a negative result reliably excludes resistance and no further testing to confirm negative results is required.

The PPV for rifampicin resistance using Xpert MTB/RIF exceeds 90% in settings or patient groups where the underlying prevalence of rifampicin resistance is >15% (table 5). In settings or patient groups where rifampicin resistance is rare, the PPV of Xpert MTB/RIF (and any other test) is adversely affected, significantly diminishing when rifampicin resistance prevalence falls below 5%.

The PPV for rifampicin resistance using Xpert MTB/RIF (or any other test) can be substantially improved by careful risk assessment in individual patients and targeted testing of risk groups: drug resistance surveillance data from 114 countries showed that the weighted proportion of MDR among previously

TABLE 4 False positive, false negative and predictive values for tuberculosis (TB) detection using Xpert MTB/RIF<sup>#</sup>

TB prevalence %	PPV %	NPV%	True positive <sup>¶</sup>	False negative <sup>¶</sup>	False positive <sup>¶</sup>	True negative <sup>¶</sup>
1	48	100	9.1	0.9	9.9	980.1
2	65	100	18.2	1.8	9.8	970.2
3	74	100	27.3	2.7	9.7	960.3
4	79	100	36.4	3.6	9.6	950.4
5	83	100	45.5	4.5	9.5	940.5
6	85	99	54.6	5.4	9.4	930.6
7	87	99	63.7	6.3	9.3	920.7
8	89	99	72.8	7.2	9.2	910.8
9	90	99	81.9	8.1	9.1	900.9
10	91	99	91	9	9	891
11	92	99	100.1	9.9	8.9	881.1
12	93	99	109.2	10.8	8.8	871.2
13	93	99	118.3	11.7	8.7	861.3
14	94	99	127.4	12.6	8.6	851.4
15	94	98	136.5	13.5	8.5	841.5
20	96	98	182	18	8	792
25	97	97	227.5	22.5	7.5	742.5

PPV: positive predictive value; NPV: negative predictive value. <sup>#</sup>: according to varying TB prevalences in a sample population of 1000 individuals; <sup>¶</sup>: sensitivity (91%) and specificity (99%) for Xpert MTB/RIF TB detection, compared with reference method (culture).

treated cases is 19.8% (95% CI 14.4–25.1), which is several times higher than the proportion of new TB cases with MDR (3.4%; 95% CI 1.9–5.0) [3]. Therefore, even in low MDR-TB prevalence settings, testing previously treated patients should result in high PPV for rifampicin resistance, allowing treatment to be initiated based on the Xpert MTB/RIF result. Testing new TB cases not at risk of MDR-TB in low MDR-TB prevalence settings will, however, result in low PPV, requiring confirmation of rifampicin resistance by phenotypic DST or LPA (and not by a second Xpert MTB/RIF test) prior to treatment initiation.

The performance of Xpert MTB/RIF has been evaluated against existing reference standards, *i.e.* microscopy and culture for TB testing and phenotypic DST for rifampicin resistance testing. None of the currently available microbiological reference methods is 100% accurate, a well-recognised constraint in TB diagnostic test development and evaluation. Emerging data seem to suggest that low-level but potentially clinically relevant rifampicin resistance linked to infrequent *rpoB* mutations may be missed by standard growth-based

TABLE 5 False positive, false negative and predictive values for rifampicin resistance using Xpert MTB/RIF<sup>#</sup>

Rifampicin resistance prevalence %	PPV %	NPV %	True positive <sup>¶</sup>	False negative <sup>¶</sup>	False positive <sup>¶</sup>	True negative <sup>¶</sup>
1	32.4	99.9	9.5	0.5	19.8	970.2
2	49.2	99.9	19	1	19.6	960.4
3	59.5	99.8	28.5	1.5	19.4	950.6
4	66.4	99.8	38	2	19.2	940.8
5	71.4	99.7	47.5	2.5	19	931
6	75.2	99.7	57	3	18.8	921.2
7	78.1	99.6	66.5	3.5	18.6	911.4
8	80.5	99.6	76	4	18.4	901.6
9	82.4	99.5	85.5	4.5	18.2	891.8
10	84.1	99.4	95	5	18	882
11	85.4	99.4	104.5	5.5	17.8	872.2
12	86.6	99.3	114	6	17.6	862.4
13	87.7	99.2	123.5	6.5	17.4	852.6
14	88.5	99.2	133	7	17.2	842.8
15	89.3	99.1	142.5	7.5	17	833
20	92.2	98.7	190	10	16	784
25	94.1	98.3	237.5	12.5	15	735

PPV: positive predictive value; NPV: negative predictive value. <sup>#</sup>: according to varying prevalences of rifampicin resistance in a sample population of 1000 individuals; <sup>¶</sup>: sensitivity (95%) and specificity (98%) for Xpert MTB/RIF rifampicin resistance, compared with reference method (culture).

methods, particularly the automated broth-based systems [97]. Sequencing, albeit limited, has largely resolved discordant results in favour of Xpert MTB/RIF, although a few truly false-positive results have been reported [17, 18, 84]. Additional data on mutation sequencing of *M. tuberculosis* strains and the clinical outcomes of patients with rifampicin resistance detected by Xpert MTB/RIF are therefore highly desirable.

#### **Use of Xpert MTB/RIF in diagnosis of paediatric TB**

Laboratory diagnosis of TB in children remains a real challenge due to the low sensitivity of sputum smear microscopy, the difficulty in collecting sufficient and high-quality specimens, and a substantial proportion of paediatric cases with extrapulmonary involvement.

Current WHO policy recommendations on the use of Xpert MTB/RIF in children are extrapolated from data on adults [6, 7], given the well-known limitations of microbiological methods in diagnosing paediatric TB. Subsequent studies have shown a significant improvement in diagnosing TB in children using the Xpert MTB/RIF assay.

Both high sensitivity (86.9%) and specificity (99.7%) of Xpert MTB/RIF in extrapulmonary paediatric samples (n=344, mainly gastric aspirates and biopsies) were reported by TORTOLI *et al.* [72], using positive culture and/or therapeutic response as a composite standard. In a large study on young South African children (including 24% with HIV co-infection), NICOL *et al.* [50] showed a sensitivity of 74.3%. Using Xpert MTB/RIF on two induced sputum specimens detected twice as many cases (75.9%) compared to sputum smear (38%) resulting in an overall Xpert specificity of 98.8%. Similar results were obtained by RACHOW *et al.* [71] using Xpert MTB/RIF for diagnosis of pulmonary TB in 164 (51.2%) older children in a high HIV prevalence setting.

#### **Use of Xpert MTB/RIF in diagnosis of extrapulmonary TB**

The diagnosis of extrapulmonary TB poses a serious challenge due to the pleomorphic presentation of the disease. Samples collected for microbiological diagnosis are often paucibacillary, resulting in a low sensitivity of smear microscopy and earlier nucleic acid amplification tests.

Several studies have now assessed the performance of Xpert MTB/RIF in the diagnosis of extrapulmonary TB [23, 27–31, 38, 39, 53–56, 59–62]. The sensitivity and specificity ranged between 77% and 95% for biopsy, urine and pus while it was lower than 50% for cavitory fluids [23]. The specificity in these specimens ranged from 97% to 100% [23].

#### **Management of patients detected by Xpert MTB/RIF**

TB patients identified by Xpert MTB/RIF without rifampicin resistance should receive appropriate first-line anti-TB treatment immediately. HIV co-infected patients detected by Xpert MTB/RIF should be managed according to WHO guidelines, including HIV clinical staging, immunological staging with CD4 count, initiation of co-trimoxazole preventive therapy and initiation of antiretroviral therapy irrespective of CD4 count [95].

Rapid DST for rifampicin is recommended by WHO [92–94]. Patients at risk of drug resistance in whom rifampicin resistance is detected by Xpert MTB/RIF should be placed on an appropriate MDR-TB regimen immediately and isoniazid added until the DST result for isoniazid is available. These patients should provide an additional sputum specimen for conventional culture and DST against other first- and second-line drugs according to WHO recommendations [93, 94], and their treatment adjusted accordingly.

Molecular tests, including Xpert MTB/RIF, are not suitable for patient monitoring as these tests detect DNA from both viable and non-viable bacilli. Conventional laboratory capacity is, therefore, required to monitor treatment response of patients detected by Xpert MTB/RIF and to conduct additional DST in patients with rifampicin resistance.

Patients whose diagnosis of TB is confirmed by Xpert MTB/RIF and who have rifampicin-susceptible TB disease should be monitored during treatment with sputum smear microscopy. No additional sputum smear microscopy examination needs to be performed for establishing baseline status. Sputum smear microscopy should be performed at completion of the intensive phase of treatment, 5 months into treatment and at the end of treatment as per WHO guidelines [98].

Treatment outcomes for patients with a positive smear culture or Xpert MTB/RIF result at the start of treatment should be categorised according to current WHO guidelines [7, 98]. Current treatment outcome definitions apply, including the outcome “cured”, *i.e.* a patient with a positive Xpert MTB/RIF test (only) at baseline can be declared cured if negative smear results during and at the end of the treatment were recorded.

Patients placed on MDR-TB treatment should be monitored by sputum culture as per current WHO guidelines. If resources permit, monthly culture throughout treatment is recommended given that this has shown the highest benefit to detect failures [99].

### Use of Xpert MTB/RIF *vis à vis* other tests

From a purely technical perspective, no test for TB is perfect. Xpert MTB/RIF limitations have been described previously. Microscopy, conventional culture and DST (both phenotypic and genotypic) all have shortcomings and limitations related to accuracy and effectiveness, operator dependency, training and resource requirements, and biosafety. WHO policy guidance on new TB diagnostics takes all these aspects into careful consideration [92], balancing test accuracy with potential harms and benefits, operational considerations, resource implications and anticipated public health impact.

As outlined previously, Xpert MTB/RIF efficiency is maximised and cost minimised by targeted testing of individuals at risk of drug resistance and/or HIV co-infection. In these patient groups, Xpert MTB/RIF clearly outperforms microscopy and should be used as the initial diagnostic test.

Xpert MTB/RIF is currently the only DST technology suitable beyond central/reference laboratory level and should be the first point of testing when MDR-TB is suspected. It is a relatively low throughput technology (maximum of 20 specimens per day in the four-module GeneXpert machine). Settings with higher patient loads should consider bigger capacity machines or the referral of specimens to central/national laboratory levels for first-line LPA or phenotypic DST (both high through-put technologies).

All DST methods currently recommended by WHO show similar accuracy for rifampicin resistance detection. All tests have poor PPV in settings with low levels of MDR-TB and good PPV in settings with high levels. In settings where rifampicin/MDR resistance is rare, resistance by any test therefore needs to be confirmed by an alternative WHO-recommended DST method. Specimens from confirmed MDR-TB patients need to undergo phenotypic DST against fluoroquinolones and kanamycin, amikacin and capreomycin to check for extensively drug-resistant-TB [93, 94].

Strategies for Xpert MTB/RIF testing of all persons suspected of having TB will be strongly dependent on available resources and the screening and diagnostic algorithms at country level. TB screening as per national guidelines should take place and pre-test screening strategies, including chest radiography, should be considered to optimise Xpert MTB/RIF efficiency and cost. Individuals with sputum smear-positive microscopy results do not need to be retested with Xpert MTB/RIF unless they belong to the risk groups for drug resistance, as described previously.

In summary, Xpert MTB/RIF does not eliminate the need for traditional bacteriological methods (direct examination, culture and DST) and for other rapid molecular methods. National programmes need to develop setting-specific, evidence-based and cost-effective algorithms designed to ensure universal access to quality diagnosis for all TB cases.

### Use of Xpert MTB/RIF in prevalence surveys and drug resistance surveillance

Upgrading laboratory infrastructure and strengthening capacity for culture and DST are among the most important indirect benefits of implementing a drug resistance survey [100] as many laboratories need considerable refurbishment and/or upgrade, training of staff, and procurement of equipment and consumables before starting a survey [101]. Although not a complete surrogate for MDR-TB, particularly in settings with low resistance levels [102], rifampicin resistance is the most important indicator of MDR-TB, with serious clinical implications for affected patients.

At least two groups of countries could benefit considerably from the use of Xpert MTB/RIF as a screening tool in drug resistance surveys. The first group is countries in which laboratories would struggle to cope with the huge workload generated by a survey while managing their routine work and maintaining high-quality standards. The second group is countries where there is no capacity to perform culture and DST. In these settings, instead of relying entirely on testing abroad, usually at a TB Supranational Reference Laboratory, with increased logistics and operational costs, Xpert MTB/RIF could be used to screen specimens and identify those requiring further testing in a specialised laboratory.

Given that most patients enrolled in drug resistance surveys are newly diagnosed with TB and at low risk of rifampicin resistance, the PPV of any test will not be adequate to identify true positives but the NPV will be sufficiently high to accurately identify true negatives. Xpert MTB/RIF could, therefore, be used as screening tool to identify those with no resistance to rifampicin, while patients with rifampicin resistance undergo further confirmatory testing with a second WHO-approved technology.

In a recent TB prevalence study, DORMAN *et al.* [86] suggested the potential use of the Xpert MTB/RIF as a single testing strategy: the diagnostic yield of *M. tuberculosis* was 2.7% (187 out of 6893) for liquid culture, 2.1% (144 out of 6893) for Xpert MTB/RIF and 1.3% (91 out of 6893) for smear microscopy. Agreement of Xpert MTB/RIF with liquid culture was 98.5% (95% CI 98.2–98.8%) and respective test failure rates (noninterpretable results) were 0.3% for Xpert MTB/RIF and 3.6% for liquid culture. Overall Xpert MTB/RIF sensitivity was 62.6% (95% CI 55.2–69.5%), specificity was 99.6% (95% CI 99.4–99.7%), PPV was 81.3% (95% CI 3.9–87.3%), and NPV was 98.9% (95% CI 98.6–99.2%) [86]. While these results are encouraging, more evidence is needed on the use of Xpert MTB/RIF in the context of prevalence surveys and other case-finding strategies in which TB prevalence and the pre-test probability of TB disease are relatively low.

### Aligning diagnostic and treatment capacity

Lack of diagnostic capacity has been a longstanding and major barrier to scaling up MDR-TB care. The advent of new TB diagnostics, and Xpert MTB/RIF in particular, allows this constraint to be largely overcome. Early implementers have reported a 30–40% increase in the number of drug-susceptible TB patients being detected after roll-out of Xpert MTB/RIF, while MDR-TB cases have increased two- to three-fold in many settings [17, 18, 71].

Providing a definitive diagnosis for the large proportion of drug susceptible TB cases currently being reported without laboratory confirmation would allow treatment to be shifted away from those who do not need it to those who do. For the  $\geq 80\%$  of MDR-TB patients estimated to arise each year but remaining undiagnosed, prompt and appropriate treatment would prevent premature death, reduce the risk for aggravating drug resistance, and curtail disease transmission. Introduction of Xpert MTB/RIF should also allow for more robust and reliable forecasting of patient numbers, one of the most pressing constraints in securing adequate availability of second-line drugs. This in turn could stimulate more investment into second-line drugs and drive down the exorbitant cost of MDR-TB treatment.

An unintended consequence of scaling up diagnostics is the risk that patients diagnosed with MDR-TB cannot access the complex, second-line drug treatment required. This raises the question of ethics, human rights and public health. Should a diagnosis not lead to appropriate treatment? Concerns about the ethics of rolling out Xpert MTB/RIF in developing countries in the presumed absence of treatment for MDR-TB have been raised [42, 87], as have the counterpoint on the ethics of not rolling out the assay in low-income countries [43, 88].

Public health, ethics and human rights should be balanced when addressing the cost, risks and benefits, and technical limitations of any new, transformational intervention. Systematic roll-out of the Xpert MTB/RIF assay complies fully with WHO guidance on ethics of TB prevention, care and control [103], as well as the WHO-endorsed “progressive realisation” approach which states that “while countries are in the process of scaling up treatment, the use of DST can be appropriate as an interim measure even when no second-line drug treatment is available, or when the only available treatment is substandard” [43]. Virtually all low-income countries have ratified the International Covenant on Economic, Social, and Cultural Rights (ICESR) governing the WHO-endorsed strategy of progressive realisation. We therefore agree with leading global ethicists that public health, ethics and human rights obligations apply equally to high TB burden low-income countries as they do to resource-rich countries and that the public health potential of the Xpert MTB/RIF assay should be considered despite cost and operational considerations [43, 103].

### Research needs

As countries start to implement Xpert MTB/RIF they will be facing operational and logistical challenges related to changes in screening and diagnostic algorithms, shifts in laboratory organisation and workload, and requirements for improved supply chain management. In addition, country-specific adaptation of the diagnostic algorithms (*e.g.* prioritisation of patient groups to be tested) may be dictated by the availability of resources. WHO has therefore recommended that roll-out of Xpert MTB/RIF be addressed in a systematic and coordinated approach to optimise the usefulness of the technology under routine programme conditions and to ensure maximum efficiency [6, 7]. In addition, WHO has recommended ongoing operational research to refine and inform future policy, in line with the requirement for dynamic policy guidance by the WHO Guidelines Review Committee [16].

More studies merely evaluating the performance of the Xpert MTB/RIF assay against conventional diagnostics in detecting pulmonary TB are not expected to challenge or change the existing evidence base. The assay has been shown repeatedly to be highly accurate, particularly if used in targeted testing as recommended by WHO. Implementation research should therefore now focus on the “how” and “when” of Xpert MTB/RIF implementation and scale-up, informed by appropriately designed studies (and using real

data) that evaluate the test's impact and cost-effectiveness when used in different algorithms and with other screening and diagnostic tests. Policy refinement will also benefit from additional data on the use of the assay in extrapulmonary and paediatric TB, in prevalence surveys and drug resistance surveillance, and in active case finding.

Xpert MTB/RIF MTB/RIF roll-out can, and should, serve as a pathfinder for implementation of future TB tests by providing national TB control programmes with data to develop long-term TB diagnostic strategies. Experiences and lessons learnt from programmatic roll-out (*i.e.* evidence for scaling up) will inform and facilitate eventual country-wide scale-up and assist other countries intending to embark on the same process.

Cost and potential disruption of health services are characteristic consequences of introducing any new public health intervention tool [88]. Rather than blocking or slowing down the introduction of new technologies [87] or waiting until ideal operational conditions are in place [89], scientific debate should focus on whether patient and public health benefits warrant implementation, even of a so-called “disruptive” intervention. Although not expected to show overall cost disadvantages, in-depth, cost-effectiveness studies on the impact of Xpert MTB/RIF in different settings would therefore be advantageous, especially since the assay will be used in varied diagnostic algorithms and underlying TB and MDR-TB epidemiology.

On a more fundamental research level, second-generation Xpert MTB/RIF tests with probes to detection resistance other than rifampicin will be most advantageous. In addition, the development of competing technologies with comparable performance and ease-of-use to the Xpert MTB/RIF assay is strongly encouraged to generate increased demand and market competition. Most pressing is the need for a robust, low-cost and safe point-of-care diagnostic for TB and drug-resistant TB. This will require dramatic increases in research investment to identify appropriate biomarkers and capitalise on technological breakthroughs to create innovative test platforms [90]. The experiences in HIV test and drug development have shown the advantages gained from innovation and solid investment in research [104], strikingly different from the TB research world that remains woefully under-funded [105].

A summary of the current evidence available on Xpert MTB/RIF [8–13, 18, 19, 27–41, 50–74, 86, 106–112] is available in the supplementary material.

## Conclusion

In the mid-1990s, when WHO declared TB a global emergency and subsequently introduced the DOTS (directly observed treatment, short course) strategy, the impact of the HIV epidemic on the dynamics of TB control (especially in Africa) was not fully realised, and no information on the public health impact of the growing problem of TB drug resistance was available. Under the assumption that MDR-TB was a rare event, good microscopy services were deemed sufficient to control TB in most settings. Indeed, many national programmes witnessed annual decreases in TB case rates following the wide implementation of microscopy services linked to the use of short-course chemotherapy under close supervision. Before the end of the 20th century, however, three events suggested that microscopy would become inadequate. The first was the magnitude of the HIV pandemic and its extraordinary impact on susceptibility to TB. The second was the growing burden and geographical spread of MDR-TB. Thirdly was the very slow decline in TB incidence in countries implementing DOTS, even when the prevalence of MDR-TB and HIV co-infection was low.

In 2006, WHO introduced the Stop TB Strategy which, in addition to the essential elements of the DOTS strategy, included measures specifically targeting (amongst others) proper care of HIV-associated TB and MDR-TB. As a result, in 2009, the World Health Assembly called for universal access to culture and DST, marking a dramatic shift in strategy. The updated Global Plan to Stop TB, 2011–2015 [22], underpinned by the Stop TB Strategy, called for massive investment in laboratory services to achieve screening for MDR in at least 20% of new TB cases and 100% of those previously treated by 2015. It also called for >50% of all smear-negative cases to be tested with molecular or culture-based methods. Data reported to WHO in 2012 clearly show that these targets are not on track. Reasons include huge gaps in funding to establish the required laboratory infrastructure, slow diagnostic policy reform at country level, and critical shortages of specialised laboratory staff.

WHO policy guidance on Xpert MTB/RIF recognises its potential in addressing some of the most pressing barriers to rapid diagnosis of TB and drug-resistant TB, and has attempted to provide the necessary information and support to enable countries to make appropriate decisions on its utilisation. The WHO guidance also explicitly highlights the resource implications of rolling out the technology, as well as the need to ensure appropriate treatment of those patients detected; however, increased demand for testing, the difficulties in providing care for drug-resistant patients, and concerns about affordability should not be the prime drivers delaying roll-out of new and innovative interventions.

Evidence on “where” to locate Xpert MTB/RIF (peripheral *versus* central laboratories) and “whom” to test (targeted *versus* general use) is growing, allowing rational and sustainable roll-out of the technology even in resource-constrained settings.

The experience of HIV testing despite inadequate treatment facilities provides a solid precedent for TB to follow: in the HIV world, moral pressure has been put on drug and diagnostics manufacturers to lower the prices of their products and to develop novel ones. Increased demand for drugs as a result of improved case detection has created scale and ultimately lowered prices, thus facilitating increased access.

As countries adjust their diagnostic algorithms to accommodate Xpert MTB/RIF roll-out, diagnostic paradigms for HIV-associated and drug-resistant TB are expected to shift significantly, away from highly centralised, complex diagnostic algorithms and referral systems (with inevitable long delays) towards simplified diagnostic approaches for at-risk patients at decentralised levels of the health system. These should be accompanied by more focused use of screening methods to increase the pre-test probability of TB prior to Xpert MTB/RIF testing, accelerated implementation of WHO screening policies for TB-HIV using Xpert MTB/RIF as the initial diagnostic test, and more focused identification of patients suspected of having to suffer from MDR-TB, using Xpert MTB/RIF as a rapid test rather than waiting for patients to fail first-line therapy before proceeding with culture and DST.

Stagnation in TB control and MDR-TB care delivery has severe consequences for TB patients, who often belong to the most vulnerable and neglected sector of society. Scientific breakthroughs such as the Xpert MTB/RIF assay (and hopefully additional new diagnostics, drugs and vaccines coming to use in the next few years) should not be withheld from these marginalised groups but deployed without undue delay, optimising patient and public health benefits.

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