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Topic: Topic for the first journal club is new technology in TB diagnosis. Our expert guest will be Dr. Jan Hajek, Clinical Assistant Professor in Department of Infectious Disease, University of British Columbia. Both papers are attached below.

Paper:
Weyer K, Mirzayev F. Rapid molecular TB diagnosis: evidence, policy-making and global implementation of Xpert®MTB/RIF. *ERJ Express*. Published on November 22, 2012 as doi: 10.1183/09031936.00157212.

Supplemental paper:
Keshavjee S, Farmer P. Tuberculosis, drug resistance, and the history of modern medicine. *NEJM* 2012. 367;10: 931-6.

ERJ Express. Published on November 22, 2012 as doi: 10.1183/09031936.00157212

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

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Summary

If tuberculosis is to be eliminated as a global health problem in the foreseeable future, the improved detection of patients, their earlier diagnosis, and the 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 specialization 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 paper we position Xpert® MTB/RIF in the tuberculosis diagnostic landscape of today 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, guidance on who to test and how, and provide perspectives on scaling up the new technology.

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-retreatment 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].

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 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 last 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 specialized 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), an automated, real-time nucleic acid amplification technology run on the multi-disease platform GeneXpert (Cepheid, Sunnyvale CA, USA). The Xpert® MTB/RIF assay represents a paradigm shift in the diagnosis of TB and drug-resistant TB by simultaneously detecting *M. tuberculosis* and rifampicin resistance-conferring mutations, in a closed system suitable for use outside conventional laboratory settings, in less than two hours, directly from sputum samples [6,7].

Objectives

This perspective 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 summarize 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 summarized to illustrate the WHO policy formulation process for new TB diagnostics. Outcomes are presented from a Global Consultation called 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 summarized. 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”, “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 (KW, WvG, GBM, RC) by examining titles, abstracts, and full articles to identify relevant studies, which are stored in the WHO database and regularly updated (last update: September 21st , 2012). Unpublished sources of data (multi-centre laboratory validation and demonstration studies coordinated by FIND (Foundation for Innovative New Diagnostics), 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 theXpert® 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 (Figure 1), together with disease-specific, single-use, disposable cartridges containing lyophilized reagents, buffers and washes. Target detection and characterization is performed in real time using a six-color laser detection device.

The Xpert® MTB/RIF cartridge for the simultaneous detection of TB and rifampicin resistance was developed within four years, following a unique collaboration between academia and industry, brokered by FIND and financially supported by the US National Institutes of Health and the Bill & Melinda Gates Foundation[8-13]. This collaboration serves as a blue-print 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 no specialized 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 (Figure 2) [8-13].

Evidence-based policy development

In 2008 WHO adopted the international GRADE process (Grades of Recommendations Assessment, Development and Evaluation; http://www.gradeworkinggroup.org) 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 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 Figure 4 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 multi-centre laboratory validation and demonstration studies coordinated by FIND [17,18], results from cost-effectiveness analyses [19] and unpublished data from 12 investigator-driven, single-centre studies (most of which subsequently published)shared with WHO under non-disclosure 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 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, attended by around 120 institutional and country representatives. Agreement was reached on interim diagnostic algorithms and the positioning of Xpert® MTB/RIF in defined risk groups (MDR 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] and providing practical suggestions for systematic roll-out of the assay to optimize use and benefits of the technology while addressing key operational research aspects in more longitudinal efforts.

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 introduction of the assay under routine TB control programme conditions (findings summarized below).

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, and

collecting 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 (Table 1) issued on 8 December 2010 [6] arose from a solid GRADE assessment of the available evidence:

1) *Analytical studies* [8-13]: The Xpert® MTB/RIF assay has analytic sensitivity of five genome copies of purified DNA, and 131 cfu/ml 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 log10 cfu/ml of *M. tuberculosis* with 15 minutes 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.
2) *Controlled clinical validation trials* [17]: the performance of Xpert® MTB/RIF was tested in 1,730 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/RIFtest identified 98.2% of sputum smear-positive TB cases (551/561) and 72.5% of those with sputum smear-negative TB (124/171). The test was specific in 604/609 patients not affected by TB (99.2%). 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% (200/205) of patients harboring rifampicin-resistant strains and 98.1% (504/514) of those with rifampicin-susceptible strains. Sequencing resolved all but two cases in favor of Xpert® MTB/RIF.
3) *Field demonstration studies* [18]: 6,648 individuals were prospectively enrolled in South Africa, Peru, India, Azerbaijan, 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% (933/1033) of the culture-confirmed TB cases, compared with 67.1% (699/1041) using microscopy. In sputum smear-negative, culture-positive TB patients Xpert® MTB/RIF test sensitivity was 76.9% (296/385) and specificity 99.0% (2846/ 2876). Sensitivity for rifampicin resistance was 94.4% (236/250) and specificity 98.3% (796/ 810).

   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 (IQR 0–1) using Xpert® MTB/RIF, compared to 1 day (0–1) for microscopy, 30 days

(23–43) for solid culture, and 16 days (13–21) for liquid culture. The median time to detection of rifampicin resistance was 20 days (10–26) for line-probe assay vs. 106 days (30–124) for phenotypic DST.

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

4) *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 around 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 around 98% and 99% respectively.

5) *Operational and logistical issues* [6,7]: 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 bio-safety requirements (as for sputum smear microcopy) and high levels of user satisfaction. Operational challenges included the requirement for an ambient temperature below 30°C (necessitating air conditioning in hot climates), and uninterrupted and stable electrical power supply (requiring generators in several sites). Storage space and conditions (below 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)Cost, affordability and cost-effectiveness analyses [6,7,19]: 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.

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, ie. 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 USD16.86 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. Second, 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 X-ray) recommended by WHO, in most countries. Third, the total cost of using Xpert® MTB/RIF to diagnose MDR-TB and to diagnose TB among people living with HIV was a small fraction (<5%) of total spending on TB control in 2010; Fourth, 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 X-ray, 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 than70 peer-reviewed publications, commentaries, viewpoints and editorials had been published [8-13, 17-19, 23-84, 86,98-100,102,103], 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; 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 (95CI96.6%-98.9%) respectively. The accuracy of Xpert® MTB/RIF in detecting paediatric TB was 74.3% (95%CI 62.4%-84.0%). Accuracy in detecting extra-pulmonary 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 under 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 (see below).

'Learning by doing': on-going technological innovation and field experience

As of June 2012, 67 low and middle-income countries had implemented the Xpert® MTB/RIF assay, with 749GeneXpert machines, 3,602 cartridge modules and 1.1 million cartridges deployed or used (Figure 5).

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% (Wendy Stevens, personal communication), very similar to unreadable microscopy results and much lower than acceptable culture contamination rates.

Changes have been made minimizing packaging, thus reducing waste and shipping costs [84]. Real-time stability studies are underway 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 (Mark Perkins, personal communication). An on-site calibration kit 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, though 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 nevertheless allows for increased advocacy for scale-up of treatment and for

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 decentralized 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 USD 16.86 to USD 9.98) was recently achieved following a novel financing agreement between the manufacturer and the Bill & Melinda Gates Foundation, the United States Agency for International Development (USAID), the Office of the United States Global AIDS Coordinator (OGAC) and UNITAID [85]. 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 done for the Global Consultation described above [86].

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, 86]. 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 cost-effective in pre-ART (antiretroviral treatment) screening and in reducing early mortality during the first six 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 [19].

Placement of Xpert® MTB/RIFin tiered laboratory services

Currently recommended TB diagnostics require different levels of laboratory sophistication due to technical complexity and biosafety concerns. Technologies to diagnose drug-resistant TB and smear-negative TB have up to now been suitable for use only at the apex of tiered laboratory services, i.e. reference laboratories at central or regional level (Table 2). A distinct advantage of Xpert® MTB/RIF is its suitability for use at *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 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 i) the prevalence of MDR or HIV-associated TB; ii) the current or estimated workload of the facility; iii) availability of adequate infrastructure; iv) availability of staff; and v) 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 [87]. One size no longer fits all, and expert laboratory input is needed to define the most cost-effective and efficient algorithms in individual countries, guided by WHO standards and procedures, and within the context of overall, integrated laboratory strengthening activities [87].

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 [88,89] or national policies, including those with HIV infection [90]. 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 [17,18,34,35,50,61]. Both diagnostic delay and treatment initiation can be significantly shortened compared to conventional approaches [87], reducing premature death and on-going

transmission. The 2012 WHO Global Report [1] also notes the still insufficient screening of HIV clients for TB and the low proportion of clients started on isoniazid preventive therapy.

HIV testing should be routinely offered to all persons suspected of having TB, based on WHO recommendations [90], ideally before investigation with Xpert® MTB/RIF. Up to 25% of clients accessing HIV services may have active TB, the vast majority of which would be missed using conventional microscopy as primary diagnostic tool [91]. 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 (IPT).

The distinct advantage of Xpert® MTB/RIFin 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 a) TB detection and b) rifampicin resistance detection (see below).

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 positive predictive value (PPV) of the assay for detecting rifampicin resistance at a low underlying prevalence (Tables 3 and 4). Where resources are limited, national TB control programmes (NTP) will have to prioritize specific groups for testing, decide whether Xpert® MTB/RIFis done 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.

11.1 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 3 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 3 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.

1.2 Predictive values of Xpert® MTB/RIF for rifampicin resistance detection

Table 4 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 above 15% (Table 4). 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 show that the weighted proportion of MDR among previously treated cases is 19.8% (95% CI: 14.4-25.1) 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 RMP resistance linked to infrequent *rpoB* mutations may be missed by standard growth-based methods, particularly the automated broth-based systems [92]. Sequencing, albeit limited, have 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.

1.3 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 extra-pulmonary 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/RIFassay.

Both high sensitivity (86.9%) and specificity (99.7%) of Xpert® MTB/RIF in extra-pulmonary 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. Nicol *et al*. in a large study on young South African children (including 24% with HIV co-infection) showed a sensitivity of 74.3% [50]. 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 older children in a high HIV prevalence (51.2%) setting.

11.4 Use of Xpert® MTB/RIF *in diagnosis of extra-pulmonary TB*

The diagnosis of extra-pulmonary 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 extra-pulmonary 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 cavitary 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 [90].

Rapid DST for rifampicin is recommended by WHO [87-89]. 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 [88,89], 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, five months into treatment and at the end of treatment as per WHO guidelines [93].

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 [7,93]. 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 [94].

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 are described above. 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 [87], balancing test accuracy with potential harms and benefits, operational considerations, resource implications, and anticipated public health impact.

As outlined above, 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 4-module GeneXpert machine). Settings with higher patient loads should consider bigger capacity machines or referring 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 need 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 XDR-TB [88,89].

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 described above.

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 [95] as many laboratories need considerable refurbishment and/or upgrade, training of staff, and procurement of equipment and consumables before starting a survey[96].Though not a complete surrogate for MDR-TB, particularly in settings with low resistance levels[97], 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.* [98] suggested the potential use of the Xpert® MTB/RIF as a single testing strategy: the diagnostic yield of *M. tuberculosis* was 2.7% (187/6893) for liquid culture, 2.1% (144/6893) for Xpert® MTB/RIF and 1.3% (91/6893) 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 (non-interpretable 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%) [98]. 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 80% or more 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 [42,99], as have the counterpoint on the ethics of *not* rolling out the assay in low-income countries[43,100].

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 tuberculosis prevention, care and control [101] 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 [43,101].

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 on-going 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 extra-pulmonary 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 [100]. Rather than blocking or slowing down the introduction of new technologies [99] or waiting until ideal operational conditions are in place [102], scientific debate should focus on whether patient and public health benefits warrant implementation, even of a so-called ‘disruptive’ intervention. Though 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 [103]. 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, 17-18, 27-41, 50-74, 98, 106-112] is available as an electronic annex (Annex 1)

Conclusions

In the mid 1990s, when WHO declared tuberculosis a global emergency and subsequently introduced the DOTS strategy, the impact of the HIV epidemic on the dynamics of TB control (especially in Africa) was not fully realized, 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 tuberculosis 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. Third 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, 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 recognizes 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 vs. central laboratories) and “whom” to test (targeted vs. 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 belong often 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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List of Figures

Figure 1. The GeneXpert® system (courtesy of FIND)

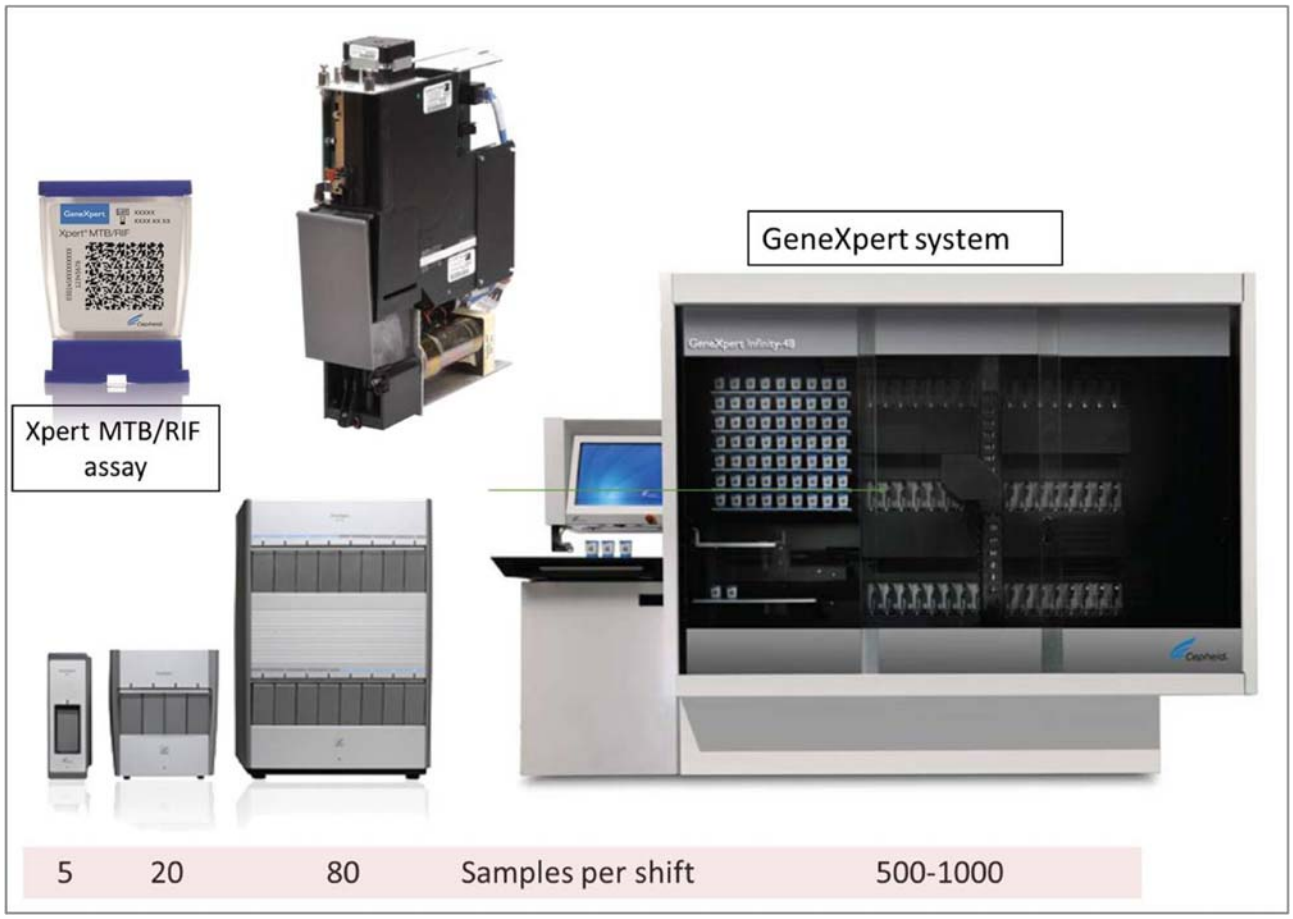


Figure 2. The Xpert® MTB/RIF assay (courtesy of FIND)

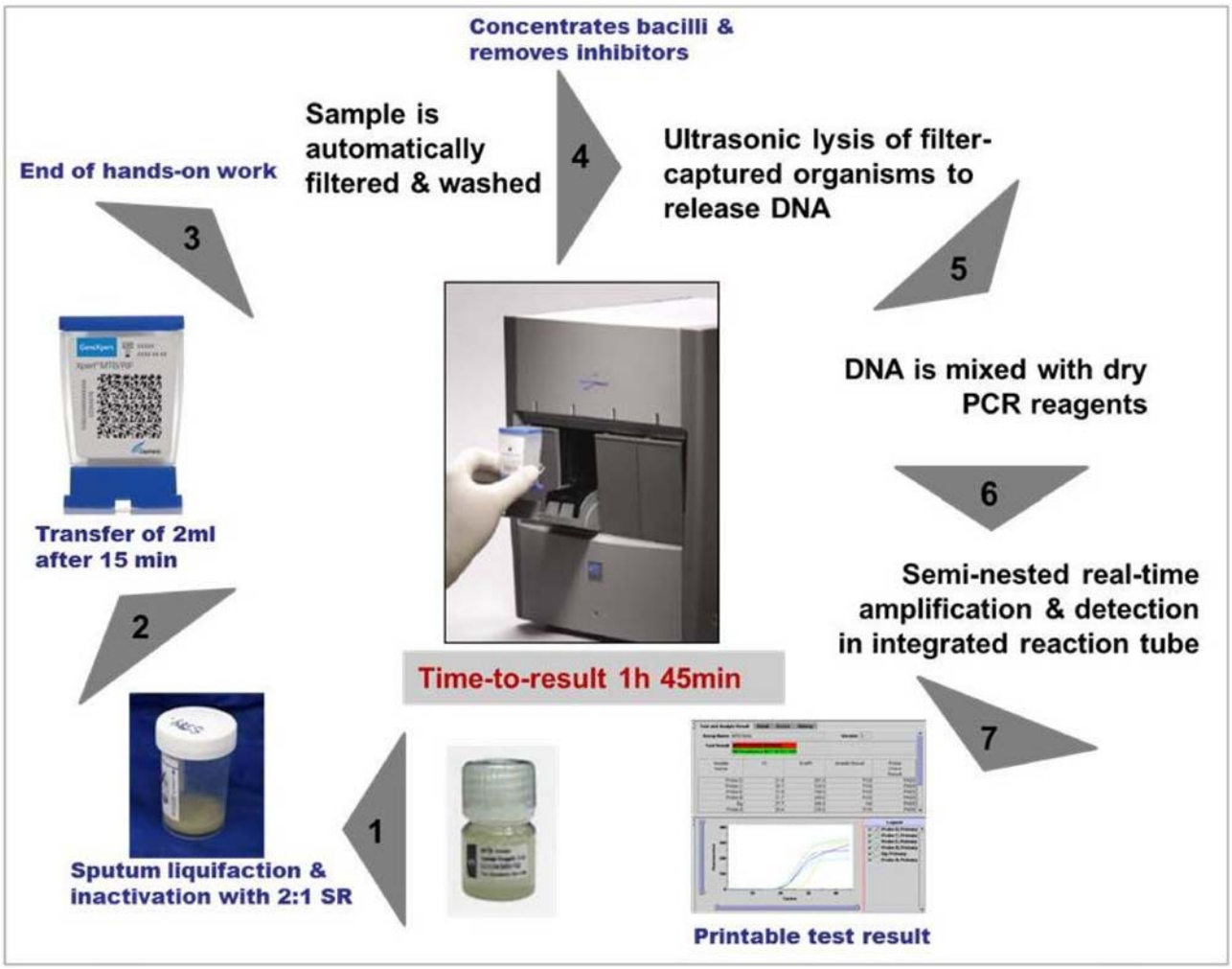


Figure 3. The WHO policy development process for TB diagnostics

Figure 4. Body of evidence required by WHO to enable TB diagnostic policy development and policy update

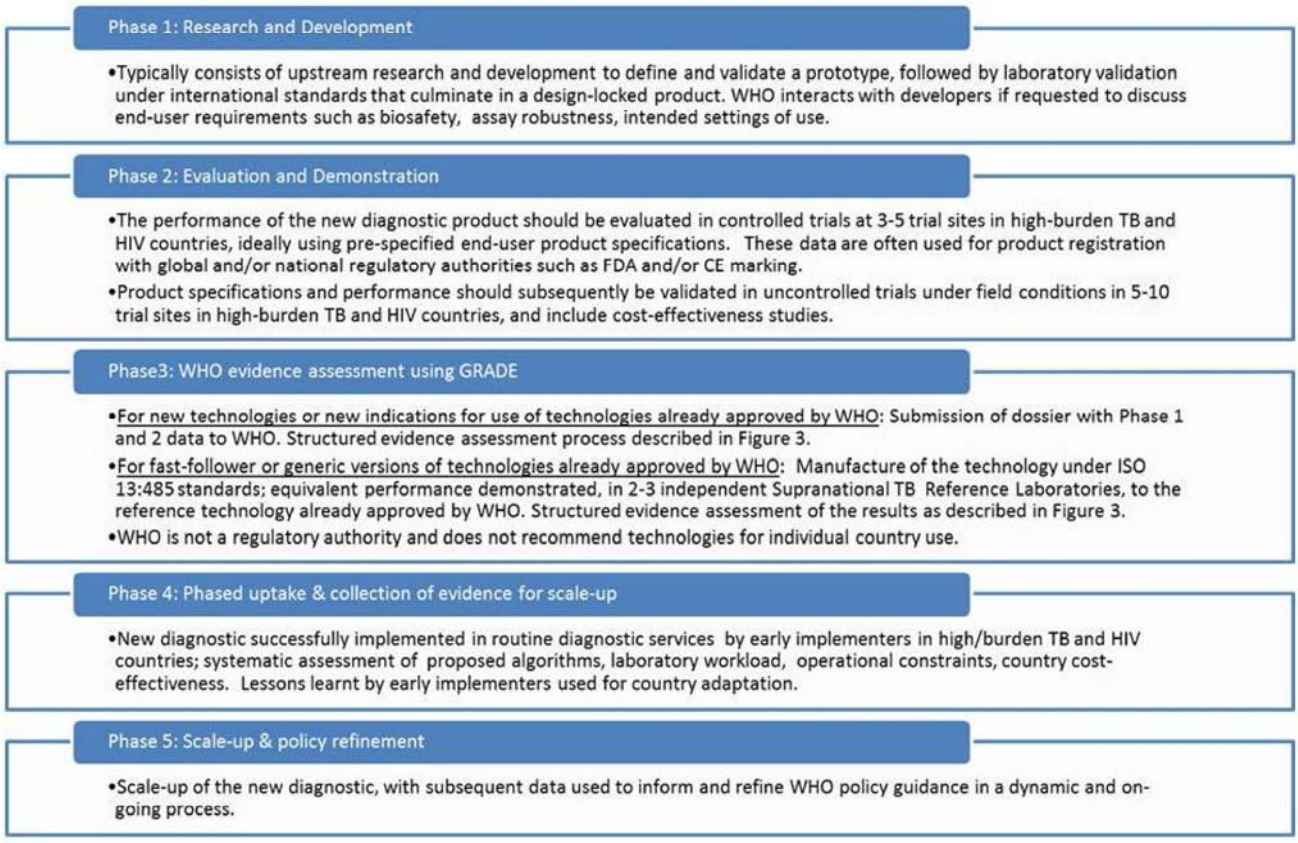
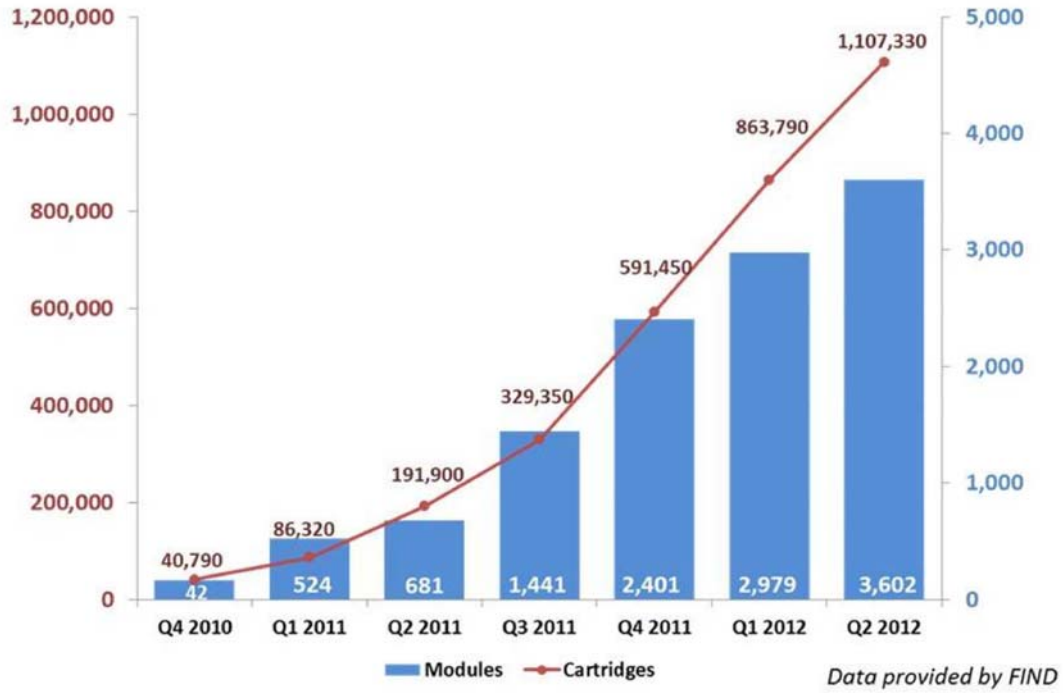


Figure 5. Global uptake of Xpert® MTB/RIF as of July 2012



As of 30 June 2012, a total of 749 GeneXpert instruments (comprising 3,602 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.

List of Tables

Table 1. WHO policy recommendations on Xpert® MTB/RIF

(Source:World Health Organization, 2011. *Policy Statement: Automated real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System*. Geneva: World Health Organization (WHO/HTM/TB/2011.4).

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:

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

2. **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 extra-pulmonary 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, for prevalence surveys and/or surveillance, and for recovering isolates for drug susceptibility testing other than rifampicin (including second-line anti-TB drugs).

Table 2.Summary of TB diagnostics evaluated by WHO, 2007-2012

APPROVED FOR USE (detailed policy guidance available athttp://www.who.int/tb/laboratory/policy_statements/en/index/html)

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 *M. 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 *M. tuberculosis* isolates.

2008: Molecular line probe assay for first-line anti-tuberculosis 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 *M. 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 *M. tuberculosis* isolates grown from culture. CRI is suitable for indirect testing on *M. 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-tuberculosis drugs (evaluated 2012)
- Loop-mediated isothermal amplification test kit for tuberculosis (evaluated 2012)

NOT APPROVED FOR USE (detailed policy guidance available athttp://www.who.int/tb/laboratory/policy_statements/en/index/html)

- Commercial serodiagnostic tests for TB diagnosis (evaluated 2011)
- Interferon-gamma release assays for detection of active TB in all settings (evaluated 2011)
- Interferon-gamma release assays as a replacement for tuberculin skin testing to detect latent TB in low- and middle-income (typically high TB and/or HIV burden) settings (evaluated 2011)

Table 3. False positive, false negative and predictive values for TB detection using Xpert MTB/RIF, according to varying TB prevalences in a sample population of 1,000 individuals

TB prevalence	PPV	NPV	True positive*	False negative*	False positive*	True negative*
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

* Sensitivity (91%) and specificity (99%) for Xpert MTB/RIF TB detection, compared with reference method (culture)

Table 4. False positive, false negative and predictive values for rifampicin resistance using Xpert MTB/RIF, according to varying prevalences of rifampicin resistance in a sample population of 1,000 individuals

Rifampicin resistance prevalence	PPV	NPV	True positive*	False negative*	False positive*	True negative*
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

* Sensitivity (95%) and specificity (98%) for Xpert MTB/RIF rifampicin resistance, compared with reference method (culture)

200 NEJM ANNIVERSARY ARTICLE

Tuberculosis, Drug Resistance, and the History of Modern Medicine

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Tuberculosis is a treatable airborne infectious disease that kills almost 2 million people every year. Multidrug-resistant (MDR) tuberculosis — by convention, a disease caused by strains of *Mycobacterium tuberculosis* that are resistant to isoniazid and rifampin, the backbone of first-line antituberculosis treatment — afflicts an estimated 500,000 new patients annually. Resistance to antituberculosis agents has been studied since the 1940s; blueprints for containing MDR tuberculosis were laid out in the clinical literature and in practice, in several settings, more than 20 years ago.[1,2] Yet today, barely 0.5% of persons with newly diagnosed MDR tuberculosis worldwide receive treatment that is considered the standard of care in the United States.[3] Those who have not received appropriate treatment continue to fuel a global pandemic that now includes strains resistant to most — and by some accounts all — classes of drugs tested.[4,5] Despite the enormity of the threat, investments to contain the epidemic and to cure infected patients have been halting and meager when compared, for example, with those made to address the acquired immunodeficiency syndrome (AIDS) pandemic. In this essay we seek to elucidate the reasons for the anemic response to drug-resistant tuberculosis by examining the recent history of tuberculosis policy.

Research in Tuberculosis — Midwife of Modern Biomedicine

On the evening of March 24, 1882, when Robert Koch completed his presentation on the infectious cause of tuberculosis, silence enveloped the crowded room at the Berlin Physiological Society.[6] A means of combating tuberculosis — a disease that in the 19th century caused, by some accounts, about 25% of all deaths in Massachusetts and New York and claimed the lives of one fourth of Europe's population — was now within reach.[7] Koch summarized the importance of his findings, for which he received the 1905 Nobel Prize, in a manuscript published in the *Berliner Klinische Wochenschrift* shortly after his announcement: "In the future the fight against this terrible plague of mankind will deal no longer with an undetermined something, but with a tangible parasite, whose living conditions are for the most part known and can be investigated further."[8]

But therapy lagged. It was not until 60 years later, in 1943, that the first effective antituberculosis agent, streptomycin, was isolated in the laboratory of Selman Waksman at Rutgers University (see timeline, available with the full text of this article at NEJM.org). In November 1944, a patient with tuberculosis received streptomycin and was declared cured of the disease.[6] Other cases of successful treatment soon followed.[9,10] The British Medical Research Council conducted the first large-scale clinical trial of streptomycin in 1948.[11] This study, said to be the world's first published drug trial that involved the randomization of participants, set the meth-

A timeline is available at NEJM.org

odologic standard for modern randomized, controlled trials. Although many patients were cured, a substantial proportion had a relapse; mycobacterial isolates cultured from the latter patients showed resistance to streptomycin.[12] That same year, two new antituberculosis agents, thiacetazone and para-aminosalicylic acid, came on the market. When either of these agents was administered with streptomycin, cure rates rose and acquired antibiotic resistance declined.[13] In 1951, isonicotinic acid hydrazide (isoniazid) was tested at Sea View Hospital in New York; it dramatically improved clinical outcomes and was soon introduced for wider use.[14] Isoniazid was followed by the development of pyrazinamide (1952), cycloserine (1952), ethionamide (1956), rifampin (1957), and ethambutol (1962).

With its high level of efficacy and ease of administration, rifampin revolutionized the treatment of tuberculosis.[15-17] But the advent of every new drug led to the selection of mutations conferring resistance to it. Resistance to rifampin was observed soon after it was first administered.[18] Laboratory data from trials revealed the rapid onset of isoniazid resistance among patients receiving monotherapy and the suppression of resistance when isoniazid was given in combination with streptomycin or para-aminosalicylic acid.[19] These observations led to the use of multidrug treatment regimens — a strategy widely used today to treat a variety of infectious diseases and cancers. Ultimately, through a series of multicountry clinical trials led by the British Medical Research Council, a four-drug regimen was recommended for use in patients with newly diagnosed tuberculosis. The backbone of such empirical regimens was the combination of isoniazid and rifampin, the most effective and reasonably well-tolerated oral agents, given for 6 to 8 months. Thus, short-course chemotherapy was born.[19]

Drug resistance, however, has remained a challenge. The early hypothesis that resistance always conferred a loss of bacterial fitness, and hence led to lower case fatality rates and decreased transmission of such strains, had been disproved by the 1950s.[19] The first national drug-resistance survey in the world, which involved 974 clinical isolates cultured from newly diagnosed cases of tuberculosis in Britain (1955–1956), showed strains that were resistant to streptomycin (2.5%), para-aminosalicylic acid (2.6%), and isoniazid (1.3%).[20] Similarly, data from the United States showed that isoniazid resistance increased from 6.3% (between 1961 and 1964) to 9.7% (between 1965 and 1968) among patients with newly diagnosed tuberculosis.[21] Between 1970 and 1990, there were numerous outbreaks of drug-resistant tuberculosis involving strains resistant to two or more drugs.[17,22,23] As early as 1970, an outbreak in New York City of highly virulent tuberculosis that was resistant to multiple drugs proved to be a grim reminder that resistance did not necessarily reduce a microbe's fitness: the index patient died; 23 of 28 close contacts had evidence of new infection, and active, drug-resistant disease developed in 6 of these 23 contacts, 5 of whom were children.[21]

Tuberculosis, whether caused by drug-susceptible or drug-resistant strains, rarely made even medical headlines, in part because its importance as a cause of death continued to decline in areas in which headlines are written. In such settings, where many of the social determinants of tuberculosis — extreme poverty, severe malnutrition, and overcrowded living conditions — became the exception rather than the norm, some public health experts declared that "virtual elimination of the disease as a public health problem" was in sight.[24] In the United States, federal funding for tuberculosis research was cut; consequently, drug discovery, development of diagnostics, and vaccine research ground almost to a halt.[17]

THE GREAT DIVERGENCE IN TUBERCULOSIS POLICY

Optimism that tuberculosis would soon be eliminated was not restricted to wealthy countries. At the 1978 International Conference on Primary Health Care in Alma-Ata (now called Almaty), Kazakhstan, delegates from around the world endorsed the goal of "health for all by the year 2000." The eradication of smallpox had been announced the previous year, and the future of international public health looked promising to many who were gathered there.

But it was not to be. By the mid-20th century, tuberculosis outcomes had diverged along the fault lines of the global economy: while tuberculosis became rare in countries where income was high, epidemics of the disease raged on in low-income settings. In 1982, the Mexican government defaulted on many of its loan payments,

triggering a debt crisis in many countries with weak economies. Increasing numbers of international health donors and policymakers, slow to contribute resources toward the ambitious Alma-Ata agenda, embraced the idea of selective primary health care: discrete, targeted, and inexpensive interventions.[25,26] Bilateral assistance withered, and poor countries became increasingly reliant on loans from international financial institutions such as the World Bank, which based its health agenda on the principles of "cost-effectiveness" and "affordable health for all" — the latter concept a nod to the Alma-Ata Declaration.[27]

Selective primary health care offered clear targets, measurable outcomes, and a high return on health investments, all of which appealed to donors worried about investing in countries that were on the brink of default.[28,29] But several leading causes of disability and death, including tuberculosis, were deemed too costly and complex to address in resource-poor settings and were largely excluded from the emerging, constricted agenda for effective health investments. "Leprosy and tuberculosis require years of drug therapy and even longer follow-up periods to ensure cure," wrote two of the architects of selective primary health care in 1979. "Instead of attempting immediate, large-scale treatment programs for these infections, the most efficient approach may be to invest in research and development of less costly and more efficacious means of prevention and therapy."[25]

But tuberculosis, which persisted in settings of poverty, could not be hidden away for long. In 1993, the World Bank began to use disability-adjusted life-years — a means of measuring the "cost-effectiveness" of a given health intervention that took into account morbidity, mortality, and age — to determine which health interventions to support.[30] As a result of this new economic calculus, short-course chemotherapy for tuberculosis was declared a highly "cost-effective" intervention and gained momentum.[31] Seizing the opportunity, the World Health Organization (WHO) shaped and promoted the DOTS (directly observed therapy, short-course) strategy, an approach that conformed to the selective primary health care agenda: simple to treat, algorithmic, and requiring no expensive inputs. According to this strategy, the diagnosis was to be made with the use of smear microscopy alone — in spite of the insensitivity and inability of this technique to detect drug resistance — and the treatment approach was to be based on the empirical use of first-line antituberculosis agents only.[32] Facility-based infection control was not part of the DOTS strategy. Despite these exclusions, DOTS was an important development in global tuberculosis policy. Increasingly, poor countries began implementing the DOTS approach; many lives were saved and many new cases averted. However, for children with tuberculosis, people with both tuberculosis and advanced disease from the human immunodeficiency virus (HIV), and the increasing proportion of patients infected with strains of tuberculosis that were already drug-resistant, the DOTS strategy provided limited options for prompt diagnosis and cure.

THE EMERGENCE OF MDR TUBERCULOSIS GLOBALLY

These shifts in tuberculosis policy — linked to the reconceptualization of this leading infectious killer of young adults and children from a disease deemed to be costly and difficult to treat to a disease deemed to be "cost-effective" to treat and slated for eradication — convey precisely what is meant by the "social construction of disease."[33] *M. tuberculosis* did not conform to the regnant disease-control strategy, and resistant strains continued to emerge and to be transmitted because empirical treatment with first-line antituberculosis drugs was ineffective for those sick with strains resistant to these drugs. HIV infection fanned epidemics of tuberculosis. In the late 1980s and early 1990s, outbreaks of MDR tuberculosis were again reported in the United States.[17] Genetic analysis of drug-resistant strains showed that airborne transmission of undetected and untreated strains played a major role in these outbreaks, disabusing practitioners of the notion that resistance stemmed solely from "sporadic pill taking."[17,34] Public health officials developed a national action plan to combat drug-resistant tuberculosis and to increase funding for relevant research.[17,35-37] The experience in New York City offered a blueprint that was quite different from the DOTS strategy; it consisted of diagnosis with the use of mycobacterial culture and fast-track drug-susceptibility testing, access to second-line antituberculosis medications, proper infection

control, and delivery of medications under direct observation.[1]

Outbreaks of MDR tuberculosis in the United States were a harbinger of the coming global pandemic. By the early-to-mid-1990s, MDR tuberculosis had been found wherever the diagnostic capacity existed to reveal it. But in contrast to the U.S. strategy, the WHO — the principal standard-setting body for many countries — continued to advocate the use of sputum-smear microscopy and first-line antituberculosis treatment alone for combating epidemics in resource-poor settings. Some international policymakers thought that treating MDR tuberculosis would be too expensive and complex — claims similar to those made about treating drug-susceptible tuberculosis before this approach was found to be "cost-effective" — and would distract attention from the newly branded (and often successful) DOTS strategy.[38] Contemporaneous experience in the United States and in several countries in the former Soviet Union suggested, however, that short-course chemotherapy was ineffective against strains shown to be resistant to precisely those drugs on which such therapy was based.[1,17,39,40]

THE LIMITS OF SHORT-COURSE CHEMOTHERAPY

The failure of short-course chemotherapy against MDR tuberculosis, though unsurprising clinically, was difficult politically. In Peru, for example, a campaign to promote the DOTS strategy had been so successful in making short-course chemotherapy available that the country's leaders elevated it as a point of national pride. Peru emerged as a crucible for debates about the treatment and management of MDR tuberculosis in poor countries.[2] In 1995, an outbreak in a shantytown in the northern reaches of Lima was identified.[41] Many patients were infected with strains found to have broad-spectrum resistance to first-line drugs. Nongovernmental organizations worked with the Peruvian Health Ministry to apply the standard-of-care treatment used in New York City and elsewhere in the United States. The strategy was modified to provide community-based care, with good results.[42] After arguing that the DOTS strategy alone could rein in the mutant bacteria, the WHO and other international public health authorities advised the Peruvian government to adopt a low-cost, standardized regimen for the treatment of MDR tuberculosis rather than protocols based on the results of drug-susceptibility testing. In the absence of tailored therapy, many hundreds of deaths occurred among some of Lima's poorest people.[43] As expected, amplification of drug resistance was documented.[44,45]

By the end of the 1990s, facing mounting evidence that MDR tuberculosis could be treated effectively in resource-poor settings,[46,47] a multi-institutional mechanism — the Green Light Committee — was created to encourage and learn from pilot projects for treating MDR tuberculosis.[2,17,48] This coincided with a grant from the Bill and Melinda Gates Foundation to scale up treatment of MDR tuberculosis in Peru and elsewhere and to change global policy.

TUBERCULOSIS POLICY AND GLOBAL HEALTH EQUITY

Drug resistance is well established as an inevitable outcome of antibiotic use; the fault lines of the MDR tuberculosis pandemic are largely man-made. The contours of global efforts against tuberculosis have always been mediated by both biologic and social determinants, and the reasons for the divergence in the rates of tuberculosis and drug resistance between rich and poor countries are biosocial.[49] As case rates dropped in wealthy countries, funding for research and implementation programs dried up, even though tuberculosis remained the world's leading infectious killer of young adults throughout the 20th century. Tuberculosis "control" in the 1990s was defined by the legacy of selective primary health care: targeted, "cost-effective" interventions packaged together, in the case of tuberculosis, as the DOTS strategy. Such protocols helped standardize tuberculosis treatment around the world — a process that was sorely needed — but they hamstrung practitioners wishing to address diagnostic and therapeutic complexities that could not be addressed by the use of sputum-smear microscopy and short-course chemotherapy or other one-size-fits-all approaches. These complexities, which now range from pan-resistant tuberculosis to undiagnosed pediatric disease, account for more than a trivial fraction of the 9 million new cases of tuberculosis and the almost 2 million deaths from this disease that occur around the globe each year.

The history of divergent policies for combat-

ing drug-resistant tuberculosis shows that decades of clinical research and effective programs in high-income settings did not lead to the deployment of similar approaches in settings of poverty. Achieving that goal demands a commitment to equity and to health care delivery.[50] The U.S. response to the outbreaks of MDR tuberculosis in New York City and elsewhere was bold and comprehensive; it was designed to halt the epidemic.[1,17] A similar response has not yet been attempted in low- and middle-income countries. Instead, selective primary health care and "cost-effectiveness" have shaped an anemic response to the ongoing global pandemic.

New diagnostics and therapeutics are urgently needed; most of the methods used currently were developed decades ago. Today, we have rapid nucleic acid–based tests for drug-resistant tuberculosis, sound models for laboratory expansion and for treatment delivery, and several drug candidates in the pipeline. To tackle tuberculosis, we also need an equity plan that takes seriously the biosocial complexity of a lethal airborne infection that has stalked us for centuries. The global AIDS effort of the past decade has shown how much can be accomplished in global health when effective diagnosis and care are matched with funding and political will. Stinting on investments or on bold action against tuberculosis — in all its forms — will ensure that it remains a leading killer of people living in poverty in this decade and the next.

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