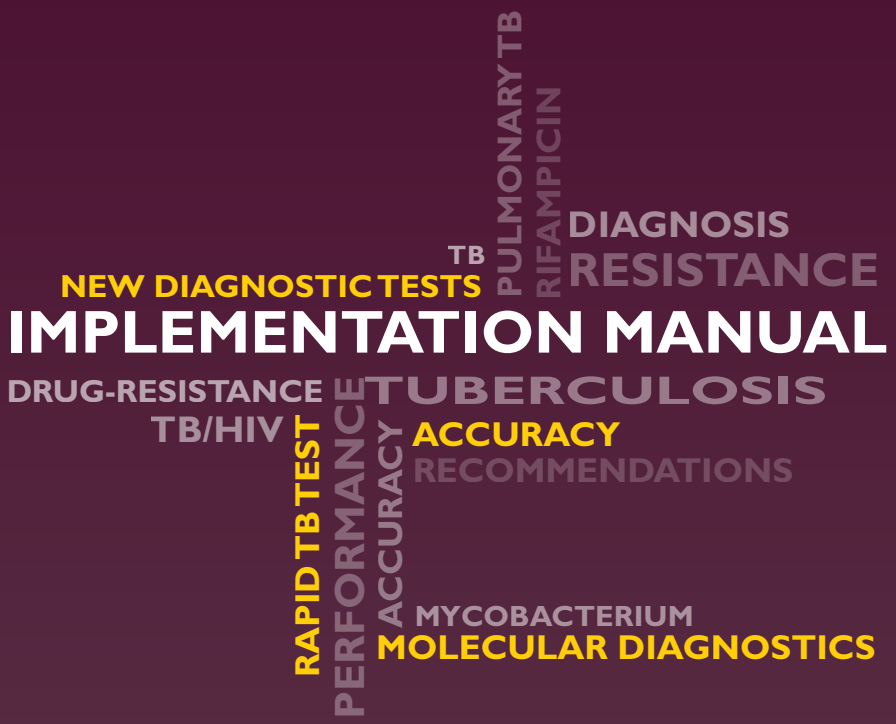
Xpert MTB/RIF
implementation manual

Technical and operational 'how-to':
practical considerations



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The first edition titled *Rapid implementation of the Xpert MTB/RIF diagnostic test* was based on the outcomes of a Global consultation on implementation and scale-up of the Xpert MTB/RIF assay convened by WHO in December 2010, together with the findings from WHO's first Expert Group Meeting on Xpert MTB/RIF, which had been convened in September 2010. This current edition of the implementation manual has been updated to reflect findings from the Expert Group Meeting convened in May 2013, and the experiences of early implementing countries and technical partners, including those experiences shared at the three annual Global Forums of implementers held by WHO in 2011, 2012 and 2013.

The initial data underlying the evidence base for the Xpert MTB/RIF assay were provided by FIND (Foundation for Innovative New Diagnostics). Subsequent evidence on the performance of Xpert MTB/RIF has been based on operational experiences and the body of work published by implementers and researchers from around the world. The development of the document was coordinated by Fuad Mirzayev, who also prepared the first draft.

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Abbreviations

AFB	acid-fast bacilli
CFU	colony-forming unit
CI	confidence interval
CrI	credible interval
CRS	composite reference standard
CSF	cerebrospinal fluid
DR-TB	drug-resistant TB
DST	drug-susceptibility testing
FIND	Foundation for Innovative New Diagnostics
GRADE	Grading of Recommendations Assessment, Development and Evaluation
LED	light emitting diode
LPA	line probe assay
MDR-TB	multidrug-resistant tuberculosis
MGIT	mycobacterial growth indicator tube
NPV	negative predictive value
NTM	non-tuberculous mycobacteria
NTP	National Tuberculosis Programme
PCR	polymerase chain reaction
PEPFAR	United States President's Emergency Plan for AIDS Relief
PPV	positive predictive value
RRDR	Rifampicin Resistance Determining Region
rpoB	gene encoding for the ß-subunit of the DNA-dependent RNA polymerase of *Mycobacterium tuberculosis*
RR-TB	rifampicin-resistant TB
STAG-TB	Strategic and Technical Advisory Group for TB
USAID	United States Agency for International Development
UNITAID	Innovative Financing to Shape Markets for HIV/AIDS, Malaria and Tuberculosis
WHO	World Health Organization
XDR-TB	Extensively drug-resistant tuberculosis

1. Background

The global priorities for tuberculosis (TB) care and control are to improve case-detection and to detect cases earlier, including cases of smear-negative disease which are often associated with coinfection with the human immunodeficiency virus (HIV) and young age, and to enhance the capacity to diagnose multidrug-resistant tuberculosis (MDR-TB). MDR-TB poses formidable challenges due to the complex requirements for diagnosis and treatment, and HIV-associated TB is often misdiagnosed due to the limitations of conventional diagnostic techniques. Alarming increases in MDR-TB incidence, the global emergence of extensively drug-resistant TB (XDR-TB), documented institutional transmission, and rapid mortality in patients with MDR-TB or XDR-TB who are coinfected with HIV have highlighted the urgent need for rapid diagnostic methods.

No single diagnostic test currently satisfies all the demands of "rapid", "affordable", and "easy". The World Health Organization (WHO) has endorsed the use of commercially available liquid culture systems and molecular line probe assays (LPAs) to rapidly detect MDR-TB; however, due to the tests' complexity and cost, as well as the need for sophisticated laboratory infrastructure and trained personnel, uptake has been limited in many resource-constrained settings.

Genotypic methods have considerable advantages in terms of scaling up the programmatic management and surveillance of drug-resistant TB, offering quicker diagnosis, standardized testing, the potential for high throughput, and having fewer requirements for ensuring laboratory biosafety. Since the development in the early 1980s of the polymerase chain reaction (PCR), molecular diagnostics have had a major impact on clinical medicine. However, despite several theoretical advantages, the use of molecular tests for TB has been limited, largely due to the complexities of DNA extraction, amplification and detection, and the biosafety concerns related to manipulating *Mycobacterium tuberculosis*. In addition, commercial nucleic acid amplification tests (NAATs) have proved to be less sensitive than microbiological culture, especially in cases of smear-negative TB. Moreover, culture largely remains necessary as a precursor to phenotypic drug-susceptibility testing (DST), and scaling up conventional culture and DST services is still slow and expensive, compounded by huge demands on laboratory infrastructure and human resources.

During 1996-2010, with support from the United States National Institutes of Health[1] and the Bill and Melinda Gates Foundation, the Foundation for Innovative New Diagnostics (FIND) partnered with Cepheid (Sunnyvale, CA, United States) and the University of Medicine and Dentistry of New Jersey to develop an automated, cartridge-based NAAT for TB that is based on the GeneXpert® multidisease platform. The GeneXpert system was launched in 2004, and it simplifies molecular testing by fully integrating and automating the three processes required for real-time PCR-based molecular testing (that is, specimen preparation, amplification and detection). The system consists of an instrument, personal computer, barcode scanner and preloaded software; single-use disposable cartridges contain lyophilized reagents, buffers and washes. Target detection and characterization is performed in real time using a six-colour laser-detection device.

The development of the Xpert® MTB/RIF assay for the GeneXpert platform was completed in 2009 and is considered an important breakthrough in the fight against TB. For the first time, a molecular test is simple and robust enough to be introduced and used outside conventional laboratory settings. Xpert MTB/RIF detects *M. tuberculosis* as well as mutation that confer rifampicin resistance using three specific primers and five unique molecular probes to ensure a high degree of specificity. The assay provides results directly from sputum in less than 2 hours. The GeneXpert system and the Xpert

MTB/RIF assay remain the only self-contained cartridge based fully automated DNA testing platform that can accurately detect both TB and resistance to rifampicin in less than 2 hours, and it is the only mature technology among a new generation of automated molecular diagnostic platforms.

In December 2010, WHO recommended the use of the Xpert MTB/RIF assay. The WHO's policy statement was issued in early 2011 and supported by a rapid implementation document (the first edition of this current document), which provided the technical "how-to" and operational considerations for rolling out the use of the assay; the implementation document also provided a simple checklist of prerequisites necessary for implementation along with key action points. An unprecedented uptake of this new technology followed the release of WHO's policy. By the end of December 2013, more than 2,000 GeneXpert instruments and more than 5 million Xpert MTB/RIF cartridges had been procured in the public sector in 98 countries eligible for concessional prices. Reports of experiences of using this technology have rapidly accumulated and have been regularly shared through peer-reviewed publications, systematic reviews, and reports from country and technical partners during several meetings of implementers that were organised by WHO.

In October 2013, WHO issued updated Policy Guidance[2], providing revised recommendations on using of Xpert MTB/RIF to diagnose pulmonary TB, paediatric TB, extrapulmonary TB and rifampicin resistance. This edition of the Xpert MTB/RIF implementation manual replaces the first edition and takes into consideration the current body of evidence and operational experiences available, in the context of the updated policy recommendations.

1 Helb, D, et al. Rapid Detection of Mycobacterium Tuberculosis and Rifampin Resistance by Use of On-Demand, Near-Patient Technology. Journal of Clinical Microbiology, 2010 48: 229–237.

2 *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children: policy update.* Geneva, World Health Organization, 2013 (available at http://www.who.int/tb/laboratory/policy_statements/en/)

2. Policy development

2.1 Procedure for developing policies

In 2008 WHO adopted the international GRADE process (Grading of Recommendations Assessment, Development and Evaluation)[3] for synthesizing and evaluating evidence. The GRADE process underpins all WHO recommendations and guidelines. The process provides a systematic means for assessing the quality of evidence used to formulate policies as well as for rating the strength the recommendations; the process aims at achieving a balance among a test performance, its risks and benefits, and its impact on patients and public health[4]. At WHO, the process of developing policies is overseen by the Guidelines Review Committee, which was specifically established for this purpose.

WHO's Global TB Programme has developed a structured, evidence-based process to facilitate the rapid development of policies and guidance on the use of new TB diagnostic tools, new diagnostic methods, and novel approaches to diagnosis using existing tools.

- The first step involves undertaking a systematic review and meta-analysis of the data where feasible, using standard methods appropriate for studies assessing diagnostic accuracy.
- The second step involves convening an Expert Group to evaluate the strength of the evidence base using the GRADE process as well as the operational and logistical considerations relevant to mainstreaming the tools or approaches into national TB control programmes; the Expert Group also identifies any gaps that need to be addressed by future research.
- The third step involves formulating WHO's policies and guidance on the use of these tools and approaches, and presenting them to WHO's Strategic and Technical Advisory Group for TB (STAG-TB); after endorsement by STAG-TB, the guidance is disseminated to Member States for implementation.

2.2 Initial policy recommendations and follow up

A meeting of the **Expert Group** was convened by WHO in September 2010. The Expert Group used the GRADE process to review data from published papers on Xpert MTB/RIF, as well as data from large multi-centre laboratory validation studies and demonstration studies coordinated by FIND, results from cost-effectiveness analyses[5] and unpublished data from 12 investigator-driven, single-centre studies. The process of evidence synthesis confirmed that there was a solid evidence base to support the widespread use of Xpert MTB/RIF to detect pulmonary TB and rifampicin resistance.

STAG-TB supported the Expert Group's recommendations[6] and advised that implementation of Xpert MTB/RIF technology should be phased in within the context of comprehensive national strategic plans addressing TB and MDR-TB. STAG-TB recommended that WHO should:

- develop a global strategy to ensure the rapid uptake of Xpert MTB/RIF using a systematic and phased approach, including developing mechanisms to monitor and assess the roll out of Xpert MTB/RIF; the strategy should ensure there is a clear plan for documenting the impact on case-detection, the scaling up of responses to MDR-TB and evaluating cost effectiveness;
- proceed with a Global Consultation to determine considerations for scaling up of the use of Xpert MTB/RIF under routine programme conditions, including developing diagnostic algorithms;

evaluating logistical considerations, and procurement and distribution procedures; implementing quality assurance plans and methods of waste disposal;

- assist countries by providing technical support and support for plans to include Xpert MTB/RIF in revised diagnostic algorithms.

At a **Global Consultation** convened by WHO during 30 November–2 December 2010, country representatives and technical partners discussed considerations for scaling up the use of Xpert MTB/RIF, and achieved broad consensus on the way forward. Key outcomes agreed at the consultation included interim diagnostic algorithms, optimal positioning of Xpert MTB/RIF at different levels of health-care system so that its use can be targeted at various risk groups, and advice on issues to be considered before the test is systematically rolled out in order to optimize its use and the benefits of the technology. The interim diagnostic algorithms were initially developed in consultation with the following Working Groups of the Stop TB Partnership: the Global Laboratory Initiative, the MDR-TB Working Group, the DOTS Expansion Working Group and the TB/HIV Working Group. The algorithms were discussed and then revised during the consultation.

Policy recommendations[7] on Xpert MTB/RIF were issued by WHO early in 2011, supported by a **Checklist for country implementation**[8] and a **Rapid Implementation document**[9].

In April 2011, WHO convened a meeting with **early implementers** of the Xpert MTB/RIF assay to refine the interim diagnostic algorithms, develop a core set of variables to determine the impact of introducing the technology on laboratory workload, and to clarify operational and logistical issues.

A second meeting of **early implementers** was convened by WHO in April 2012 to give participants the opportunity to share their experiences of introducing the assay under routine programmatic conditions.

A **third Global Forum of Xpert MTB/RIF Implementers**[10] was convened in April 2013 in association with the 5th GLI Partners Meeting, during which countries and their technical partners shared information about the lessons that had been learnt and the challenges encountered during scale-up, with a focus on evidence of the test's impact and how to link scaled-up diagnosis with scaled-up access to treatment.

2.3 Policy update

Since WHO's initial recommendations were made in 2010, evidence on the performance and use of Xpert MTB/RIF has rapidly accumulated. Given the amount of additional data that has emerged since 2010, an update of WHO's initial policy and guidance was warranted. WHO's Global TB Programme therefore commissioned three systematic reviews, including reviews of the utility of Xpert MTB/RIF in diagnosing TB and rifampicin resistance in pulmonary, extrapulmonary and paediatric TB. A review of published studies on the affordability and cost effectiveness of Xpert MTB/RIF was also performed.

In May 2013, an **Expert Group** convened by WHO reviewed the expanded body of evidence presented in the systematic reviews and applied GRADE process to the evidence. Based on the outcomes of the review and the recommendations of the Expert Group, which were presented to and supported by STAG-TB[11] in June 2013, an updated policy statement was issued in October 2013.[12]

2.4 Summary of WHO's 2013 policy recommendations

2.4.1 Using Xpert MTB/RIF to diagnose pulmonary TB and rifampicin resistance in adults and children

- Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in adults suspected of having MDR-TB or HIV-associated TB (**strong recommendation**, high-quality evidence).
- Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in children suspected of having MDR-TB or HIV-associated TB (**strong recommendation**, very low-quality evidence).
- Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in **all adults** suspected of having TB (**conditional recommendation** acknowledging resource implications, high-quality evidence).
- Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in **all children** suspected of having TB (**conditional recommendation** acknowledging resource implications, very low-quality evidence).
- Xpert MTB/RIF may be used as a follow-on test to microscopy in adults suspected of having TB who are not at risk of MDR-TB or HIV-associated TB, especially when further testing of smear-negative specimens is necessary (**conditional recommendation** acknowledging resource implications, high-quality evidence).

Remarks

These recommendations apply to the use of Xpert MTB/RIF for specimens of processed and unprocessed sputum.

These recommendations also apply to specimens of gastric lavage and aspirate from adults and children, the recommendation for adults is based on the generalization of data from children.

These recommendations support the use of a single sputum specimen for diagnostic testing, acknowledging that processing multiple specimens increases the sensitivity of Xpert MTB/RIF but also has resource implications.

Children suspected of having pulmonary TB but who have had a single negative result by Xpert MTB/RIF should undergo further diagnostic testing, and a child for whom there is a high clinical suspicion for TB should be treated even if an Xpert MTB/RIF result is negative or if the test is not available.

Conventional microscopy and culture remain essential for monitoring therapy and for performing DST for anti-TB agents other than rifampicin (including for isoniazid and second-line anti-TB agents).

Expanding the scope of the use of Xpert MTB/RIF and its placement in diagnostic algorithms will have significant implications for operational implementation, and its use should be phased in within the context of national strategic plans for TB.

Emerging data have shown that Xpert MTB/RIF detects some rifampicin-resistant strains that are identified as susceptible by phenotypic DST. Sequencing these discordant results usually resolves in favour of Xpert MTB/RIF, and patients missed by phenotypic DST have poor treatment outcomes on first-line treatment.

2.4.2 Using Xpert MTB/RIF to diagnose extrapulmonary TB and rifampicin resistance in adults and children

- Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test for cerebrospinal fluid (CSF) specimens from patients suspected of having TB meningitis (**strong recommendation** given the urgency of rapid diagnosis, very low-quality evidence).
- Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture or histopathology) for testing specific nonrespiratory specimens (lymph nodes and other tissues) from patients suspected of having extrapulmonary TB (**conditional recommendation**, very low-quality evidence).

Remarks

Individuals suspected of having extrapulmonary TB but who have had a single negative result from Xpert MTB/RIF should undergo further diagnostic testing, and those for whom there is a high clinical suspicion for TB (especially children) should be treated even if an Xpert MTB/RIF result is negative or if the test is not available.

For CSF specimens, Xpert MTB/RIF should be preferentially used instead of culture if the sample volume is low or if additional specimens cannot be obtained in order to make a quick diagnosis. If sufficient volume of material is available, concentration methods should be used to increase the yield.

Pleural fluid is a suboptimal sample for the bacterial confirmation of pleural TB regardless of the method used. A pleural biopsy is the preferred sample. The sensitivity of Xpert MTB/RIF in testing samples of pleural fluid is very low. Nevertheless, any individual with a positive result from pleural fluid tested by Xpert MTB/RIF should be treated for pleural TB; those with a negative result from Xpert MTB/RIF should have other tests.

Conventional microscopy and culture are essential for monitoring therapy and for performing DST for anti-TB agents other than rifampicin (including for isoniazid and second-line anti-TB agents).

Emerging data have shown that Xpert MTB/RIF detects some rifampicin-resistant strains that are found to be susceptible by phenotypic DST. Sequencing these discordant results usually resolves in favour of Xpert MTB/RIF, and patients missed by phenotypic DST have poor treatment outcomes on first-line treatment.

These recommendations do not apply to samples of stool, urine or blood, given the lack of data on the utility of Xpert MTB/RIF for these specimens.

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3 *GRADE Working Group* (http://www.gradeworkinggroup.org, accessed 10.12.2013)

4 *WHO handbook for guideline development*. Geneva, World Health Organization, 2012 (available at http://apps.who.int/iris/bitstream/10665/75146/1/9789241548441_eng.pdf)

5 Vassall A et al. Rapid diagnosis of tuberculosis with the Xpert MTB/RIF assay in high burden countries: a cost-effectiveness analysis. PLoS Medicine, 2011, 8:e1001120 (doi: 10.1371/journal.pmed.1001120).

6 *Strategic and Technical Advisory Group for Tuberculosis (STAG-TB): report of the tenth meeting*. Geneva, World Health Organization, 2010 (available at http://www.who.int/tb/advisory_bodies/stag_tb_report_2010.pdf?ua=1).

7 *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. Policy statement*. Geneva, World Health Organization, 2011 (available at http://whqlibdoc.who.int/publications/2011/9789241501545_eng.pdf)

8 *Prerequisites to country implementation of Xpert MTB/RIF and key action points at county level*. Geneva, World Health Organization, 2011 (available at http://whqlibdoc.who.int/hq/2011/WHO_HTM_TB_2011.12_eng.pdf)

9 *Rapid implementation of the Xpert MTB/RIF diagnostic test: technical and operational „How-to"; practical considerations*. Geneva, World Health Organization, 2011 (available at http://whqlibdoc.who.int/publications/2011/9789241501569_eng.pdf)

10 http://www.stoptb.org/wg/gli/meetings.asp

11 *Strategic and Technical Advisory Group for Tuberculosis (STAG-TB): report of the thirteenth meeting*. Geneva, World Health Organization, 2013 (available at http://www.who.int/tb/advisory_bodies/STAG_report2013.pdf?ua=1).

12 *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extrapulmonary TB in adults and children: policy update*. Geneva, World Health Organization, 2013 (available at http://www.who.int/tb/laboratory/policy_statements/en/).

3. Evidence base

3.1 Evidence available at the end of 2010

Initial data from published papers, along with data from large, multi-centre laboratory validation studies and demonstration studies coordinated by FIND, and unpublished data from investigator-driven single-centre studies were reviewed in late 2010 by WHO.

Results from **analytical studies**[13] showed that the Xpert MTB/RIF assay has analytic sensitivity for five genome copies of purified DNA, and 131 cfu/ml of *M. tuberculosis* spiked into sputum. The molecular beacons that target the *rpoB* gene cover all the mutations found in more than 99.5% of all rifampicin-resistant strains. There is no cross-reactivity with nontuberculous mycobacteria (NTM), and TB and rifampicin resistance were correctly detected in the presence of nontuberculous DNA or a mix of susceptible strains and resistant strains. When the sample reagent is added in a 2:1 ratio to sputum it kills more than 6 $\log_{10}$ cfu/ml of M. tuberculosis within 15 minutes of exposure, rendering more than 97% of smear-positive samples negative as assessed using Löwenstein–Jensen culture. No detectable infectious aerosols were generated during inoculation and testing.

Results from **controlled clinical validation trials**[14] involving 1730 individuals suspected of having TB or MDR-TB who were prospectively enrolled in 4 distinctly diverse settings showed that 92.2% of culture-positive patients were detected by a single direct Xpert MTB/RIF test. The sensitivity of a single Xpert MTB/RIF test in smear-negative culture-positive patients was 72.5% and this increased to 90.2% when three samples were tested. The specificity of Xpert MTB/RIF was 99%.

Results from **field demonstration studies**[15] involving 6673 individuals prospectively enrolled from 6 distinctly different settings confirmed these findings.

- **Test accuracy** was retained; a single Xpert MTB/RIF test directly on sputum detected 99% of smear-positive patients and 80% of patients with smear-negative disease. The overall sensitivity of a single, direct Xpert MTB/RIF test in culture-positive cases was 91%; in comparison, the sensitivity of a single direct smear test was 59.5% in culture positive cases. Rifampicin resistance was detected with 95.1% sensitivity and 98.4% specificity. Although HIV co-infection substantially decreased the sensitivity of microscopy (to 47%), it did not significantly affect the performance of Xpert MTB/RIF.
- **Mean time to detection** was less than 1 day for Xpert MTB/RIF, 1 day for microscopy, 17 days for liquid culture and more than 30 days for solid culture. Rifampicin resistance was detected in less than 1 day with Xpert MTB/RIF compared with an average of 75 days for phenotypic DST. When Xpert MTB/RIF results were not used to direct therapy, smear-negative TB patients started treatment after a median of 58 days compared with a median of 4 days when Xpert MTB/RIF results were used.
- **The operational aspects** assessed confirmed the robustness of Xpert MTB/RIF when used under varying conditions of temperature and humidity, and also confirmed that minimal training is required for personnel, and that there were high levels of user satisfaction. Storing cartridges in high-volume settings was a concern given that there is often a lack of space. The amount of waste generated was considerably more than was generated by microscopy. Xpert MTB/RIF requires an uninterrupted and stable electrical power supply, and annual calibration of the modules, which may pose problems in rural or remote settings.

Results from 12 single-centre evaluation studies that used varying designs and included different study populations reported that the sensitivity of Xpert MTB/RIF in detecting TB ranged from 70% to 100% in culture-positive patients to around 60% in patients with smear-negative disease. The specificity ranged from 91% to 100%. The pooled crude sensitivity for TB detection was 92.5%; the pooled crude specificity was 98%. The average sensitivity for detecting rifampicin resistance was 98%; the average specificity was about 99%.

3.2 Evidence base as of February 2013

Additional evidence[16] has been published since WHO's initial recommendations were made in 2010. There have been several reviews of the published evidence including a Cochrane review in January 2013, which evaluated the literature available until the end of December 2011. A significant number of additional studies were published in 2012 and 2013, and the review commissioned by WHO included all of the evidence on the use of Xpert MTB/RIF published until the end of February 2013.

The reviews focused on four specific areas of interest:

- using Xpert MTB/RIF to diagnose pulmonary TB and rifampicin resistance in adults;
- using Xpert MTB/RIF to diagnose extrapulmonary TB in adults and children;
- using Xpert MTB/RIF to diagnose pulmonary TB and rifampicin resistance in children;
- the affordability and cost effectiveness of Xpert MTB/RIF for diagnosing TB.

Using Xpert MTB/RIF to diagnose pulmonary TB and rifampicin resistance in adults

A total of 27 unique studies involving 9558 participants were included in the systematic review. Two of the 27 studies were multicentre international studies (one with five distinct study centres and the other with six). Two of the 27 studies evaluated Xpert MTB/RIF in primary care clinics where the results were used to begin treatment on the same day. Sixteen studies (59%) were performed in low-income or middle-income countries.

When used as an initial diagnostic test replacing smear microscopy, Xpert MTB/RIF achieved a pooled sensitivity of 88% (95% credible interval [CrI], 84-92%) and a **pooled specificity of 99%** (95% CrI, 98-99%), (22 studies, 9008 participants).

When used as an add-on test following a negative smear microscopy result, Xpert MTB/RIF yielded a pooled sensitivity of 68% (95% CrI, 61-74%) and pooled specificity of 99% (95% CrI, 98-99%), (23 studies, 7151 participants).

When used in cases of smear-positive, culture-positive TB, the pooled sensitivity of Xpert MTB/RIF was 98% (95% CrI, 97-99%) (23 studies, 1952 participants).

When used in people living with HIV, the pooled sensitivity of Xpert MTB/RIF was 79% (95% CrI, 70-86%), (7 studies, 1789 participants), for people without HIV infection, the pooled sensitivity was 86% (95% CrI, 76-92%), (7 studies, 1470 participants).

When used to detect rifampicin resistance, Xpert MTB/RIF achieved a **pooled sensitivity of 95%** (95% CrI, 90-97%), (17 studies, 555/2624 total specimens) and a **pooled specificity of 98%** (95% CrI, 97-99%), (24 studies, 2414 specimens).

Xpert MTB/RIF was highly accurate in distinguishing TB from NTM in clinical specimens: among 180 specimens with NTM, Xpert MTB/RIF had a positive result in only 1 specimen that grew NTM (14 studies, 2626 participants).

Using Xpert MTB/RIF to diagnose extrapulmonary TB in adults and children

A total of 15 published studies and 7 unpublished studies (5922 samples) were included in the review. The majority of studies were performed

in settings with high-burden of TB. Due to the heterogeneity of the specimen types included in the studies, pre-specified subgroups of specimens (pleural fluid, lymph node specimens [tissue and aspirate combined], other tissues and CSF) with a comparison against culture and against a composite reference standard (CRS) were included in the meta-analysis.

Using culture as the reference standard, the pooled sensitivity of Xpert MTB/RIF in **lymph node tissues or aspirates** was 84.9% (95% confidence interval [CI], 72.1-92.4%) (14 studies, 849 samples); the pooled sensitivity in **gastric fluid** was 83.8% (95% CI, 65.9-93.2%) (12 studies, 1258 samples); and in **other tissue specimens** it was 81.2% (95% CI, 67.7-89.9%) (12 studies, 699 specimens). In CSF, the pooled sensitivity of Xpert MTB/RIF compared against culture as a reference standard was 79.5% (95% CI, 62.0-90.2%) (16 studies, 709 specimens); compared against a CRS it was 55.5% (95% CI: 44.2-66.3%) (6 studies, 512 specimens). In **pleural fluid**, the pooled sensitivity of Xpert MTB/RIF compared against culture was 43.7% (95% CI, 24.8-64.7%) (17 studies, 1385 specimens); a pooled sensitivity against a CRS was 17.0% (95% CI, 7.5-34.2%) (7 studies, 698 specimens). The pooled specificity was always greater than 98.7% if Xpert MTB/RIF was tested against a CRS. The data for other types of specimens (such as ascitic fluid, pericardial fluid, urine, blood and stool) were limited and therefore were not considered for analysis by subgroup.

Using Xpert MTB/RIF to diagnose pulmonary TB and rifampicin resistance in children

A total of 16 studies (12 published, 4 unpublished) were included in the review. All studies were performed at higher levels of care, and the children included in the studies were mainly inpatients.

The diagnosis of pulmonary TB was evaluated in 13 studies that included 2603 participants. The overall pooled sensitivity of Xpert MTB/RIF compared against culture as the reference standard in children suspected of having TB was 66% (95% CrI, 52-77%) in 10 studies where expectorated sputum or induced sputum was used; the pooled sensitivity was 66% (95% CrI, 51-81%) in 7 studies where gastric lavage aspirates were used. The pooled specificity of Xpert MTB/RIF compared against culture as the reference standard was at least 98% with narrow confidence intervals. The pooled sensitivity of Xpert MTB/RIF in specimens from culture-negative paediatric patients compared against a clinical TB reference standard was very low at 4% for specimens of expectorated or induced sputum (8 studies) and 15% for samples from gastric lavage or aspiration (3 studies); the credible intervals were wide, indicating a substantial potential for over-diagnosis of TB in children using clinical TB as the reference standard. The sensitivity of Xpert MTB/RIF in detecting rifampicin resistance in specimens from children was 86% (95%CrI, 53-98%).

Affordability and cost effectiveness of using Xpert MTB/RIF to diagnose tuberculosis

Twelve published papers were identified that compared the costs of current diagnostic algorithm for diagnosing TB and MDR-TB with the costs of using Xpert MTB/RIF as the initial diagnostic test or as a follow-on test to microscopy. The setting for the majority of analyses was South Africa; two studies included other countries in sub-Saharan Africa (Botswana, Lesotho, Namibia, Swaziland and Uganda); one study included countries in the former Soviet Union; and one global analysis included all countries. Seven of the 12 studies were analyzed costs and 5 were cost-effectiveness analyses. Wide variations in the methods used, the underlying assumptions, and the intended use of Xpert MTB/RIF made a systematic review impossible, however given these limitations, the review concluded that:

- using Xpert MTB/RIF to diagnose TB and MDR-TB is cost effective compared with current practices when applied to all individuals suspected of having TB and for HIV-positive individuals suspected of having TB;
- the cost of performing one Xpert MTB/RIF test was US$ 15–39, depending on

the cost of the cartridge and where the machine was placed;

- using Xpert MTB/RIF could be cost saving for TB patients;
- using Xpert MTB/RIF is more costly than current practices, but the increased costs represent only a small share of funding available for TB-control programmes.

13 For details see the original policy document (available at: http://www.who.int/tb/laboratory/mtbrifrollout/en/index.html)

14 Boehme C et al. Rapid molecular detection of tuberculosis and rifampin resistance. New England Journal of Medicine, 2010, 363:1005–1015.

15 Boehme C et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet , 2011, 377:495–1505.

16 Further details available in the Expert Group Meeting report at http://www.who.int/tb/laboratory/policy_statements/en/.

4. Positioning the test and selecting a site

A GeneXpert instrument can be positioned anywhere, from a peripheral clinic, mobile laboratory or doctor's office to a high-throughput reference laboratory. The selection of a site will depend on the testing workload and the efficiency of referral networks, and should take into consideration the infrastructure requirements, the human resources capacity and running costs.

In order to meet infrastructure requirements and optimize the throughput of an instrument and running costs, machines are often placed above the peripheral level, which requires establishment of reliable specimen or patient-referral networks. In general, while proficiency in conducting Xpert MTB/RIF is needed at the reference laboratory level in order to monitor and support a country's laboratory network, the Xpert MTB/RIF testing should not be placed solely in centralized reference laboratories since patients gain the greatest benefit from the test when it is placed as close as possible to the point of care.

Once the GeneXpert instrument is available, Xpert MTB/RIF assay does not require additional laboratory equipment, but the sophisticated nature of the device requires that certain conditions and infrastructure be present to ensure its efficient use. These considerations may limit where it can be positioned (see section 7.1 for details). The device needs:

- a stable and continuous electrical supply to avoid interruptions to the procedure and the subsequent loss of results, waste of cartridges and possible damage to or failure of the modules;
- to be secured against theft, particularly the accompanying computer;
- an ambient temperature of 15–30 °C in the room where the instrument is placed;
- adequate storage space for the cartridges with an ambient temperature of 2–28 °C;
- trained staff to perform the test;
- biosafety precautions similar to those needed for direct smear microscopy and as described in WHO's 2012 Tuberculosis laboratory biosafety manual.[17]

When choosing where to place the Xpert MTB/RIF testing, the following issues should be considered:

1. the groups to be targeted for testing with Xpert MTB/RIF;
2. the diagnostic pathway or algorithm to be used, and the availability of other screening or diagnostic tests in the facilities and laboratory network;
3. the current or estimated case-load of the facility that cares for the targeted patient groups;
4. the existence and structure of the network for specimen transport and patients' referral;
5. the possibility of referring specimens for further susceptibility testing when rifampicin resistance has been detected;
6. the availability of adequate infrastructure;
7. the availability of dedicated personnel who can be trained, perform testing and keep equipment in good order;
8. the funding available for capital and running costs (as described in section 7.3);
9. the availability of sufficient capacity to appropriately treat patients with TB and rifampicin-resistant TB who are detected.

17 *Tuberculosis laboratory biosafety manual*. Geneva, World Health Organization, 2012. WHO/HTM/TB/2012.11 (available at http://apps.who.int/iris/bitstream/10665/77949/1/9789241504638_eng.pdf)

5. Testing and managing patients

5.1 Selecting individuals to be tested

The decision about whether to perform an Xpert MTB/RIF assay should be made by a health-care professional who conducts a thorough **risk assessment** for the likelihood of TB for each individual presenting at the health centre. To facilitate the decision on whether the Xpert MTB/RIF should be used and at which stage of the diagnostic process, these individuals can be assigned into several groups presented here.

WHO's updated policy document continues to strongly advise that Xpert MTB/RIF be used as **the initial diagnostic test in both adults and children who are at risk of MDR-TB or HIV-associated TB**, and that these two groups should be prioritized for testing with Xpert MTB/RIF when resources are limited.

Group A

This group includes individuals (both adults and children) suspected of having TB who are considered to be at risk of harbouring drug-resistant TB bacilli (these risk groups should be defined according to national policies or as defined in WHO's Guidelines for the Programmatic Management of Drug-resistant TB[18]).

It also includes both adults and children who have been treated with anti-TB drugs and in whom TB has again been diagnosed, that is, all retreatment categories (failure, return after loss to follow-up, return after relapse).

Xpert MTB/RIF should be used as the initial diagnostic test in these individuals rather than conventional microscopy, culture and DST.

A country or setting with high prevalence of rifampicin resistant TB (RR-TB) may also decide to use Xpert MTB/RIF for all smear-positive cases to rapidly detect rifampicin resistance.

Group B

Individuals (adults and children) suspected of having HIV-associated TB should ideally be offered HIV testing routinely, preferably before investigation with Xpert MTB/RIF. HIV testing should be performed according to national guidelines.

Among adults and adolescents living with HIV, a person suspected of having TB is defined as anyone who reports any one of the following symptoms: current cough, fever, weight loss or night sweats.[19] Among children living with HIV, TB should be suspected in any child who has any one of the following symptoms: poor weight gain, fever, current cough or a history of contact with someone who has TB.[20]

Xpert MTB/RIF should be used as the initial diagnostic test rather than conventional microscopy, culture and DST in all persons living with HIV who have signs or symptoms of TB, in persons who are seriously ill and suspected of having TB regardless of their HIV status, and in those whose HIV status is unknown but who present with strong clinical evidence of HIV infection in settings where there is a high prevalence of HIV or among members of a risk group for HIV.

Policy recommendations also allow the use of Xpert MTB/RIF as a **follow-on test to microscopy in adults** who are not considered to be at risk of either MDR-TB or HIV-associated TB. This recommendation acknowledges that evidence from systematic reviews has shown significant diagnostic superiority of Xpert MTB/RIF over microscopy when compared to culture.[21] It is **conditional**, however, taking into account significant resource implications if all individuals with negative sputum smear results are routinely tested with Xpert MTB/RIF. Thus, this recommendation is not to be used as a rule suggesting that all smear-negative individuals should be tested with Xpert MTB/RIF but rather as a possible, well justified strategy in some settings.

Group C

This group includes adults suspected of having TB but who are not at risk of MDR-TB or HIV-associated TB (that is, adults who are HIV-negative or whose HIV status is unknown and who are not a member of a risk group for HIV or who live in a setting with a low prevalence of HIV).
These individuals may receive an Xpert MTB/RIF test as an initial diagnostic test for TB. When resource limitations do not allow Xpert MTB/RIF to be used for all individuals, sputum-smear examination may be conducted first; using Xpert MTB/RIF for smear-negative individuals will identify TB cases missed by smear microscopy.

The updated WHO policy document recommends using the Xpert MTB/RIF assay for **all individuals (adults and children) suspected of having TB**. These recommendations are conditional acknowledging significant resource implications should programmes decide to test everyone suspected of having TB (a sizeable group in many countries) using the Xpert MTB/RIF assay. The recommendation for adults is based on stronger body of evidence than for children, however given the diagnostic difficulties in paediatric TB diagnosis, children may be prioritized for Xpert MTB/RIF testing if resources are limited.

Group D

This group includes all individuals suspected of having TB (adults and children). **Xpert MTB/RIF may be used as an initial diagnostic test for TB.** This can result in more bacteriologically confirmed patients and shortened time to treatment[22]. Resource limitations may affect the ability of national programmes to undertake Xpert MTB/RIF testing in all individuals in this group.

In many settings, the majority of individuals suspected of having TB will not have risk factors for MDR-TB or HIV-associated TB. Therefore, careful consideration should be given to the resource implications and cost effectiveness of routinely offering Xpert MTB/RIF testing. Smear microscopy may be placed first in the diagnostic algorithm, with Xpert MTB/RIF used as a follow-on test for those who have negative smear microscopy results but who are suspected of having TB with the aim of finding TB cases missed by smear microscopy. While Xpert MTB/RIF is more expensive than conventional microscopy, using it as the initial diagnostic test will increase the number of patients with bacteriologically confirmed TB, given the higher sensitivity of Xpert MTB/RIF especially in settings with a high prevalence of HIV. Such an algorithm may require additional screening using either chest X-ray (if it is accessible and affordable) or further clinical assessment as a pre-test screening tool to reduce the numbers of individuals to be tested, given

that in most settings the vast majority (e.g. ~90%) of individuals suspected of having TB would have a negative result from smear microscopy. Setting-specific operational research is needed to understand the cost effectiveness of using smear microscopy or chest X-ray, or both, before Xpert MTB/RIF.

5.2 Test performance

As with any other diagnostic test, the performance of Xpert MTB/RIF depends on the prevalence of the target conditions (TB disease and rifampicin resistance) in the population tested, and on the reference standard used.

5.2.1 Accuracy of the reference standard

Culture is regarded as the best reference standard for active TB, and was the reference standard used in the systematic review on use of Xpert MTB/RIF in pulmonary TB. Phenotypic culture-based DST methods using WHO's recommended critical concentrations were the reference standard for rifampicin resistance.[23]

Three studies have raised concerns about phenotypic DST methods, in particular using the automated BACTEC MGIT (mycobacterial growth indicator tube) 960 Mycobacterial Detection System (Becton Dickinson, Franklin Lakes, NJ, United States) to detect rifampicin resistance. One study involved several TB Supranational Reference Laboratories (Van Deun 2009) and reported that the BACTEC 460 system and the BACTEC MGIT 960 system missed certain strains associated with low-level rifampicin resistance.[24] Another study (Williamson 2012) used Xpert MTB/RIF and gene sequencing, and identified four patients (three with clinical information available) whose TB isolates contained mutations in the *rpo*B gene but appeared to be rifampicin-susceptible according to MGIT 960 system. In that study, 2/49 (4.1%) patients whose isolates did not have apparent mutations of the *rpo*B gene, experienced treatment failure compared with 3/3 (100%) patients whose isolates did have the *rpo*B gene mutations but had been found to be susceptible to rifampicin using phenotypic methods[25].

A study involving retreatment patients (Van Deun 2013) found that several *rpo*B mutations conferring low-grade resistance were often missed by rapid phenotypic DST, particularly with the MGIT 960 system but also to a lesser extent by conventional (solid media) DST. The authors suggested that this may be the reason why molecular DST for rifampicin resistance is perceived to have insufficient specificity.[26] Although the study involved retreatment patients, the results appear to hold also for individuals newly diagnosed with TB (Van Deun, personal communication, 2013).

Therefore, determining specificity of a molecular DST method using only phenotypic DST as a reference may underestimate the specificity of the molecular method of DST. In light of these findings, it is unclear whether and to what extent Xpert MTB/RIF might outperform phenotypic DST methods for detecting rifampicin resistance.

WHO will continue to collect and evaluate data on this issue, and will formally review the accuracy of phenotypic resistance standards for DST once sufficient data become available.

5.2.2 Using Xpert MTB/RIF to detect TB

Given the high sensitivity of Xpert MTB/RIF in detecting TB (88%), the negative predictive value (NPV) is greater than 98% both in settings with a low prevalence of TB and in those with a high prevalence of TB – that is, a negative result accurately excludes TB in most situations. Typically, in high-burden settings, between 10% and 20% of persons with respiratory symptoms will have culture-confirmed TB. In such settings the vast majority of patients with a negative result from Xpert MTB/RIF will not have TB. However, the ability of any diagnostic test using sputum specimens to detect TB depends on the quality of the specimen collected; therefore, an individual with a negative result from Xpert MTB/RIF could still have TB. An individual still suspected of having TB after a negative Xpert MTB/RIF test may,

therefore, require further clinical management and another diagnostic test, including a repeated Xpert MTB/RIF test using a different sputum specimen.

The specificity of Xpert MTB/RIF for detecting TB is very high (99%), and false-positive results are likely to be linked to the detection by Xpert MTB/RIF of dead M. tuberculosis bacilli that would not be detected by culture, which is the present reference standard. Given that the specificity of Xpert MTB/RIF is not 100%, the positive predictive value (PPV) of Xpert MTB/RIF testing is adversely affected in settings with a low prevalence of disease or in populations with a low prevalence. Testing for TB is not usually implemented in a general, asymptomatic population but in individuals suspected of having TB following some form of screening involving, for example, symptom assessment or chest X-ray. Such screening procedures increase the prevalence of TB in the group tested, and improve the PPV of the test, making concerns related to false-positive results less relevant.

5.2.3 Using Xpert MTB/RIF to detect rifampicin resistance

Given the high sensitivity of Xpert MTB/RIF in detecting rifampicin resistance (95%), the NPV (the NPV for rifampicin resistance is the proportion of cases diagnosed as rifampicin-susceptible that are truly susceptible) is greater than 98% both in settings with a low prevalence of rifampicin resistance and those with a high prevalence of rifampicin resistance. Therefore a negative result accurately excludes the possibility of rifampicin resistance and, usually, no further testing is required to confirm negative results. In rare instances, when a patient is strongly suspected of having MDR-TB even after a negative result from Xpert MTB/RIF, a follow-up test may be done using phenotypic culture-based DST to detect rifampicin resistance that is conferred by regions outside of the *rpo*B region detected by Xpert MTB/RIF. Administrative errors are often more frequent than technical errors, and an unexpected result suggesting susceptibility to rifampicin could belong to a specimen from a different patient. Follow-up testing using Xpert MTB/RIF on a fresh specimen may be done if in doubt.

The specificity of Xpert MTB/RIF in detecting rifampicin resistance is very high (98%), and increasing evidence has shown that the infrequent occurrence of so-called **false-positive results may be linked to the detection by Xpert MTB/RIF of strains that are truly resistant to rifampicin, but which are not detected by the phenotypic culture-based DST, the present reference standard**. Such strains appear to have clinically relevant mutations in the region conferring resistance to rifampicin, causing disease for which first-line treatment is likely to fail. A study by Van Deun and colleagues[27] showed that an epidemiologically-significant proportion of rifampicin-resistant strains (10-13%) in patients who have experienced their first treatment failure and in relapsed patients may be missed by rapid phenotypic DST.

The PPV for detecting rifampicin resistance (the PPV for rifampicin resistance is the proportion of cases diagnosed as rifampicin-resistant that are truly resistant) using Xpert MTB/RIF exceeds 90% in settings or groups of patients where the underlying prevalence of rifampicin resistance is greater than 15%, and the PPV is probably even higher considering the limitations of the present reference standard, as mentioned above. In settings or groups where rifampicin resistance is rare, the PPV is adversely affected but it **can be greatly improved by undertaking a careful risk assessment of individual patients and targeting testing carefully** to increase the pre-test probability of rifampicin resistance.

It is important to differentiate between new cases of TB and previously treated cases of TB; previously treated cases are much more likely to have MDR-TB. According to drug resistance surveillance data from 114 countries, the global weighted proportion of MDR-TB among previously treated cases is 20% (95% CI, 13-26%), which is several times higher than the proportion of new cases with MDR-TB (3.7% ; 95% CI, 2.1-5.2%).[27] **Therefore, even in settings with a low prevalence**

of MDR-TB, testing *previously treated* TB cases with Xpert MTB/RIF will result in a high PPV for the detection of rifampicin resistance.

5.3 Interpreting results from Xpert MTB/RIF

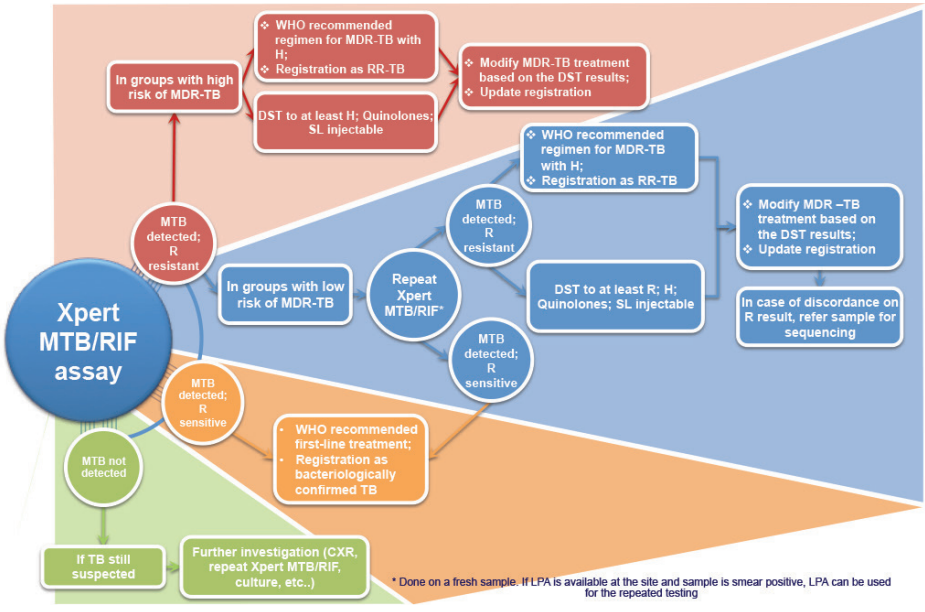
To complete any diagnostic algorithm, the test results need to be interpreted appropriately. Accurately interpreting results allows health-care workers and clinicians to make correct decisions about the interventions needed in relation to **patient management** and **registration**, and to any **additional laboratory work-up** that may be required. It is therefore important to train health-care staff how to interpret and follow-up any new test being introduced.

The interpretation of Xpert MTB/RIF results and follow-on steps will depend on both the result and the risk group from which the patient originated, based on the risk assessment as described in section 5.1. All patients identified as having TB by Xpert MTB/RIF should be initiated on the appropriate WHO-recommended treatment regimen as soon as possible. The prompt treatment initiation will have a positive effect on patients' outcomes, and a treatment regimen can be refined later if additional results become available.

As shown in Figure 1, an Xpert MTB/RIF result can indicate that *M. tuberculosis* (MTB) was not detected, MTB was detected and was not resistant to rifampicin (that is, it is rifampicin susceptible), or that MTB was detected and it was resistant to rifampicin. A small proportion of tests may result in an error or invalid result; these tests need to be repeated.

When Xpert MTB/RIF does not detect *M. tuberculosis*, the disease can be ruled out in most cases unless there is still a strong suspicion of TB

Figure 1. Interpreting results from Xpert MTB/RIF tests



CXR [chest X-ray], DST [drug-susceptibility testing], H [isoniazid], LPA [line probe assay], MDR-TB [multidrug-resistant TB], MTB [Mycobacterium tuberculosis], R [rifampicin], RR-TB [rifampicin-resistant TB]

(special attention is required in people living with HIV who have signs and symptoms of TB) that may warrant further investigation (such as a chest X-ray, culture, another Xpert MTB/RIF test, or a trial of antibiotics). The ability of any diagnostic test to detect TB depends on the quality of the specimen collected.

When Xpert MTB/RIF detects *M. tuberculosis* without rifampicin resistance, the patient should be referred for the appropriate WHO-recommended first-line regimen and registered as a case with susceptible bacteriologically confirmed TB. Further testing by phenotypic DST is not required.

When Xpert MTB/RIF detects *M. tuberculosis* with rifampicin resistance, decisions about subsequent steps depend on the patient's risk group.

- In patients from a group considered to be **at high risk of MDR-TB**, a WHO-recommended regimen for MDR-TB with the addition of isoniazid should be initiated; the patient should be registered as having bacteriologically confirmed rifampicin-resistant TB (RR-TB), and another sputum sample should be taken immediately and prior to starting treatment; these additional specimens should be sent for phenotypic DST for at least isoniazid, fluoroquinolones and second-line injectables. Confirmatory testing of rifampicin resistance using another testing technology is not necessary in such cases (given the high PPV for rifampicin resistance in this group). When the DST results are available, treatment can be modified if necessary and the patient's registration can be updated accordingly. Treatment modifications may include stopping isoniazid if resistance has been found, changing the quinolone and/or second-line injectable, or, in the case of XDR-TB, placing the patient on an appropriately designed regimen that includes group V drugs. The patient's registration should be modified to reflect any new information, and the case should be notified according to national regulations.
- In patients considered to be **at low risk of MDR-TB**, rifampicin resistance may be unexpected and clinicians may be hesitant to enrol patients on a treatment regimen requiring second-line drugs (mostly because of the treatment length and concerns about toxicity). An unexpected Xpert MTB/RIF result may be attributed to the PPV for rifampicin resistance in a group that has a low underlying prevalence, or may result from nonsystematic or random errors at the preanalytical or postanalytical stages of testing (these errors are relatively frequent even in quality-assured laboratories). These include clerical errors made when information about specimens or test results is recorded, or administrative errors that result in specimens being mixed up, etc. **An immediately repeated Xpert MTB/RIF test on a fresh specimen can be useful** in excluding preanalytical and postanalytical errors and improving a clinician's confidence when deciding on treatment.

When the result of a second Xpert MTB/RIF test identifies TB but not rifampicin resistance (an expected result in an individual at low risk of MDR-TB), a WHO-recommended first-line regimen should be prescribed, and the patient should be registered as having susceptible, bacteriologically confirmed TB. Further testing by phenotypic DST is not required.

When the result of a second Xpert MTB/RIF test on a fresh specimen again shows rifampicin resistance, a WHO-recommended regimen for MDR-TB with the addition of isoniazid may be started without any further delay. In this case the patient should be registered as having bacteriologically confirmed rifampicin-resistant TB, and an additional specimen should be taken for phenotypic DST to re-confirm resistance to rifampicin and also to test for susceptibility to isoniazid, fluoroquinolones and second-line injectables. When DST results are available, the treatment regimen and patient registration should be adjusted as appropriate. Treatment modifications may include stopping isoniazid if resistance has been found, changing

quinolone and/or second-line injectable, or, in cases where XDR-TB has been detected, placing the patient on an appropriately designed regimen that includes group V anti-TB agents. The patient's registration should be modified to reflect new information and the case should be notified according to national regulations.

In cases where discordant results are obtained from Xpert MTB/RIF and phenotypic DST or LPA, the available culture isolate should be referred to a reference laboratory for DNA sequencing; while awaiting the results, a clinical decision should be made whether to continue the MDR-TB regimen. The detection of a change in the amino acid sequence of the rifampicin resistance determining region (RRDR) should be considered confirmation of clinically significant rifampicin resistance.

The management of patients with MDR-TB should follow international standards of care as outlined in WHO's *Guidelines for Programmatic Management of Drug-resistant Tuberculosis*.[28] The Xpert MTB/RIF assay is **not suitable** for monitoring a patient's response to treatment. Conventional microscopy and culture are required for monitoring MDR-TB patients during treatment.

5.4 Diagnostic algorithms

National programmes need to develop setting-specific, evidence-based and cost-optimized algorithms designed to ensure universal access to high quality TB, MDR-TB and HIV-related TB diagnosis. Implementation of Xpert MTB/RIF testing should be managed by Ministries of Health within the context of national plans for the appropriate management of TB, MDR-TB and HIV-associated TB; the implementation should include the development of country-specific screening and diagnostic strategies, means for ensuring timely access to quality-assured first-line and second-line anti-TB drugs, and appropriate care-delivery mechanisms.

The settings in which Xpert MTB/RIF is used and the algorithms for using the test should be guided by the country-specific or region-specific epidemiology of TB, HIV and MDR-TB, by the available resources, anticipated cost effectiveness of the algorithm. The algorithm should also take into account all screening and diagnostic tools available in the country and their characteristics. Testing costs should also be measured against the costs of treatment, the benefits to patient and public health, including direct financial savings associated with decreased delays in diagnosis and reduced transmission associated with providing early and appropriate treatment.

The adoption of Xpert MTB/RIF does not eliminate the need for conventional TB microscopy, culture and DST. Microscopy or culture, or both, remain necessary for monitoring treatment since it is unlikely that any currently available test that uses DNA detection will be suitable for monitoring treatment. In addition, conventional culture and DST will be required to detect resistance to anti-TB agents other than rifampicin. Because Xpert MTB/RIF detects resistance only to rifampicin, countries with documented or suspected cases of XDR-TB should establish or expand their capacity for conventional culture and DST for second-line anti-TB agents, and to ensure that testing for second-line drugs is quality assured and based on WHO's policies and guidance.

Ministries of health and national TB programs should actively obtain information on the adoption of Xpert MTB/RIF by **private-sector laboratories** and other private health-care providers, seek information about their intended use, and enforce notification of all TB cases detected in the private sector using Xpert MTB/RIF. In settings where private sector providers are widely used by TB patients, these providers should be made aware of the availability of Xpert MTB/RIF, and which groups should have priority for testing using Xpert MTB/RIF; referrals from these providers should be actively monitored. Collaboration among private providers and national TB programmes may be mutually beneficial, allowing private providers to access concessional prices and national TB programmes to ensure that patients detected in the private sector are duly reported and subsequently registered for appropriate treatment.

Careful overview of the advantages and limitations of currently available TB diagnostics and DST methods is necessary in designing the most appropriate diagnostic algorithm. Inclusion of Xpert MTB/RIF into the diagnostic algorithm should take into account both **the selection of individuals to test** (also in relation to other available TB testing technologies) and the **interpretation of the Xpert MTB/RIF results** and management decisions that should follow each possible result, therefore mapping and joining these two processes into one diagnostic algorithm. Diagnostic algorithms can be different for each country or particular setting and depend on epidemiology, other available technologies, financial and human resources.

Pre-test screening strategies can be useful to reduce the number of individuals who ultimately undergo Xpert MTB/RIF testing in a TB case finding diagnostic algorithm. Two examples of such strategies are symptom and chest X-ray screening, both of which representing additional costs and requiring additional efforts depending on the setting.[29]

A brief overview of the advantages and limitations of different TB diagnostics is given below to assist in developing an appropriate diagnostic algorithm.

Sputum-smear microscopy

Microscopy is suitable for laboratories at peripheral and higher levels and it can be done safely under minimal biosafety conditions. It is inexpensive but has limited sensitivity, which is further reduced in HIV-positive individuals. Microscopy identifies acid fast bacilli not M. tuberculosis, which may affect its specificity in settings with a low burden of TB or places with a high prevalence of NTM. Microscopy cannot distinguish between viable and non-viable organisms, or between susceptible organisms and resistant. Microscopy is used to monitor patients' responses to anti-TB therapy and light-emitting diode (LED) fluorescence microscopy is recommended.[30] An extensive quality assurance programme must be implemented for microscopy to control for human error and sustain high quality performance.

Culture methods

Conventional culture (either solid or liquid) is suitable for national or regional laboratories. Manipulation of both solid cultures and liquid cultures requires the highest biosafety measures in the TB laboratory, and results are inevitably delayed due to the slow growth of mycobacteria. The use of both solid culture and liquid culture is recommended by WHO, and liquid culture is regarded as the gold standard for detecting TB; liquid culture results are also available more rapidly than results from solid culture. All positive cultures must be speciated to confirm *M. tuberculosis*. Culture is required to monitor the response of patients with MDR-TB to anti-TB therapy.

Phenotypic drug susceptibility testing

Phenotypic DST is suitable for national or regional laboratories. Being a conventional culture-based method, phenotypic DST requires the highest biosafety measures in the TB laboratory, and therefore is usually available only at national or higher-level regional laboratories. DST for second-line anti-TB agents should be done on all *M. tuberculosis* isolates with confirmed multidrug resistance. Phenotypic DST for second-line anti-TB agents is required to confirm or exclude XDR-TB.

Molecular line probe assay (LPA)

Due to its complexity and biosafety requirements, LPA is suitable only for national or regional level laboratories. LPA requires at least three separate rooms, and dedicated equipment, consumables and reagents in each room to minimize DNA cross-contamination. WHO recommends LPA to detect resistance to rifampicin and isoniazid only on smear-positive sputum specimens and *M. tuberculosis* isolates. Its sensitivity for detection of isoniazid resistance is sub-optimal. LPA can be used as a diagnostic test for MDR-TB, however,

conventional culture (solid or liquid) is required to monitor treatment response (culture conversion) for patients with MDR-TB. Conventional second-line DST is required to detect XDR-TB because using LPA for detection of resistance to second-line anti-TB agents is not currently recommended by WHO due to sub-optimal performance of the test.

5.5 Monitoring patients during treatment

Molecular tests, including Xpert MTB/RIF, are not suitable for monitoring patients during treatment because these tests detect DNA from both viable and non-viable bacilli. The management of patients with HIV-associated TB and drug-resistant TB also requires concurrent clinical laboratory capacity (for example biochemistry, haematology, general microbiology) to monitor treatment and associated comorbid conditions.

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 except in cases of extrapulmonary TB. No additional sputum-smear microscopy examination needs to be performed to establish their baseline status. For these patients, sputum-smear microscopy should be performed when the intensive phase of treatment has been completed, 5 months into treatment and at the end of treatment, following WHO guidelines.

Treatment outcomes for patients with a positive result from smear microscopy, culture or Xpert MTB/RIF at the start of treatment should be categorized according to the current WHO guidelines. All current treatment outcome definitions should be applied, including the outcome "Cured" – that is, a patient with a positive Xpert MTB/RIF test at baseline can be declared cured if a negative smear result is recorded at the end of treatment.

Patients whose TB and rifampicin resistance have been confirmed by Xpert MTB/RIF and who have been placed on an MDR-TB treatment regimen should be monitored by sputum-smear microscopy and culture, following WHO's current guidelines. If resources permit, monthly culture is recommended throughout treatment, given that this has been shown to have the greatest benefit in detecting treatment failure.

5.6 Using Xpert MTB/RIF in drug resistance surveys

Lack of laboratory capacity for culture and DST and the absence of referral systems at lower levels of networks are among the most important problems hindering implementation of drug resistance surveys[31], which measure the frequency of drug resistance among a representative sample of TB patients. If carefully planned and implemented, the use of Xpert MTB/RIF in drug resistance surveys could greatly reduce logistical issues, transportation costs, and laboratories' workloads. Though not a complete surrogate for MDR-TB, particularly in settings with low levels of rifampicin resistance[32] rifampicin resistance is the most important indicator of MDR-TB.

At least two groups of countries could benefit considerably from the use of Xpert MTB/RIF in drug resistance surveys. The first group is countries in which laboratories would struggle to cope with the workload generated by a survey while managing their routine work and maintaining high standards of quality. 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, which increases logistical issues and incurs further operational costs – Xpert MTB/RIF could be used to detect rifampicin-resistant specimens requiring further testing in a specialized laboratory.

Most patients enrolled in drug resistance surveys are at a low risk of rifampicin resistance. Given the high NPV of the test for detecting rifampicin resistance in such populations, Xpert MTB/RIF will accurately identify those whose disease is not resistant, reliably screening them out. Patients in whom rifampicin resistance is detected would be a relatively small group, thus presenting a

lower workload for the central laboratory or TB Supranational Reference Laboratory. Strains from this group would require further testing to confirm resistance to rifampicin and to detect resistance to isoniazid and selected second-line drugs (fluoroquinolones and injectable agents).

5.7 Using Xpert MTB/RIF in TB prevalence surveys

TB prevalence surveys[33] are important for obtaining a direct measurement of the absolute burden of disease caused by TB. TB prevalence surveys are population-based surveys that measure the number of people with TB disease in a sample. The number of people with active TB disease in the general population is relatively low (usually less than 1%), hence surveys typically involve large population sample sizes and require screening participants with interviews and chest X-rays and subsequent bacteriological testing of all participants with symptoms or chest X-ray abnormalities. Therefore, prevalence surveys entail a substantial workload for the laboratories involved, and this capacity is not always available in countries where the survey is planned or the quality of the testing is not assured.

The sensitivity of Xpert MTB/RIF in smear-negative culture-positive specimens is approximately 68%. For the purpose of TB prevalence surveys Xpert MTB/RIF cannot generally be considered a suitable replacement for culture to accurately estimate the prevalence of bacteriologically confirmed pulmonary TB. Nevertheless, when estimating the prevalence of bacteriologically confirmed pulmonary TB in surveys based on Xpert MTB/RIF testing, statistical adjustments can be made to account for the known diagnostic performance of Xpert MTB/RIF. Because Xpert MTB/RIF does not require advanced or additional infrastructure within the culture laboratory supporting the survey, it may facilitate the conduct of TB prevalence surveys. Furthermore, experience in several TB prevalence surveys have revealed several serious challenges that arise when large numbers of smear-positive samples are subsequently not confirmed by culture to be M. tuberculosis. Thus, the use of Xpert MTB/RIF on all smear-positive samples should help to rapidly identify samples with NTM (by identifying and excluding those with M. tuberculosis). This will ensure that individuals with TB receive appropriate treatment and prevent unnecessary treatment of those without TB. Survey participants with prominent symptoms or radiological abnormalities may also benefit from being tested with Xpert MTB/RIF in cases in which the culture has been contaminated or showed no growth. Appropriate operational research is, however, required before any definitive recommendations can be made on the use of Xpert MTB/RIF in TB prevalence surveys.

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18 *Guidelines for the programmatic management of drug-resistant tuberculosis*. Emergency Update 2008. Geneva, World Health Organization, 2008 (WHO/HTM/TB/2008.402).

19 Guidelines for intensified TB case finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings. Geneva, World Health Organization, 2011 (http://whqlibdoc.who.int/publications/2011/9789241500708_eng.pdf).

20 HIV prevalent settings are defined as countries, sub-national administration units (e.g., districts, counties), or selected facilities (e.g., referral hospitals, drug rehabilitation centres) where the adult HIV prevalence rate among pregnant women is ⩾ 1% or in which the HIV prevalence among TB patients is⩾ 5%.

21 Using the Xpert MTB/RIF assay to detect pulmonary and extrapulmonary tuberculosis and rifampicin resistance in adults and children. Expert Group meeting report: October 2013. Geneva, World Health Organization, 2014 (available at http://www.who.int/tb/laboratory/policy_statements/en/)

22 Theron, G et al. Feasibility, Accuracy, and Clinical Effect of Point-of-care Xpert MTB/RIF Testing for Tuberculosis in Primary-care Settings in Africa: a Multicentre, Randomised, Controlled Trial. Lancet 2013. (doi:10.1016/S0140-6736(13)62073-5).

23 *Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs*. Geneva, World Health Organization, 2008. WHO/HTM/TB/2008.392.

24 Van Deun A et al. Mycobacterium tuberculosis strains with highly discordant rifampin susceptibility test results. Journal of Clinical Microbiology. 2009, 47: 3501-3506.

25 Williamson DA et al. An evaluation of the Xpert MTB/RIF assay and detection of false-positive rifampicin resistance in Mycobacterium tuberculosis. Diagnostic Microbiology and Infectious Diseases. 2012; 74: 207-209.

26 Van Deun A, et al. Rifampicin drug resistance tests for tuberculosis: challenging the gold standard. Journal of Clinical Microbiology. 2013;51: 2633-2640.

27 *Global tuberculosis report 2012*. Geneva, World Health Organization. (WHO/HTM/TB/2012.6).

28 *Guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update*. Geneva, World Health Organization, (WHO/HTM/TB/2011.6) (available at http://whqlibdoc.who.int/publications/2011/9789241501583_eng.pdf).

29 *Systematic screening for active tuberculosis: principles and recommendations*. Geneva, World Health Organization, 2013. (WHO/HTM/TB/2013.04) (available at http://apps.who.int/iris/bitstream/10665/84971/1/9789241548601_eng.pdf)

30 *Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis, WHO policy statement* (available at http://whqlibdoc.who.int/publications/2011/9789241501613_eng.pdf?ua=1)

31 Weyer, K, et al. Rapid Molecular TB Diagnosis: Evidence, Policy-making and Global Implementation of Xpert(R)MTB/RIF. The European Respiratory Journal, 2012. (doi:10.1183/09031936.00157212).

32 Smith SE, et al. Global isoniazid resistance patterns in rifampin-resistant and rifampin-susceptible tuberculosis. International Journal of Tuberculosis and Lung Disease 2012; 16(2):203-205

33 *Tuberculosis Prevalence Surveys: a Handbook*. Geneva, World Health Organization, 2011. (available at http://www.who.int/tb/advisory_bodies/impact_measurement_taskforce/resources_documents/thelimebook/en/index.html)

6. Case definitions and patient registration

The definitions and the reporting framework for TB were revised in 2013[34]. These changes were driven primarily by a need to clarify how to register TB patients detected using molecular techniques: these changes to definitions and reporting have been reflected throughout this edition of the implementation manual. Additionally, the most important definitions relevant to this manual are presented below.

6.1 TB case

- **A bacteriologically confirmed TB case** is a person from whom a biological specimen has tested positive by smear microscopy, culture or WHO-approved rapid diagnostics, such as the Xpert MTB/RIF assay. All such cases should be notified, regardless of whether TB treatment has started.
- **A clinically diagnosed TB case** is a person who does not fulfil the criteria for bacteriological confirmation but has been diagnosed with active TB by a clinician or other medical practitioner who has decided to treat the patient with a full course of anti-TB treatment. This definition includes cases diagnosed on the basis of X-ray abnormalities or suggestive histology and extrapulmonary cases that do not have laboratory confirmation. Clinically diagnosed cases who are subsequently found to be bacteriologically positive (before or after starting treatment) should be reclassified as bacteriologically confirmed.

6.2 Classification based on type of drug resistance

Cases are classified based on the results of DST of clinical isolates confirmed to be M. tuberculosis as described below.

- **Monoresistance:** resistance to only one first-line anti-TB drug.
- **Polydrug resistance:** resistance to more than one first-line anti-TB drug (other than both isoniazid and rifampicin).
- **Multidrug resistance (known as MDR-TB):** resistance to at least both isoniazid and rifampicin.
- **Extensive drug resistance (known as XDR-TB):** multidrug resistance plus resistance to any fluoroquinolone and at least one of three second-line injectable drugs (amikacin, capreomycin or kanamycin).
- **Rifampicin resistance (known as RR-TB):** resistance to rifampicin detected using phenotypic or genotypic methods with or without resistance to other anti-TB drugs. This category includes any resistance to rifampicin, whether there is only resistance to rifampicin (monoresistance), or there is multidrug resistance, polydrug resistance or extensive drug resistance.

These categories are not mutually exclusive. For instance, when enumerating rifampicin-resistant TB, mono-resistance to rifampicin is included as well as MDR-TB and XDR-TB. While it has been the practice until now to limit the definitions of monoresistance and polydrug resistance only to first-line drugs, future treatment regimens may make it important for surveillance to extend these definitions to fluoroquinolones, second-line injectable agents as well as to any other anti-TB agents for which reliable DST becomes available.

6.3 Registration of TB cases diagnosed using Xpert MTB/RIF

All TB cases diagnosed by Xpert MTB/RIF and found to be rifampicin-susceptible, irrespective of their smear results, should be registered as **bacteriologically confirmed TB cases**. The Xpert

MTB/RIF result should be entered in the revised Basic TB Management Unit (BMU) register. Reporting separately the bacteriologically confirmed cases diagnosed only by Xpert MTB/RIF may help the national TB programme monitor the use and the yield of this technology. If results from Xpert MTB/RIF are not available then the procedures for registering TB cases diagnosed using conventional TB diagnostic tests remain unchanged.

All TB cases diagnosed by Xpert MTB/RIF and found to be rifampicin-resistant should be entered in the register at the Basic TB Management Unit and in laboratory registers as **rifampicin-resistant TB** (denoted as RR-TB) and also noted as **Xpert MTB/RIF-positive with rifampicin resistance**. If isoniazid resistance is detected by conventional or molecular techniques, the case should be registered as MDR-TB. If results from Xpert MTB/RIF are not available, the procedures for registering patients diagnosed with MDR-TB using conventional TB diagnostic tests remain unchanged.

Patients found to have a RR-TB or MDR-TB strain at any point should be started on a WHO-approved second-line treatment regimen. These cases are excluded from the main TB cohort when calculating treatment outcomes, and are included only in the analysis of the cohort receiving second-line anti-TB treatment. A separate register is used to monitor these patients.

34 *Definitions and reporting framework for tuberculosis – 2013 revision*. Geneva, World Health Organization, 2013 (WHO/HTM/TB/2013.2).(available at http://apps.who.int/iris/bitstream/10665/79199/1/9789241505345_eng.pdf).

7. Practical considerations

This part of the document presents practical considerations for introducing the Xpert MTB/RIF assay. The considerations are based on experiences reported to WHO, and reports from countries and partners that were presented and discussed in a series of implementers' meetings convened by WHO in 2011, 2012 and 2013.

7.1 Operational considerations

7.1.1 Checklist: key prerequisites to be met before implementing the Xpert MTB/RIF

Category	Prerequisite
Epidemiological data	1. Data must be available on the prevalence of MDR-TB and HIV-associated TB to allow for decision-making on prioritizing placement of the technology and optimizing the use of Xpert MTB/RIF in targeted patient groups.
Diagnostic policy reform	2. Plans should be made to modify diagnostic algorithms as part of the national TB programme's strategy to introduce Xpert MTB/RIF.
Laboratory network	3. Sufficient capacity and appropriate referral networks (in both the public and private sectors) must be available to provide quality assured laboratory services with: a) culture and DST to determine resistance to first-line and second-line anti-TB drugs at the central level (at least); these laboratories must be quality assured through an established link with a TB Supranational Reference Laboratory; b) sputum-smear microscopy for TB testing and for monitoring responses to treatment; c) culture to monitor responses to treatment for MDR-TB.
Laboratory workload	4. There must be a sufficient potential number of specimens to be tested using Xpert MTB/RIF in the facility where the technology will be placed to justify implementation of the test and to ensure that utilization of the new technology will be optimal (the capacity of the GeneXpert IV instrument is 12-16 a day or 3000-4000 tests annually).
Infrastructure	5. The electricity supply must be stable in the facilities where the test will be implemented or sufficient measures must be taken to the supply remains uninterrupted supply (for example an uninterrupted power supply unit may be used with additional batteries, a generator or solar panels) 6. Premises must be secure to prevent theft of the GeneXpert unit and the computer/laptop. 7. Adequate storage space must be provided for the cartridges, which must be stored at the recommended temperature range (2-28°C). 8. Appropriate measures must be taken to prevent ambient temperatures from rising above 30°C or falling below 15°C in the room where the equipment will be installed (for example some form of the ventilation, or air conditioning may be necessary).
Biosafety	9. Biosafety requirements must be implemented similar to those for direct sputum-smear microscopy.

Personnel	10. Each site will need 1-2 staff with basic computer literacy and knowledge of laboratory registers; these staff will need to be trained to perform the test and maintain the equipment.
Treatment capacity	11. Sufficient capacity to treat patients of identified with TB and MDR-TB should be available and treatment should follow WHO's recommendations.
Financing	12. Funding should be secured from national budget, donors or partners.
Procurement	13. Importation procedures must allow for reliable procurement of both equipment and consumables (through either regulatory registration or waiver), and the exchange of modules for calibration, swap or repair in case of module failure. Developing efficient, integrated supply chains and distribution systems will ensure a regular supply of consumables with sufficient shelf-life.

7.1.2 Checklist: Key actions necessary for implementation of Xpert MTB/RIF

Category	Action
Policy reform	1. Incorporate Xpert MTB/RIF testing into the national diagnostic strategy and algorithms; this should include placing Xpert MTB/RIF at the appropriate level of the diagnostic network. Appropriate pre-test screening strategies should be identified where necessary.
Logistics	2. Identify adequate premises for the equipment (as described in prerequisites 5-10 above). 3. Allocate storage space for cartridges (as described in prerequisite 7). 4. Identify procedures for cartridge disposal (for example, incineration) as part of each laboratory's waste disposal plan.
Procurement	5. Register the GeneXpert system and Xpert MTB/RIF assay OR obtain a waivr for importation. 6. Forecast needs based on the expected demand and period of implementation. 7. Calculate the first and subsequent orders for the period of implementation. 8. Quantify the buffer stock necessary to cover at least 3 months of expected workload, taking into account the shelf-life of cartridges, and possible delays in procurement and importation. 9. Place an order for equipment and cartridges directly with the manufacturer or with a certified distributor; insist on preferential pricing where relevant.
Financing	10. Secure sustainable funding from the national budget, donors or partners to ensure the continued use of Xpert MTB/RIF testing, as well as continued training, maintenance and calibration.
Training	11. Identify and train staff to perform the Xpert MTB/RIF assay. 12. Train clinicians and other health-care workers on the utility of Xpert MTB/RIF and the groups of patients that should be targeted and referred for testing; train clinicians and health-care workers how to interpret test results. Offer refresher training for healthcare workers about how to collect a good quality specimen from patients. 13. Train staff to ensure that patients are referred in a timely manner and receive proper treatment, train staff in infection control measures and contact tracing.

Reporting	14. Adapt request and reporting forms and patient registers to include results from Xpert MTB/RIF. 15. Develop systems for reporting to the clinic on the same day that results become available.
Verification	16. Verify that the GeneXpert platform is working as expected by testing known positive and negative specimens at the time of installation and after each calibration of the modules.
Maintenance	17. Order remote calibration cartridges before the end of each year of testing, and perform calibration in a timely manner. 18. Order an extended warranty or budget appropriately for potential repairs.
Monitoring	19. Setup a system for monitoring the implementation of Xpert MTB/RIF.

7.1.3 Testing capacity

Figure 2. GeneXpert instruments with 1, 2, 4 and 16 modules

The range of GeneXpert instruments includes systems with 1, 2, 4, 16, 48 or 80 modules. Modules function independently so that batching is not required and individual tests can be started at different times. Results become available for each test in less than 2 hours, so a GeneXpert instrument with four modules (that is, the GeneXpert IV instrument) has the capacity to perform **up to 16 tests in an 8-hour working day**. Experience from sites using the platform has shown that during the initial 6-12 months of use, while laboratory staff and clinicians grow accustomed to the test, throughput may be only up to 8 tests a day; therefore, cartridge orders should be planned carefully to prevent cartridges from expiring before they are used.

For sites that initially expect low throughput but are unsure whether this will increase later, a GeneXpert IV can be ordered with fewer than four modules, leaving the remaining bays empty. This allows for the possibility of increasing throughput later by installing additional modules, which can be ordered separately from the manufacturer. If additional instruments are required in a laboratory to increase throughput, instruments may be spliced together with a cable to allow data to be stored on a single computer.

7.1.4 Operation and storage conditions

Xpert MTB/RIF cartridges and the specimen reagent should be stored at 2–28 °C, following the manufacturer's recommendations, although it has been stated by the manufacturer that the cartridges are stable if kept at 2-45 °C for less than 6 weeks at 75% relative humidity. The cartridges are bulky when packed and require substantial storage space. An average household refrigerator can hold the supplies needed for 2 weeks at a laboratory performing 12–16 tests per day. The shelf life of the Xpert MTB/RIF cartridges may pose challenges in relatively inaccessible areas that have complex customs clearance procedures. When an order is placed, the shelf life of the cartridges being purchased should be requested from the manufacturer. Planning is essential to prevent stock-outs and cartridges from expiring before they are used; orders should be based on the number of cartridges that have been used, the shelf-life of the cartridges, the lead-time for delivery and the expected time needed to clear customs.

The manufacturer's recommended ambient operating temperature for the GeneXpert instrument is between 15 °C and 30 °C, which is not different from the operating temperatures recommended for a wide range of other laboratory equipment, household appliances, computers and mobile phones. The room where the test is performed may need air conditioning or heating to ensure that the ambient temperature is maintained in the recommended range. Ignoring the recommended temperature range may increase error rates because extreme temperatures interfere with thermo-cycling during the test.

7.1.5 Biosafety

Both the preparation of specimens and the running of the Xpert MTB/RIF test r**equire the same biosafety** conditions as are used for conventional direct sputum-smear microscopy[35].

7.1.6 Calibration and maintenance

Since April 2012, new GeneXpert instruments have an initial 2-year warranty that is conditional upon modules being regularly calibrated. If the machine is not calibrated after first year, the second year of the warranty is invalidated. The warranty covers repairs of the instrument and any parts. An optional extension of the warranty may be purchased annually or as a 3-year extension (preferential prices are available from the manufacturer for the countries listed in *Annex 1*).

The GeneXpert modules require **annual calibration**. A remote calibration option uses a kit containing special cartridges that can be run on each module (without specimens) when calibration is due. During this run, which lasts about 20 minutes, the instrument will be automatically calibrated. However, in some cases remote calibration will not be sufficient, and at the end of the run the user will be informed that a module needs to be exchanged (or swapped); in this case, a replacement module will be sent from Cepheid and the original module must be returned for calibration. In 2014, the calibration kit could be ordered at the preferential price of USD$ 450 per kit; each kit is sufficient to calibrate up to 4 modules. Delivery costs can be minimized by ordering calibration lit at the same time as test cartridges are ordered.

A detailed commercial sales contract and customer support plan should be negotiated with the supplier, guaranteeing an ample and continual supply of cartridges, facilitating customs clearance, maintenance and calibration, and repair and replacement, as necessary.

7.1.7 Power supply

The GeneXpert instrument requires a **stable electric power supply**: even a short-term interruption in power may cause results to be lost, cartridges to be wasted, and the need to obtain another specimen. An unstable supply of electricity may also damage the electronics of the instrument and the computer, which may not be covered by the manufacturer's warranty. Therefore, a power line stabilizer and an uninterrupted power supply unit (UPS) are recommended for the GeneXpert instrument.

If the electricity is unstable and if power outages occur, then it is important to have an uninterrupted supply unit with additional batteries; the cost of the solution to the problem of power outages will vary depending on the setting and the duration of outages. The budget for the UPS unit will increase significantly if the unit will also act as a back up source of power for a longer period; the cost of and UPS depends on the duration of time that back-up will be needed, the power consumption of the diagnostic instrument and the capacity of the internal battery in the unit. For example, if power outages never exceed a few minutes, a small unit will be sufficient to ensure that the test cycle is not interrupted, and will prevent cartridges from being wasted and protect the equipment. When longer outages are possible, it is prudent to have a supply unit with external battery packs that can provide power for both the instrument and the computer for the average duration of the test – that is, 2 hours. The cost for such a unit depends on the choice of the unit and the availability of local solutions using external batteries and power inverters.

Several countries have reported back-up solutions ranging from custom-made battery arrays with a power inverter to solar-power installations[36].

7.1.8 Reporting results

A printer may be installed to print the test results if required for medical filing or test reports. Mechanisms for **rapidly reporting** results from Xpert MTB/RIF to clinicians and for providing timely access to appropriate treatment must be established so that patients benefit from early diagnosis. Electronic systems for reporting results using text or SMS messaging have been developed by various organizations, including Abt Associates (GXAlert)[37] and Interactive Research and Development (XpertSMS)[38]. The GeneXpert instruments can also be interfaced with most laboratory information systems and the delivery of results can be followed with an SMS message or other means of delivering the results.

7.1.9 Quality assurance

The Xpert MTB/RIF assay includes several internal quality controls that verify specimen processing, success of PCR and cartridge integrity.

Each cartridge includes a sample processing control (SPC), which contains non-infectious spores in the form of a dry spore cake that is included in each cartridge to verify adequate processing of MTB to:

- verify that lysis of MTB has occurred if the organisms are present,
- verify that the specimen processing is adequate,
- detect specimen associated inhibition of the real-time PCR assay.

SPC should be positive in a negative sample and SPC can be negative or positive in a positive sample.

The Probe Check Control is a check undertaken before the start of the PCR reaction. The system measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability.

There is currently no consensus on the need nor the requirements for performing any additional periodic blinded testing using quality assurance panels. As an interim recommendation to fill this gap, the Global Laboratory Initiative recommends the following minimum requirements for quality assurance of the Xpert MTB/RIF assay.

Instrument verification

Each module in the GeneXpert instrument should be evaluated as being "fit for purpose" through verification with known positive or negative material prior to commencing routine testing of clinical specimens. A single verification test should be performed per module upon instrument installation and following calibration of instrument modules.

Verification panels are now routinely distributed by Cepheid with each new instrument and

with recalibrated modules. The format of the verification panels is a card containing 5 Dried Culture Spots (DCS) of a known concentration of whole inactivated *Mycobacterium tuberculosis* (Rifampicin sensitive) bacilli.

Performance indicator monitoring

Each instrument should be monitored using the following minimum set of indicators to evaluate proper use:

- number of tests performed per month per module,
- number and proportion of MTB positive results,
- number and proportion of MTB positive rifampicin resistant results,
- number and proportion of errors (disaggregated by type of error),
- number and proportion of indeterminate results,
- number and proportion of invalid results.

7.2 Preferential pricing and eligible countries

The manufacturer makes Xpert MTB/RIF cartridges available to the public sector in eligible countries at the cost of **US$ 9.98** each, plus shipping (*Annex 1*). The buyers eligible for preferential pricing in eligible countries are defined as follows:

- governments or government-funded Institutions such as ministries of health, national or regional TB centres, government-associated hospitals, institutions associated with the armed forces, the prison services;
- non-governmental organizations and United Nations related organizations working for or in eligible countries, such as International Organization for Migration and United Nations High Commissioner for Refugees;
- non-profit welfare organizations helping eligible countries to improve diagnosis such as Médecins Sans Frontières, and other humanitarian organizations such as UNICEF, Save the Children and the International Committee of the Red Cross;
- donor agencies such as UNITAID, PEPFAR, the United States Agency for International Development, the Global Fund to Fight AIDS, Tuberculosis and Malaria, and Government agencies based outside the country but that support local implementation such as the United States Centers for Disease Control and Prevention CDC (USA);
- non-profit private organizations whose mission is in keeping with humanitarian principles, such as private charities or private non-profit hospitals and clinics.

The preferential price for cartridges is a result of a unique buy-down arrangement agreed among the manufacturer, the United States Government (through USAID and PEPFAR), the Bill and Melinda Gates Foundation and UNITAID.

In early 2014, the negotiated price of a GeneXpert 4-module unit for the public sector in eligible countries excluding shipping was:

a. GeneXpert System 4-module unit with desktop computer – **US$ 17000**
b. GeneXpert System 4-module unit with laptop computer – **US$ 17500**

The manufacturer should be contacted directly to obtain prices for other variants – for example a 4-module instrument shell containing 2 modules, or a 16-module instrument.

In 2014, a calibration kit that can be used for up to 4 modules could be ordered for **US$ 450** plus shipping. The manufacturer has also updated the preferential pricing for module exchange:
– for 1 module, US$ 900
– for 2 modules, US$ 1200
– for 3 modules, US$ 1500
– for 4 modules, US$ 1800.

For the 4-module instrument, shipping costs average close to US$ 1000 and about US$ 1 per cartridge, but both costs vary according to

the distance and the number of units or cartridges being shipped.

Contact details
Cepheid HBDC SAS, Vira Solelh,
81470 Maurens-Scopont, France
Telephone: +33 563 825 333
Fax +33 563 825 301
E-mail: ordershbdc@cepheidhbdc.com

7.3 Implementation costs

7.3.1 Sample budget

Table 1 provides a sample calculation of a budget based on purchasing a 4-module GeneXpert instrument that will run at full capacity during an 8-hour work day. This sample budget will need to be customized according to each setting's requirements. As described in *Section 7.1.6*, the throughput of machines during the initial 6-12 months of use may be significantly below maximum capacity, while laboratory staff and clinicians grow accustomed to the new technology.

Other costs not included in the table but that should be considered and budgeted for if applicable are:

- customs and other clearance fees for imported goods;
- the costs of transportation from the port of entry to the final destination in the country;
- Infrastructure costs, and costs for security, appropriate space and air conditioning or heating, where relevant;
- Training for test operators and health-care workers at different levels of the health-care system, including clinicians.

7.4 Public health impact of Xpert MTB/RIF

The most frequently used test for detection of TB, sputum-smear microscopy, presents a low-cost option, but it seriously lacks sensitivity. As a result, health services miss many TB patients or identify them only when their disease is at an advanced stage. The diagnostic accuracy of smear microscopy depends on the proficiency of the personnel conducting the test and the rigour of the quality assurance programme. Smear microscopy cannot be used to identify drug resistance, and it doesn't differentiate between *M. tuberculosis* and NTM. Conventional solid culture and liquid culture methods for DST are slow, requiring weeks or months to generate results, and demand high proficiency from personnel as well as costly infrastructure to ensure sufficient biosafety conditions in the containment laboratory.

New TB diagnostics, and Xpert MTB/RIF in particular, largely alleviate many of these constraints. Xpert MTB/RIF provides higher sensitivity than microscopy, has a sensitivity close to solid culture, and is highly specific. The infrastructure requirements for the Xpert MTB/RIF assay are the same as for smear microscopy. The experience of those who have been early adopters of the Xpert MTB/RIF assay shows an increase in the number of bacteriologically-confirmed TB patients diagnosed by around 30-40% when compared with sputum-smear microscopy. Moreover, reports from some settings with a high prevalence of HIV have shown an up to threefold increase in case detection[39].

Diagnosing drug resistance remains a particular challenge for laboratory systems in many low-income and middle-income countries. The capacity to diagnose resistant TB is limited in the places where it is needed most. As a result, only a fraction of the estimated cases of MDR-TB have a laboratory-confirmed diagnosis. The use of more sensitive and rapid diagnostics will increase the number of patients who are reliably diagnosed with TB and drug-resistant TB, and may place a higher demand on health services. Additional human and financial resources will be needed to address the increase in workload and to allow these new tests to be used efficiently and for the convenience of patients.

Table 1. Sample annual itemized budget

Row label	Category	Item	Cost, number of days, tests or cartridges	Comment [a]
A	Equipment	GeneXpert 4 module unit with laptop	US$ 17 500.00	US$ 17 000 if desktop computer is selected; preferential pricing for selected countries (see *Annex 1*).
B		Shipping	US$ 1 000.00	Average cost, actual cost depends on destination
C		Uninterrupted power supply unit and external batteries	US$ 1 200.00	Local purchase; price depends on the market and back-up capacity of UPS
D		Printer	US$ 200.00	Local purchase, price depends on the market, optional
E	Maintenance	Calibration kit after 1st year	US$ 450.00	Can be included in shipment with test cartridges to reduce price of shipping
F		Annual warranty after 2nd year (includes calibration kit)	US$ 2 900.00	3-year extended warranty available for US$ 6 900
G	Consumables	Cost per cartridge	US$ 9.98	Preferential pricing for selected countries (see Annex 1)
H		Shipment cost per cartridge	US$ 1.20	Average cost, actual cost depends on destination
I		Number of working days per year	250	Number can vary depending on local context
J		Average number of tests per instrument /day: Year 1	6	Number may vary depending on working hours
K		Average number of tests per instrument /day: Year 2 and beyond	12	Number may vary depending on working hours
L		Number of cartridges to order: year 1	1 500	I*J
M		Number of cartridges to order: year 2 and beyond	3 000	I*K
N	Human resources and technical assistance costs	Annual salary for technician		To be added; depends on the country
O		Training and monitoring		To be added; depends on the country
	Installation costs: year 1		US$ 19 900.00	A+B+C+D
	Running costs: year 1		US$ 17 220.00	E+((G+H)*(L))+(N+O)
	Running costs: year 2 and beyond		US$ 36 440.00	F+((G+H)*(M))+(N+O) (Extended warranty purchased at end of year 2 for year 3, etc.)

a The calculations for selected items are described using the letters assigned to the rows

The introduction of early and rapid diagnosis creates opportunities for improvement in TB programmes, provided that test results are responded to rapidly. For example, if treatment delays continue to occur despite the rapid availability of results because there are other problems within the programme, then the introduction of new diagnostic platforms will have little effect on outcomes for patients and the programme. The implementation of new, more accurate and rapid diagnostics can result in a swift increase in the number of patients diagnosed with TB and drug-resistant TB, which may exert pressure on managers who are unprepared or programmes that lack adequate capacity. Improving diagnostic capacity requires the strengthening of treatment capacity. If efforts to improve treatment capacity are neglected or delayed, then treatment will lag and the problem of having patients on waiting lists, which is already prevalent in many affected countries, may be exacerbated. This problem touches on numerous ethical, financial and public-health issues, and it is important to highlight several frequently overlooked points.

- Diagnosing TB or drug-resistant TB does not increase the number of people with TB or drug-resistant TB, it merely helps to identify them; these people are in the community already, transmitting disease even when they have not been diagnosed.
- Even when treatment is not available for all who need it, diagnosing patients with TB and resistant TB is ethically sound and has more advantages than disadvantages[40]. Providing a diagnosis in the absence of treatment can ensure that individuals with resistant TB are not inappropriately treated with first-line anti-TB agents, which can harm both the patient and public health; providing a diagnosis may help individuals make life plans, inform their behaviour in terms of infection control; diagnosing patients will also provide information on the actual burden of disease in an area, and may stimulate policy-makers and donors to scale-up treatment services.
- If more patients have a bacteriological diagnosis then fewer patients will be treated empirically, which means fewer individuals will be given unnecessary treatment or receive incorrect or suboptimal treatment.

Although the availability of new diagnostics reduces the need for conventional laboratory services, sputum-smear microscopy and culture laboratories are still needed within TB programmes to monitor treatment. However, reductions in the demand for these services will help to optimize the workload, and culture laboratories will be able to focus on DST for first-line and second-line anti-TB agents other than rifampicin.

The widespread introduction of new diagnostic testing platforms will allow TB to be diagnosed early and accurately. Less advanced forms of TB will be diagnosed; treatment delays will be reduced; disease transmission will decrease; case–fatality rates will decrease; adverse sequelae will be prevented; and patients' outcomes will improve.

35 *Tuberculosis laboratory biosafety manual*. Geneva, World Health Organization, 2012 (WHO/HTM/TB2012.11) (available at http://apps.who.int/iris/bitstream/10665/77949/1/9789241504638_eng.pdf).

36 *Solar energy powers GeneXpert IV Dx system for detection of tuberculosis and rifampicin resistance in district/sub-district public health care settings in Uganda*. Global Health Delivery online, 2011 (http://www.ghdonline.org/uploads/Uganda-GX-solar_for-website_190420111-SOLAR_3.pdf accessed 11.12.2013).

37 *Gx Alert open source software*, 2014 (http://www.gxalert.com accessed 11.12.2013)

38 *Xpert SMS automated reporting of GeneXpert results*. Karachi, Interactive Research and Development, 2014. (http://irdresearch.org/xpert-sms-automated-reporting-of-genexpert-results accessed 11.12.2013).

39 Rachow, A et al. Increased and Expedited Case Detection by Xpert MTB/RIF Assay in Childhood Tuberculosis: a Prospective Cohort Study. *Clinical Infectious Diseases*. 2012, 54: 1388–1396. doi:10.1093/cid/cis190.

40 *Guidance on ethics of tuberculosis prevention, care and control*. Geneva, World Health Organization, 2010 (WHO/HTM/TB/2010.16) (available at http://whqlibdoc.who.int/publications/2010/9789241500531_eng.pdf).

8. Monitoring and evaluation

Monitoring and evaluation of Xpert MTB/RIF implementation is necessary to **ensure the effective and efficient use of resources** and also to measure the impact of Xpert MTB/RIF in order to guide and justify further scale-up.

8.1 Routine monitoring

At the site level, monitoring the use of Xpert MTB/RIF ensures that established diagnostic algorithms are being followed, detects whether a particular instrument module is functioning suboptimally or whether any users require additional training, and allows supplies to be effectively managed. Site-level information should be shared with the supervising regional or national reference laboratory; this will allow the relevant laboratory to provide guidance on any actions that need to be undertaken to improve effectiveness, efficiency or user performance, and to strengthen the supply-management process to prevent stock-outs or cartridges from expiring by exchanging cartridges among sites.

The key data that are recommended to be collected monthly or quarterly fall into three main categories.

1. Monitoring the groups of patient tested and the test results:

- the number of Xpert MTB/RIF tests performed, disaggregated by reason for testing (that is, by the group of either TB patients or individuals suspected of having TB);
- the number of tests with MTB DETECTED, Rif resistance NOT DETECTED;
- the number of tests with MTB DETECTED, Rif resistance DETECTED;
- the number of tests with MTB DETECTED, Rif resistance INDETERMINATE;
- the number of tests with MTB NOT DETECTED;
- the number of tests that had invalid results, no results or other errors.

2. Monitoring the operation of the GeneXpert platform and the performance of users:

- the number and types of various errors. Identifying the most frequent types of errors can help troubleshoot the process, given that certain errors may be associated with the technique used to process specimens; other errors may be related to mechanical problems with the instrument's modules or other issues, such as room temperature;
- the number of errors occurring by instrument module. If a particular module produces more errors over time compared with other modules, it may require repair;
- the number of errors occurring by user. If a particular user has an unusually high number of errors, further investigation of the specific error types is warranted, since some errors may be caused by the technique used to process specimens;
- the number of tests lost due to power outages or surges;
- the number, duration, and causes of routine interruptions in the Xpert MTB/RIF testing service. Common causes of service interruptions include cartridge stock-outs, expired cartridges, no staff available, instrument breakdown, and computer breakdown;
- the number of instrument modules not functioning and the duration (in days) of module failure during the reporting period;
- the number of instrument modules overdue for calibration at the end of the reporting period.

Remote monitoring tools that automatically send results via the Internet to a central country focal

point greatly facilitate performance monitoring. Cepheid expects to roll out its remote monitoring platform in June 2014. In early 2014, other tools that serve this purpose include those developed by Abt Associates (GXAlert)[41] and Interactive Research and Development (XpertSMS)[42].

3. Monitoring supply management:

- the number of cartridges in stock at the beginning of the reporting period;
- the number of cartridges received during the reporting period;
- the number of cartridges used during the reporting period;
- the number of cartridges that were lost or damaged;
- the number of cartridges in stock at the end of the reporting period ;
- whether there were any stock-outs during the reporting period, the duration of stock-out (in days);
- Number of cartridges that expired before being used.

8.2 Measuring the impact

In order to understand the impact of Xpert MTB/RIF on case detection, the management of patients, and other laboratory processes, additional data need to be collected from other sources in the laboratory or at the district level or treatment-facility level. Because impact can be assessed only using a comparator, baseline data from a year before Xpert MTB/RIF was introduced are needed.

Sites introducing Xpert MTB/RIF usually observe a significant increase in the number of bacteriologically confirmed TB cases. In order to measure this increase, the following data should be collected:

- the number and proportion of incident cases (both new and relapsed) confirmed by microscopy, culture, or Xpert MTB/RIF, or a combination of these, during the reporting period after the introduction of Xpert MTB/RIF;
- the number and proportion of incident cases (both new and relapsed) confirmed by microscopy or culture, or both, during an analogous reporting period before the introduction of Xpert MTB/RIF.

Some sites and countries have reported that the introduction of Xpert MTB/RIF has not resulted in an overall increase in TB case notification. This is usually found in settings where a large number of patients have been diagnosed based on a clinical assessment. Diagnosing TB based on the clinical evidence alone can result in patients being falsely diagnosed with TB and receiving unnecessary treatment. If an increase in the proportion of bacteriologically confirmed cases is observed, it can provide assurance that the risk of misdiagnosis and unnecessary treatment has been reduced.

Unless culture and phenotypic DST or LPA were already widely in use, sites introducing Xpert MTB/RIF will observe increases in the number of rifampicin-resistant cases detected. To evaluate the impact of Xpert MTB/RIF on the diagnosis of rifampicin-resistant cases and MDR-TB cases, and to ensure that patients with detected resistance receive appropriate follow-up testing and treatment, the following data should be collected and monitored:

- the number and proportion of individuals found to have rifampicin-resistant TB by any method during the reporting period, and the number and proportion found to have rifampicin-resistant TB during an analogous reporting period before Xpert MTB/RIF was introduced, disaggregated by patient group;
- the number and proportion of rifampicin-resistant cases detected by Xpert MTB/RIF that received further phenotypic DST during the reporting period;
- the number and proportion of rifampicin-resistant cases detected by Xpert MTB/

RIF that were initiated on a WHO-recommended treatment regimen for MDR during the reporting period.

In order to understand the impact of introducing Xpert MTB/RIF on the number of other diagnostic tests being performed, the following data should be collected, as applicable, and compared with baseline data from an analogous period:

- the number of smear-microscopy tests performed for diagnosis and for treatment follow-up;
- the numbers of culture tests performed for diagnosis and for treatment follow-up;
- the number of DST performed.

Other aspects of implementation – in particular data on cost-effectiveness and the impact on diagnostic delays and time to treatment initiation – are best collected by operational research studies rather than as part of the routine processes for monitoring and evaluation.

41 GxAlert open source software, 2014 (http://www.gxalert.com accessed 11.12.2013)
42 XpertSMS automated reporting of GeneXpert results. Karachi, Interactive Research and Development, 2014 (http://irdresearch.org/xpert-sms-automated-reporting-of-genexpert-results accessed 11.12.2013).

9. Collaboration and coordination

The introduction of Xpert MTB/RIF into a country must be led by the Ministry of Health or the equivalent agency. In-country coordination is essential to optimize the use of resources, streamline activities, and ensure that sound technical advice is delivered and appropriate approaches are used. It is also fundamental to ensure that there is collaboration among national TB programme, HIV/AIDS programme, and public or private laboratory services.

To avoid duplicating efforts, WHO and its partners provide global-level coordination of the roll out of Xpert MTB/RIF. A dedicated web site has been established[43] to map uptake of the Xpert MTB/RIF test; the web site also collects information from national TB programmes and implementing partners about the siting of the instruments and plans for additional procurement. Countries and partners embarking on the roll out of Xpert MTB/RIF are encouraged to share their activities and plans to ensure that information on the web site is as comprehensive and up to date as possible.

9.1 Knowledge sharing

In April 2011, WHO convened a meeting of early implementers of the Xpert MTB/RIF assay to refine the proposed diagnostic algorithms, develop a core set of variables to be used to determine the impact of introducing the technology on a laboratory's workload, and to clarify operational and logistical issues. A second meeting of early implementers followed in April 2012 during which participants shared experiences of introducing the assay under routine programmatic conditions.

A third Global Forum of Xpert MTB/RIF Implementers was convened in April 2013, in association with the fifth annual meeting of the Global Laboratory Initiative and its partners. During this meeting countries and their technical partners shared information about the lessons that had been learnt and the challenges encountered during scale-up; the discussions focused on providing evidence of the impact of scale-up and on linking scale up in diagnosis with improving access to treatment. The results of the testing strategies from the roll-out phase and the subsequent refinement of the strategies will be used to inform future efforts to scale-up Xpert MTB/RIF to the country level.

A dedicated task force at the Global Laboratory Initiative has developed a training package[44] consisting of modules about the background, use and maintenance of the Xpert MTB/RIF assay; the modules include information about all of the steps necessary to implement the technology. The training package also includes specific modules about how to interpret results, and a clinical guidance module to help care providers correctly interpret and use the test results.

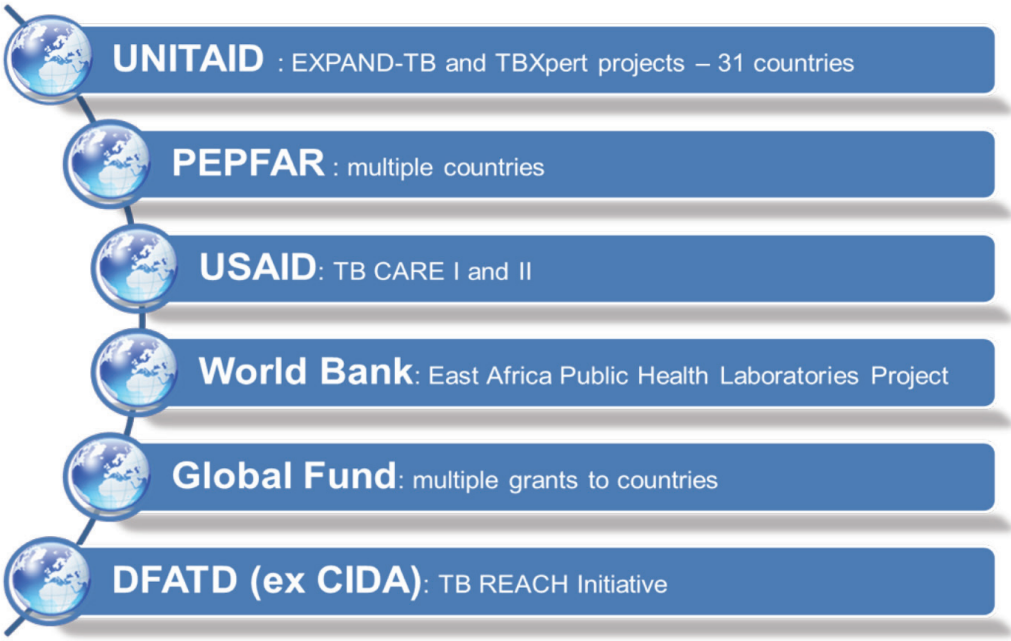
WHO maintains a periodically updated list of published evidence and commentary on Xpert MTB/RIF, which is categorized by topic[45].

9.2 Donors supporting the roll-out of Xpert MTB/RIF

It is essential that the introduction of Xpert MTB/RIF is coordinated at the country level. Technical agencies and donors need to work within the framework of national TB programmes and HIV/AIDS programmes to assist in implementing Xpert MTB/RIF testing. Increase in the number of cases of TB and MDR-TB detected will require increases in the capacity for patient management and provision of anti-TB drugs.

It is necessary to ensure that cases of MDR-TB are accurately reported and forecast in order to guarantee an uninterrupted supply of quality assured medicines. In addition, sustained and prolonged technical assistance will be urgently required to rapidly increase the capacity to deliver care for patients with MDR-TB.

Figure 3. Donors supporting the roll-out of Xpert MTB/RIF



Many international donors have been active in supporting countries during the implementation of Xpert MTB/RIF testing. The Global Fund for AIDS, Tuberculosis and Malaria, UNITAID[46, 47], US President's Emergency Plan for AIDS Relief and the United States Agency for International Development are some of the largest supporters of this technology in affected countries. Until August 2012, the high cost of Xpert MTB/RIF tests was a barrier to its introduction in low-income and middle-income countries. Since then, a total of 145 countries are benefitting from a 40% price reduction on the cartridges obtained by UNITAID, PEPFAR, USAID, and the Bill and Melinda Gates Foundation.

43 *WHO monitoring of Xpert MTB/RIF roll-out: country and partner plans*. Geneva, World Health Organization, 2013 (http://www.stoptb.org/wg/gli/assets/documents/map/2/atlas.html accessed 11.01.2014).

44 *Global Laboratory Initiative*. Geneva, Stop TB Partnership (http://www.stoptb.org/wg/gli/ accessed 11.01.2014).

45 Published evidence and commentary on the Xpert MTB/RIF assay. Geneva, World health Organization 2014 (http://www.stoptb.org/wg/gli/assets/documents/map/XpertPublications.pdf accessed 30.01.2014).

46 *Expand-TB project briefing note* (http://www.who.int/tb/publications/factsheet_expand_tb.pdf accessed 11.01.2014).

47 *TBXpert project briefing note* (http://www.who.int/tb/publications/TBXpert_briefing_note.pdf accessed 11.01.2014).

Annex 1. Countries eligible for preferential pricing on equipment and consumables

The following countries are eligible for preferential pricing.[a]

Afghanistan	Dominica	Malawi	Seychelles
Albania	Dominican Republic	Malaysia	Sierra Leone
Algeria	Ecuador	Maldives	Solomon Islands
Angola	Egypt	Mali	Somalia
Antigua and Barbuda	El Salvador	Mauritania	South Africa
Argentina	Eritrea	Mauritius	South Sudan
Armenia	Estonia	Mexico	Sri Lanka
Azerbaijan	Ethiopia	Micronesia, Federated States of	Sudan, the
Bangladesh	Fiji	Mongolia	Suriname
Belarus	Gabon	Montenegro	Swaziland
Belize	Gambia, The	Morocco	Syrian Arab Republic
Benin	Georgia	Mozambique	Tajikistan
Bhutan	Ghana	Myanmar	Thailand
Bolivia, Plurinational State of	Grenada	Namibia	The former Yugoslav republic of Macedonia
Bosnia and Herzegovina	Guatemala	Nauru	Timor-Leste
Botswana	Guinea, Equatorial	Nepal	Togo
Brazil	Guinea-Bissau	Nicaragua	Tonga
Bulgaria	Haiti	Niger, the	Tunisia
Burkina Faso	Honduras	Nigeria	Turkmenistan
Burundi	India	Pakistan	Tuvalu
Cambodia	Indonesia	Palau	Uganda
Cameroon	Iraq	Panama	Ukraine
Cape Verde	Jamaica	Papua New Guinea	United Republic of Tanzania
Central African Republic	Jordan	Paraguay	Uruguay
Chad	Kazakhstan	Peru	Uzbekistan
Chile	Kenya	Philippines	Vanuatu
China	Kiribati	Republic of Moldova	Venezuela, Bolivarian Republic of
Colombia	Kosovo	Romania	Viet Nam
Comoros	Kyrgyzstan	Russian Federation	West Bank and Gaza Strip
Congo, Republic of the	Lao People's Democratic Republic	Rwanda	Western Sahara

Costa Rica	Latvia	Saint Kitts and Nevis	Yemen
Côte d'Ivoire	Lebanon	Saint Lucia	Zambia
Croatia	Lesotho	Saint Vincent and the Grenadines	Zimbabwe
Cuba	Liberia	Samoa	
Democratic People's Republic of Korea	Libyan Arab Jamahiriya	Sao Tome and Principe	
Democratic Republic of the Congo	Lithuania	Senegal	
Djibouti	Madagascar	Serbia	

a This is the list shown on the manufacturer's website as of 11 February 2014.

Annex 2. Standard Operating Procedure (SOP) for processing extrapulmonary specimens (CSF, lymph nodes and other tissues) for Xpert MTB/RIF assay

Content

1. Scope

This standard operating procedures (or SOP) describes methods for processing specimens of cerebrospinal fluid (CSF), lymph nodes and tissues for testing using the Xpert MTB/RIF assay and for purposes of culturing Mycobacterium tuberculosis culture on solid media or liquid media.

2. Definitions and abbreviations

BSC, biological safety cabinet
CSF, cerebrospinal fluid
ID, patient's specimen identification, usually laboratory number
LJ, Löwenstein–Jensen
NALC, N-acetyl-L-cysteine
NaOH, sodium hydroxide
PBS, phosphate buffer 0.067mol/ litre, pH 6.8
RCF, relative centrifugal force

3. Procedure

3.1 Principle

WHO has issued recommendations about using of Xpert MTB/RIF to diagnose extrapulmonary TB and to detect rifampicin resistance:

- Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test for CSF specimens from patients suspected of having TB meningitis (strong recommendation given the urgency of rapid diagnosis, very low-quality evidence).
- Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture, and histopathology) for testing specific nonrespiratory specimens (lymph nodes and other tissues) from patients suspected of having extrapulmonary TB (conditional recommendation, very low-quality evidence).

In order to reach a quick diagnosis using CSF specimens, Xpert MTB/RIF should be preferentially used instead of culture if the specimen volume is low or if additional specimens cannot be obtained. If a sufficient volume of material is available, concentration methods should be used to increase yield.

Individuals suspected of having extrapulmonary TB but who have had with a single negative

result from Xpert MTB/RIF should undergo further diagnostic testing; the processing of their tissue specimens (lymph nodes and other tissues) for Xpert MTB/RIF should include a decontamination step to enable specimens to be cultured concurrently.

Pleural fluid is a suboptimal specimen for the bacterial confirmation of pleural TB using any method. A pleural biopsy provides the preferred specimen.

These recommendations do not apply to specimens of stool, urine or blood, given the lack of data on the utility of Xpert MTB/RIF on these specimens.

3.2 General considerations

Important points about specimen processing procedures

- All specimens should be processed as soon as possible, to obtain optimal culture recovery of M. tuberculosis. Longer transportation times of specimens should not affect the use of Xpert MTB/RIF.
- Ensure that the Xpert MTB/RIF cartridge and any culture media to be inoculated are labelled correctly and clearly.
- Tissues must be processed within a biological safety cabinet, given the risk of producing aerosols while grinding and homogenizing samples.
- CSF samples are paucibacillary and can be processed using the same precautions as those used for sputum EXCEPT when they are concentrated by centrifugation.
- It is important to use Safe Working Practices to avoid contamination by bacteria other than tubercle bacilli and especially to avoid cross-contamination with tubercle bacilli from other specimens.
- When a sufficient volume of sample is available, culture should be performed concurrently with Xpert MTBR/RIF testing.
- Exposure time to decontamination reagents must be strictly controlled for samples requiring decontamination.
- Decontaminate samples for culture using either 4% NaOH or NaOH-NALC depending on usual practice. The example below uses 4% NaOH.

3.3 Specimen processing

The Xpert MTB/RIF assay can be used directly on CSF specimens and homogenized extrapulmonary specimens (from biopsies of lymph nodes or other tissues) or on decontaminated specimens if culture is performed concurrently.

Whenever possible, specimens should be transported and stored at 2–8 °C prior to processing (the maximum time for storage and processing is 7 days).

3.3.1 Lymph nodes and other tissues (for Xpert MTB/RIF only)

1. Using sterile pair of forceps and scissors, cut the tissue specimen into small pieces in a sterile mortar (or homogenizer or tissue grinder).

2. Add approximately 2 ml of sterile phosphate buffer (PBS).

3. Grind the solution of tissue and PBS using a mortar and pestle (or homogenizer or tissue grinder) until a homogeneous suspension has been obtained.

4. Use a transfer pipette to transfer approximately 0.7 ml of the homogenized tissue specimen to a sterile, conical screw-capped tube.

NOTE: Avoid transferring any clumps of tissue that have not been properly homogenized.

5. Use a transfer pipette to add a double volume of the Xpert MTB/RIF Sample Reagent (1.4 ml) to 0.7 ml of homogenized tissue.

6. Vigorously shake the tube 10 to 20 times or vortex for at least 10 seconds.

7. Incubate for 10 minutes at room temperature, and then shake the specimen vigorously again for another 10–20 times or vortex for at least 10 seconds.

8. Incubate the specimen at room temperature for an additional 5 minutes.

9. Using a fresh transfer pipette, transfer 2 ml of the processed sample to the Xpert MTB/RIF cartridge.

10. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

3.3.2 Lymph nodes and other tissues (nonsterile collections for Xpert MTB/RIF and culture)

1. Using a sterile pair of forceps and scissors, cut the tissue sample into small pieces in a sterile mortar (or homogenizer or tissue grinder).

2. Add approximately 2 ml of sterile PBS.

3. Grind the solution of tissue and PBS with a mortar and pestle (or homogenizer or tissue grinder) until a homogeneous suspension has been obtained.

4. Use a sterile transfer pipette to add the suspension to a 50 ml conical tube.

5. Add an equal volume of 4% NaOH and tighten the screw-cap.

6. Vortex thoroughly to homogenize the suspension.

7. Let the tube stand for 15 minutes at room temperature.

8. Fill the tube to within 2 cm of the top (that is, to the 50 ml mark on the tube) with PBS.

9. Centrifuge at 3000 g for 15 minutes.

10. Carefully pour off the supernatant through a funnel into a discard can containing 5% phenol or other mycobacterial disinfectant.

11. Resuspend the deposit in approximately 1–2 ml PBS.

12. Use another sterile transfer pipette to inoculate deposit into liquid media and/or onto two slopes of egg-based medium labelled with the specimen's identification number.

13. Label an Xpert/MTB/RIF cartridge with the specimen's identification number.

14. Using a transfer pipette, transfer approximately 0.7 ml of the homogenized tissue specimen to a conical, screw-capped tube to be used for the Xpert MTB/RIF test.

NOTE: Avoid transferring any clumps of tissue that have not been properly homogenized.

15. Using another transfer pipette, add a double volume of the Xpert MTB/RIF Sample Reagent (1.4 ml) to 0.7 ml of homogenized tissue.

16. Vigorously shake 10–20 times or vortex for at least 10 seconds.

17. Incubate for 10 minutes at room temperature, and then shake the specimen vigorously again for another 10–20 times, or vortex for at least 10 seconds.

18. Incubate the specimen at room temperature for an additional 5 minutes.

19. Using a fresh transfer pipette, transfer 2ml of the processed specimen to the Xpert MTB/RIF cartridge.

20. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

3.3.3 Lymph nodes and other tissues (sterile collection for Xpert MTB/RIF and culture)

1. Using a sterile pair of forceps and scissors, cut the tissue specimen into small pieces in a sterile mortar (or homogenizer or tissue grinder).

2. Add approximately 2 ml of sterile PBS.

3. Grind the solution of tissue and PBS with a mortar and pestle (or homogenizer or tissue grinder) until a homogeneous suspension has been obtained, and add PBS to adjust to a final volume of approximately 2 ml.

4. Using a sterile transfer pipette, transfer the suspension to a 50 ml conical tube.

5. Use another transfer pipette to inoculate suspension into liquid media and/or onto two slopes of egg-based medium labelled with the specimen's identification number.

6. Label an Xpert/MTB/RIF cartridge with the specimen's identification number.

7. Using a transfer pipette, transfer approximately 0.7 ml of the homogenized tissue specimen to a conical, screw-capped tube to be used for the Xpert MTB/RIF testing.

NOTE: Avoid transferring any clumps of tissue that have not been properly homogenized.

8. Using a transfer pipette, transfer a double volume of the Xpert MTB/RIF Sample Reagent (1.4 ml) to 0.7 ml of homogenized tissue.

9. Vigorously shake the tube 10–20 times or vortex for at least 10 seconds.

10. Incubate for 10 minutes at room temperature, and then shake the specimen vigorously again for another 10–20 times or vortex for at least 10 seconds.

11. Incubate the sample at room temperature for an additional 5 minutes.

12. Using a fresh transfer pipette, transfer 2 ml of the processed sample to the Xpert MTB/RIF cartridge.

13. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

3.3.4 CSF

The preferred processing method for CSF in Xpert MTB/RIF depends on the volume of specimen available for testing.

NOTE: *Blood-stained and xanthochromic CSF specimens may cause false-negative results from Xpert MTB/RIF.*

If there is more than 5 ml of CSF

1. Transfer all of the specimen to a conical centrifuge tube, and concentrate the specimen at 3000 g for 15 minutes.

2. Carefully pour off the supernatant through a funnel into a discard can containing 5% phenol or other mycobacterial disinfectant.

NOTE: Concentrated CSF should be decanted within a biological safety cabinet

3. Resuspend the deposit to a final volume of 2 ml by adding the Xpert MTB/RIF sample reagent.

4. Label an Xpert/MTB/RIF cartridge with the specimen's identification number.

5. Using a fresh transfer pipette, transfer 2 ml of the concentrated CSF specimen to the Xpert MTB/RIF cartridge.

6. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

If there is 1–5 ml of CSF

1. Add an equal volume of sample reagent to the CSF.

2. Add 2 ml of the sample mixture directly to the Xpert MTB/RIF cartridge.

3. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

If there is 0.1–1ml of CSF

1. Resuspend the CSF to a final volume of 2 ml by adding the Xpert MTB/RIF sample reagent.

2. Add 2 ml of the sample mixture directly to the Xpert MTB/RIF cartridge.

3. Load the cartridge into the GeneXpert instrument following the manufacturer's instructions.

If there is less than 0.1 ml

1. This is an insufficient sample for testing using the Xpert MTB/RIF assay.

4. Related documents

1. *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance in adults and children. Policy update.*
It is available at:
http://www.who.int/tb/laboratory/policy_statements/en/

2. The full report of the Expert Group meeting is available at:
http://www.who.int/tb/laboratory/policy_statements/en/

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