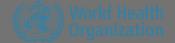2011

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Automated Real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System

Policy Statement

World Health Organization

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Contents

EXECUTIVE SUMMARY

Background

Earlier and improved tuberculosis (TB) case detection - including smear-negative disease, often associated with HIV co-infection - as well as expanded capacity to diagnose multidrug-resistant tuberculosis (MDR-TB) are global priorities for TB control. Conventional laboratory methods are slow and cumbersome and novel technologies for rapid detection are therefore the focus of TB research and development. With support from NIH, the Foundation for Innovative New Diagnostics (FIND) has partnered with Cepheid, Inc. (Sunnyvale, CA) and the University of Medicine and Dentistry of New Jersey (UMDNJ, Newark, NY) to develop a TB-specific automated, cartridge-based nucleic amplification assay (Xpert MTB/RIF) based on the GeneXpert multi-disease platform, currently unique in its simplification of molecular testing with fully integrated and automated sample preparation, amplification and detection required for real-time polymerase chain reaction. Xpert MTB/RIF detects *M. tuberculosis* as well as rifampicin resistance-conferring mutations directly from sputum, in an assay providing results within two hours. Data from published papers, large multi-centre laboratory validation and demonstration studies coordinated by FIND, and unpublished data investigator-driven, single-centre studies were recently reviewed by WHO with the aim to develop policy guidance on Xpert MTB/RIF use.

Summary of results

Results from **analytical studies** showed that the Xpert MTB/RIF assay has analytic sensitivity of five genome copies of purified DNA, and 131 cfu/ml of *M. tuberculosis* spiked into sputum. The molecular beacons which target the *rpoB* gene cover all the mutations found in >99.5% of all rifampicin resistant strains. There is no cross-reactivity with non-tuberculous mycobacteria, and TB and rifampicin resistance were correctly detected in the presence of non-tuberculous DNA or mixed susceptible and resistant strains. The sample reagent added in a 2:1 ratio to sputum was shown to kill >6 $\log_{10}$ cfu/ml of *M. tuberculosis* with 15 minutes of exposure, and to render >97% of smear-positive samples negative by LJ culture. The Xpert inoculation procedure and sample testing generated no detectable infectious aerosols.

Results from **controlled clinical validation trials** involving 1,730 individuals suspected of TB or MDR-TB prospectively enrolled in four distinctly diverse settings showed that 92.2% of culture-positive patients were detected by a single direct Xpert MTB/RIF test. Sensitivity of a single Xpert MTB/RIF test in smear-negative/culture-positive patients was 72.5% and increased to 90.2% when three samples were tested. Xpert MTB/RIF specificity was 99%. Xpert MTB/RIF detected rifampicin resistance with 99.1% sensitivity and excluded resistance with 100% specificity.

Results from **field demonstration studies** involving 6,673 individuals prospectively enrolled in six distinctly different settings confirmed these findings:
Test accuracy was retained, with a single Xpert MTB/RIF test directly from sputum detecting 99% of smear-positive patients and >80% of patients with smear-negative disease. HIV co-infection substantially decreased the sensitivity of microscopy, but did not significantly affect Xpert MTB/RIF performance. Rifampicin resistance was detected with 95.1% sensitivity and 98.4% specificity.
Mean time to detection was <1 day for Xpert MTB/RIF, 1 day for microscopy, 17 days for liquid culture and >30 days for solid culture. Rifampicin resistance was detected in <1 day with Xpert MTB/RIF vs 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 period of 58 days, compared to a median of 4 days when Xpert MTB/RIF results were used.
Operational aspects assessed confirmed robustness of Xpert MTB/RIF under varying temperature and humidity conditions, minimal training required of personnel, and high levels of user satisfaction. Storage of cartridges in high-volume settings was a concern given lack of adequate space. Waste generated was considerable more than for microscopy. Xpert MTB/RIF requires

uninterrupted and stable electrical power supply and annual validation of the system, which may pose a problem in rural/remote settings.

The negative predictive value (NPV[i]) of Xpert MTB/RIF assay is over 99% in settings with both low and high prevalence of rifampicin resistance, i.e. a negative result accurately excludes the possibility for rifampicin resistance.

The positive predictive value (PPV[ii]) for rifampicin resistance using Xpert MTB/RIF depends on underlying prevalence of rifampicin resistance. In settings or patient groups where rifampicin resistance is rare, the PPV of Xpert MTB/RIF testing is adversely affected. The PPV of Xpert MTB/RIF can be greatly improved by careful risk assessment in individual patients and targeted Xpert MTB/RIF testing.

Cost-effectiveness modeling indicated that the use of Xpert MTB-RIF significantly increased TB case-finding (by roughly 30%) when used as a replacement or add-on test to microscopy. Use of Xpert MTB/RIF as replacement for conventional culture and DST also significantly increased MDR case-finding (roughly three-fold). The capital and running costs of Xpert MTB/RIF are substantially greater than those of microscopy, though similar to the cost for performing culture and drug susceptibility testing.

Results from single-centre evaluation studies with varying design and study populations reported sensitivity in detecting TB ranging from 70% to 100% in culture-positive patients and around 60% in those with smear-negative disease. Specificity ranged from 91% to 100%. Pooled crude sensitivity for TB detection was 92.5% and pooled crude specificity was 98%. Average rifampicin sensitivity and specificity were around 98% and 99% respectively.

Conclusions

The WHO evidence synthesis process confirmed a solid evidence base to support widespread use of Xpert MTB-RIF for detection of TB and rifampicin resistance. It is therefore recommended that:

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

2) Xpert MTB/RIF may be used as a follow-on test to microscopy in settings where MDR and/or HIV is of lesser concern, especially in smear-negative specimens (conditional recommendation, recognising major resource implications).

Xpert MTB/RIF technology does, however, <u>not</u> eliminate the need for conventional microscopy culture and DST, which are required to monitor treatment progress and to detect resistance to drugs other than rifampicin. In settings or patient groups where rifampicin resistance is rare, Xpert MTB/RIF results indicating rifampicin resistance should be confirmed by conventional DST or LPA.

In addition, several operational conditions need to be met for successful implementation of Xpert MTB/RIF - stable electrical supply, a maximum ambient operating temperature of 30°C for the GeneXpert device, security against theft, dedicated trained personnel, adequate storage space, annual calibration of the instrument by a commercial supplier, and biosafety precautions similar to those for direct sputum microscopy should all be in place.

A Global Consultation called by WHO in December 2010 discussed the implementation considerations for scale-up of Xpert MTB/RIF and achieved broad consensus on the way forward. Key outcomes of the consultation were agreement on interim diagnostic algorithms, the positioning of Xpert MTB/RIF in risk groups at different levels of health services, and

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[i] NPV for rifampicin resistance: the proportion of diagnosed rifampicin-susceptible cases that are truly susceptible
[ii] PPV for rifampicin resistance: the proportion of diagnosed rifampicin-resistant cases that are truly resistant

implementation considerations for programmatic roll-out of Xpert MTB/RIF to optimize use and benefits of the technology.

Finally, a key consideration is the need for rapid access to appropriate treatment and care for all TB and MDR-TB patients who will be identified by the introduction of Xpert MTB/RIF. Implementation of Xpert MTB/RIF technology should therefore be phased in within the context of comprehensive national TB and MDR-TB strategic plans.

Acknowledgements

Declarations of Interest

Expert Group members were asked to submit completed Declaration of Interest (DOI) forms. These were reviewed by the WHO Legal Department prior to the Expert Group meeting. DOI statements were summarised by the co-chair of the Expert Group meeting (Karin Weyer, WHO-STB) at the start of the meeting.

Three expert group members (Richard Anthony, Daniela Cirillo, Doris Hillemann) declared FIND (Foundation for Innovative New Diagnostics) support for unrelated research activities. These declarations were deemed to be insignificant.

Frank Cobelens declared contractual agreements with FIND to conduct cost-effectiveness studies of the Xpert MTB/RIF assay as well as a BMGF consultancy to conduct an impact assessment of the Xpert MTB/RIF. This was deemed to be significant. Therefore, Frank Cobelens was present as observer to the meeting, did not participate in the GRADE evaluation process, and did not contribute to the meeting discussions where recommendations were developed.

FIND participants provided technical clarifications and were present as observers only to the sessions where technical and operational performance of the Xpert MTB/RIF assay was discussed. They were asked to recuse themselves once the meeting proceeded to sessions on GRADE evaluation and recommendations. Neither FIND participants nor other observers provided comments on the final document.

AUTOMATED REAL-TIME NUCLEIC ACID AMPLIFICATION TECHNOLOGY FOR RAPID AND SIMULTANEOUS DETECTION OF TUBERCULOSIS AND RIFAMPICIN RESISTANCE: Xpert MTB/RIF SYSTEM

1.   Background

Earlier and improved tuberculosis (TB) case detection - including smear-negative disease - as well as expanded capacity to diagnose multidrug-resistant tuberculosis (MDR-TB) are global priorities for TB control.   MDR-TB poses formidable challenges due to its complex diagnostic and treatment requirements, while HIV-associated TB largely goes undetected due to the limitations of current diagnostic techniques. Alarming increases in MDR-TB, the global emergence of extensively drug-resistant TB (XDR-TB), documented institutional transmission, and rapid mortality in MDR-TB and XDR-TB patients with HIV co-infection have highlighted the urgency for rapid screening methods.

Conventional methods for mycobacteriological culture and drug susceptibility testing (DST) are slow and cumbersome, requiring sequential procedures for isolation of mycobacteria from clinical specimens, identification of *Mycobacterium tuberculosis* complex, and *in vitro* testing of strain susceptibility to anti-TB drugs. During this time patients may be inappropriately treated, drug-resistant strains may continue to spread, and amplification of resistance may occur. Novel technologies for rapid detection of anti-TB drug resistance have therefore become a priority in TB research and development.

Since the development in the early 1980s of the polymerase chain reaction (PCR) - the first and most familiar method to amplify nucleic acid sequences - molecular diagnostics have been widely expected to have 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 *M. tuberculosis* organisms. In addition, commercial nucleic acid amplification tests (NAAT) proved to be significantly less sensitive than microbiological culture, especially for smear-negative TB. Moreover, culture largely remained necessary as a precursor to drug-susceptibility testing, while scale-up of conventional culture and drug-susceptibility services remained slow and expensive, compounded by huge demands on laboratory infrastructure and human resources.

Over the past five years, and with support from NIH, the Foundation for Innovative New Diagnostics (FIND) has partnered with Cepheid, Inc. (Sunnyvale, CA) and the University of Medicine and Dentistry of New Jersey (UMDNJ, Newark, NY) to develop an automated, cartridge-based NAAT for TB based on the GeneXpert multi-disease platform. The GeneXpert system was launched in 2004 and simplifies molecular testing by fully integrating and automating the three processes (sample preparation, amplification and detection) required for real-time PCR-based molecular testing. The GeneXpert platform is currently the only one of its kind and uses a cartridge containing lyophilised reagents, buffers and washes. Target detection and characterization is performed in real time using a six-colour laser detection device.

The Xpert MTB/RIF system is a recently developed TB-specific application, designed for the GeneXpert platform, to detect *M. tuberculosis* as well as rifampicin resistance-conferring mutations directly from sputum, in an assay providing results within two hours. Testing involves three manual steps: the addition of sample treatment reagent to liquefy and inactivate the bacteria in the sputum, transfer of 2ml of liquefied sputum into the cartridge, and loading of the cartridge into the device for the assay. All further steps are automated. The assay can be performed directly on a clinical sputum

specimen or on a decontaminated concentrated sputum pellet. Testing procedures are outlined below:

Figure 1: Assay Procedure for the Xpert MTB/RIF Test. Two volumes of sample treatment reagent are added to each volume of raw sputum or three volumes to each volume of decontaminated pellet. The mixture is shaken, incubated at room temperature for 15 minutes, and shaken again. Next, a sample of 2 to 3 ml is transferred to the test cartridge, which is then loaded into the instrument. All subsequent steps occur automatically. The user is provided with a printable test result, such as "MTB detected; RIF resistance not detected."

The Xpert MTB/RIF assay is based on hemi-nested real-time PCR amplifying the *rpoB* gene target. Molecular beacons using novel fluorophors and quenchers are used to detect hybridization to each of the five amplified target regions of the gene. *Bacillus globigii*, a spore-forming soil organism, is used as a full process control, acting as quality check for bacterial trapping, bacterial lysis, DNA extraction, amplification, and probe detection.

The Xpert MTB/RIF assay has been designed to be robust and easy to use. Xpert MTB/RIF cartridges and the specimen reagent should be stored at 2-28°C as per the manufacturer's recommendations. The cartridges are pre-loaded with all reagents necessary for sample processing, DNA extraction, amplification, and laser detection of target amplicon binding to the molecular beacons. The ambient operating temperature for the GeneXpert instrument is currently limited to a maximum of 30°C.

The assay was designed to be safe to use in microscopy laboratories where biological safety cabinets are usually not available. The sample treatment reagent is mycobactericidal, rapidly killing *M. tuberculosis* during the liquefaction process, and studies have confirmed that no aerosolation of mycobacteria occur while running the device.

GeneXpert instruments with varying capacities are available. The instruments assessed in the studies described below are four-module devices with capacity of running a maximum of 20 tests per day.

Both the GeneXpert platform and the Xpert MTB/RIF assay are under multiple patents. The GeneXpert platform is FDA approved. The assay is manufactured under ISO 13485:2003 certification and approved by the Regulatory Authority in Europe (CE-Marked), with FDA approval pending.

The GeneXpert platform and the Xpert MTB/RIF assay are currently the only mature technology representing a new generation of automated molecular diagnostic platforms. Others are at prototype stage and expected to become available in due course. These will need to be subjected to the same level of validation and expert review before their implementation can be recommended by WHO.

2. Methods

2.1 Evidence synthesis

In September 2010, an Expert Group was convened by the Stop TB Department of the World Health Organization (WHO) to assess all available data on Xpert MTB/RIF with a view towards policy recommendations on its use. Data from four published papers, large multi-centre laboratory validation and demonstration studies coordinated by FIND, and unpublished data from 12 investigator-driven, single-centre studies were made available to WHO to assess assay performance, the feasibility of programmatic implementation, and issues to be addressed in future research.

In accordance with current WHO standards for evidence assessment in the formulation of policy recommendations, the GRADE system[1] (http://www.gradeworkinggroup.org) was used for the evidence synthesis process, providing a systematic, structured framework for evaluating both the accuracy and the patient/public health impact of new interventions.

The following review questions were pre-defined in consultation with the WHO Expert Group:

1. What is the overall diagnostic accuracy of Xpert MTB/RIF for the diagnosis of TB and rifampicin resistance?

2. What is the diagnostic accuracy of Xpert MTB/RIF for the diagnosis of smear-negative TB?

GRADE evidence profiles were prepared to assess the diagnostic accuracy of the Xpert MTB/RIF for the detection of pulmonary TB and the detection of rifampicin resistance compared with conventional culture and DST as reference tests. Using the GRADE framework, sensitivity and specificity results were interpreted as proxy measures for patient-important outcomes based on the relative importance/impact of false-positive and false-negative results: Poor sensitivity would result in false-negative results where TB and MDR-TB patients would be missed with negative consequences for morbidity, mortality and transmission of disease. Poor specificity would result in false-positive results where patients without TB or MDR-TB would be prescribed unnecessary treatment, with negative consequences such as serious adverse events related to second-line anti-TB drug use.

For each outcome, the GRADE quality of evidence was initially regarded as 'high' since all studies were cross-sectional or cohort studies, prospectively enrolling patients suspected with TB and/or MDR-TB. The quality of evidence and study limitations were also assessed using six GRADE criteria: (1) study design; (2) risk of bias; (3) directness; (4) inconsistency; (5) imprecision; and (6) publication/reporting bias. It was noted that the majority of studies did not enroll children and that studies were conducted in largely urban settings due to the need for quality-assured culture and DST as reference methods. As the intended use of the Xpert MTB/RIF test is at district and sub-district levels, the quality of evidence was downgraded by one point to 'moderate'.

The system used to establish the strength and ranking of the recommendations involved assessing and balancing each question according to: (1) desirable and undesirable effects; (2) quality of available evidence; (3) values and preferences related to intervention in different settings; and (4) cost considerations for different epidemiological settings.

The findings and recommendations from the Expert Group Meeting were presented to the WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) in September 2010. STAG-TB acknowledged the transforming potential of this new technology and the solid evidence base to support its widespread use for detection of TB and rifampicin resistance[2]. STAG-TB also acknowledged the need for urgent access to this innovation in individuals at risk of MDR-TB and HIV-associated TB in resource-constrained settings. STAG-TB subsequently recommended that WHO:

1. Proceed with detailed policy guidance on the use of Xpert MTB/RIF;

2. Develop a global strategy for rapid uptake of Xpert MTB/RIF in a systematic and phased approach, including mechanisms to monitor and assess the roll-out of Xpert MTB/RIF, with a clear plan to document the impact on case detection, MDR-TB response, scale-up and cost-effectiveness;

3. Proceed with a Global Consultation on the implementation considerations for scale-up of Xpert MTB/RIF under routine programme conditions (including diagnostic algorithms, logistics, procurement and distribution, quality assurance, and waste disposal);

4. Assist countries with technical support and planning for inclusion of Xpert MTB/RIF in revised diagnostic algorithms.

2.2   Decision-making during the Expert Group meeting

The Expert Group meeting was co-chaired by the STB secretariat and a GRADE evidence synthesis expert. Decisions were based on consensus (preferred option) and by voting (with simple majority rule) if consensus was not achieved. Concerns and opinions by Expert Group members were noted and included in the final meeting report[3]. The detailed meeting report was prepared by the STB secretariat and underwent several iterations (managed by the STB secretariat) before final sign-off by Expert Group members.

Draft WHO policy guidance based on the consensus recommendations was subsequently prepared by the STB secretariat and presented to STAG-TB. Final policy guidance was circulated to the Expert Group and STAG-TB members following a similar iterative process as described above.

The final policy guidance document was approved by the WHO Guidelines Review Committee (GRC), having satisfied the GRC requirements for guideline development.[iii]

[iii] GRC statement: This guideline was developed in compliance with the process for evidence gathering, assessment and formulation of recommendations, as outlined in the WHO Handbook for Guideline Development (current version).

3.   Scope of the policy guidance

This document provides a pragmatic summary of the evidence and recommendations related to Xpert MTB/RIF, and should be read in conjunction with the detailed findings from the *Xpert MTB/RIF Expert Group Meeting Report 2013*[3], the *WHO Framework for Implementing TB Diagnostics, 2010*[4] and the *WHO Rapid Implementation of Xpert MTB/RIF, 2011* document[5]. These documents are available on http://www.who.int/tb/dots/laboratory/policy/en and provide guidance on the implementation of currently approved WHO diagnostic tools and methods, within the local context of country infrastructure, available resources, TB/MDR-TB epidemiology, and TB policy reform.

None of the existing TB diagnostic tools are mutually exclusive and they can be implemented in various combinations in country screening and diagnostic algorithms, which are highly setting and resource specific. Expert laboratory input is therefore needed to define the most cost-effective and efficient algorithms in individual countries, guided by WHO standards and procedures, and within a framework of overall, integrated laboratory strengthening activities.

This policy guidance should be used to support implementation of Xpert MTB/RIF technology for TB diagnosis and rifampicin resistance detection within National TB Programmes (NTPs), and is intended to be used by NTB Managers and Laboratory Directors, in coordination with external laboratory consultants, donor agencies, technical advisors, laboratory technicians, laboratory equipment procurement officers, warehouse managers, private-sector service providers, other relevant government sectors, and implementing partners involved in country-level TB laboratory strengthening. Individuals responsible for programme planning, budgeting, resource mobilization, and training activities for TB diagnostic services may also benefit from using this document.

Review of additional data from implementation sites will be done annually, with ongoing refinement of the policy guidance based on more extensive field evaluation of the new technology after implementation, including country-specific cost-effectiveness and cost-benefit analyses.

Date of review: 2015

4.   Evidence base for policy formulation

4.1   Analytic studies

Limits of detection (LOD) studies using bacterial genomic DNA and whole organisms spiked into buffer or uninfected sputum showed the Xpert MTB/RIF assay to have an analytic sensitivity of five genome copies of purified DNA and 131 cfu/ml of *M. tuberculosis* spiked into sputum. The specificity of the assay, tested with a large variety of non-tuberculous mycobacteria, fungi and viruses, showed the assay to correctly exclude all non-tuberculous isolates.

The molecular beacons were designed based on published and experimental data covering 4,115 mutations in the RRDR region from 4,072 strains of *M. tuberculosis* and covered the mutations found in >99.5% of all rifampicin resistant strains. TB and rifampicin resistance were correctly detected even in the presence of DNA from non-tuberculous mycobacteria or mixed susceptible and resistant strains.

Two important biosafety features were confirmed: First, the sample reagent added in a 2:1 ratio to sputum was shown to kill 10^7 log cfu/ml of *M. tuberculosis* with 15 minutes of exposure at room temperature and to render >97% of smear-positive specimens negative by conventional culture.

Second, aerosol studies while running the device spiked with high concentrations of mycobacteria did not detect any infectious aerosols generated.

4.2   Multi-centre clinical validation studies

Controlled clinical trials were conducted by FIND in four geographically diverse settings with varying underlying TB, HIV and MDR-TB prevalence (Azerbaijan, India, Peru, South Africa), prospectively evaluating 1,730 individuals suspected of either drug-susceptible or MDR-TB. A total of 4,386 sputum specimens from 1,462 eligible patients were tested by Xpert MTB/RIF as well as microscopy and two conventional culture methods.

Detection of pulmonary TB
The proportion of culture-positive patients detected with a single, direct Xpert MTB/RIF test was 92.2% vs. 96.0% for testing two specimens (one direct and one pellet from a concentrated specimen) and 97.6% for testing three (one direct plus two pellets). No significant difference was observed between tests on untreated sputum and those on decontaminated pellets.

In smear-negative, culture-positive patients, the addition of a second Xpert MTB/RIF test increased sensitivity by 12.6% from 72.5% to 85.1%, and a third test further increased sensitivity by 5.1% to 90.2%. There was no significant variation in sensitivity across sites. A single, direct Xpert MTB/RIF test identified a greater proportion of culture-positive patients than did a single LJ culture.

HIV status was known for 976 (40.2%) of patients. Among 392 HIV-positive patients with pulmonary TB, the sensitivity of Xpert MTB/RIF was 93.9%, compared to 98.4% in HIV-negative patients.

Among 105 patients with culture-negative results treated for TB based on clinical symptoms and showing improvement, 29.3% had a positive Xpert MTB/RIF result.

The specificity of Xpert MTB/RIF, confirmed by clinical and microbiological follow-up of TB suspects, was 99%.

Detection of rifampicin resistance
Rifampicin resistance is a reliable proxy for MDR-TB in most settings, and the Xpert MTB/RIF technology probes for five beacons in the *rpo*B gene comprising the vast majority of rifampicin resistance-conferring mutations. Xpert MTB/RIF correctly detected rifampicin resistance in 209 of 211 patients (99.1% sensitivity) and in all 506 patients with rifampicin susceptibility (100% specificity), when compared against phenotypic testing and sequencing results to resolve 15 discordant results. Overall, 97.6% (200/205) of rifampicin resistant cases were confirmed to have multi-drug resistance on phenotypic testing.

Indeterminate results
The rate of indeterminate Xpert MTB/RIF results was 3.7%, lower than the rate of culture contamination. Valid results were obtained in 92.8% in which enough material remained for the test to be repeated.

4.3   Demonstration studies

Demonstration studies coordinated by FIND involved 6,673 mostly adult subjects presenting with symptoms suggestive of pulmonary TB from nine sites in Azerbaijan, India, Peru, the Philippines, South Africa and Uganda. The patient populations were very diverse, with prevalence of HIV ranging from <1% to 100% and MDR prevalence ranging from 2% to 54%.

Given the need for reference standards, access to quality-assured conventional culture and DST was pre-requisite and participating centres were therefore largely based in urban or peri-urban areas. All sites categorized patients into MDR risk patients (previously treated, non-converting patients, treatment failures, symptomatic contacts of confirmed MDR cases) and TB case detection patients (others).

Patients were divided into four categories for analysis: smear-positive/culture-positive; smear-negative/culture-positive; smear-negative/culture-negative without treatment (no TB); and smear-negative/culture-negative with treatment based on clinical and radiological findings (clinical TB).

Detection of pulmonary TB

High sensitivity of Xpert MTB/RIF was confirmed in these studies, with a single test directly on sputum detecting 99% of smear-positive/culture-positive patients and >80% of patients with smear-negative disease. In comparison, a single, direct smear performed on the same sputum specimen as Xpert MTB/RIF had a sensitivity in culture-positive cases of 59.5% (95CI 56% - 63%). There was no significant difference in specificity (99%; 95CI 98% - 99%).

A single, direct Xpert MTB/RIF test (sensitivity 90.8%; 95CI 88% - 93%) identified a similar proportion of overall culture-positive patients than did a single LJ culture (sensitivity 89.8%; 95CI 87% - 92%).

Among HIV-positive patients with culture-confirmed pulmonary TB, the sensitivity of Xpert MTB/RIF was 86% compared with 92% in HIV-negative patients. HIV co-infection did not significantly affect Xpert MTB/RIF performance to detect smear-negative/culture-positive patients; in comparison, the sensitivity of smear microscopy was significantly reduced (to 47% compared to 65% in HIV-negative patients).

Among 120 TB suspects who were culture-negative but were treated for TB on the basis of clinical symptoms and improved on TB treatment, 19.2% had positive Xpert MTB/RIF results.

Detection of rifampicin resistance

Rifampicin resistance is determined by the Xpert MTB/RIF software on the basis of late or absent probe signals. The sensitivity and specificity for detecting rifampicin resistance using the initial software was 98.4% and 96.1% respectively. During the early phase of the Field Demonstration studies two false-positive rifampicin resistance calls by Xpert MTB/RIF were reported from South Africa. These two cases were found to be susceptible by line probe assay (used as reference standard) and the absence of mutations was confirmed by sequencing and susceptibility confirmed by phenotypic DST.

A root cause analysis showed that the false calls resulted from some of the molecular beacons generating their signal slightly later than the limit imposed by the software. A review of test results from other sites identified a small number of similar looking artifacts.

Since assay specificity for rifampicin resistance was considered crucial, especially for low MDR prevalence settings, the decision was made to implement a software change for all settings. With these changes, sensitivity for rifampicin detection was slightly reduced to 95.1% and specificity increased to 98.4%.

Rifampicin mono-resistance was uncommon (1.4%) and the overall predictive values for MDR using rifampicin resistance detection were high (positive predictive value 92.9%; negative predictive value 98.9%). There were no reported amplicon contamination events.

Indeterminate results

Indeterminate Xpert MTB/RIF results were low at 2.5% and repeated testing led to a valid result in 97% of patients. In comparison, the overall contamination rate for culture was 4.7%.

The mean times to TB detection were <1 day for Xpert MTB/RIF, 1 day for microscopy, 17 days for liquid culture and >30 days for solid culture. Rifampicin resistance was detected in <1 day with Xpert MTB/RIF vs an average of 75 days with conventional phenotypic testing.

Time to treatment initiation
The impact of speed on utility of results was documented in the Field Demonstration studies: 0.5% of Xpert MTB/RIF results were not reported to the health services, compared to 1.9% of microscopy results, 14% of culture results and 20% of conventional DST results.

During the validation phase when Xpert MTB/RIF was not used to direct therapy, smear-negative TB patients started treatment after a median of 58 days; during the implementation phase when Xpert MTB/RIF was used to direct treatment, the median time to initiation fell to 4 days.

4.4   Unpublished single-centre evaluation studies

Unpublished single-centre evaluation studies yielded similar performance results on 4,575 tests with the Xpert MTB/RIF assay, with reported sensitivity in detecting TB ranging from 70% to 100% in culture-positive patients and around 60% in those with smear-negative disease. Specificity ranged from 91% to 100%. Pooled crude sensitivity for all Xpert MTB/RIF tests performed was 92.2% and pooled crude specificity was 98%. Average rifampicin sensitivity and specificity were around 98% and 99% respectively.

4.5   Operational and programmatic aspects

Robustness
Technical problems over a one-year period (11 instruments and 44 modules) included module failure (one site, one module), module not detected (two sites, one module each), cartridge stuck inside the module (one site, one module), error message due to high temperature (one site), and error in print-out of results (three sites). All technical problems were resolved quickly, mostly with remote support.

Operating temperatures routinely recorded by the GeneXpert instrument showed that the maximum operating temperature recommended by the manufacturer (30˚C) was exceeded in several sites, without adversely affecting instrument or test performance. However, all GeneXpert instruments were new and long-term data on the robustness of the device under prolonged periods of temperatures exceeding 30˚C are not yet available.

No DNA contamination events were recorded, despite 50% of operators not having followed instructions to clean instrument surfaces with bleach and ethanol on a daily basis.

Positive (known smear-positive sputum) and negative controls (Xpert sample reagent buffer) controls were run upon receipt of new lots. No lot-to-lot variability or quality issues with cartridges or sample reagent buffer were observed.

Training needs
The training duration per laboratory was two days. Operator skill sets prior to this training did not include any experience with molecular tests. Most operators were microscopists and computer knowledge was basic in most cases.

Training was done using a combination of the user manual and PowerPoint slide sets developed by FIND. Training covered a) how to handle the computer and operate the GeneXpert software; b) the

sample processing step and associated biosafety; c) the concept of DNA contamination, including the need for cleaning with bleach and disposal of cartridges without re-opening; and d) trouble-shooting.

One-day web-based training was provided by the manufacturer for more experienced operators at two sites and proficiency achieved without any problems. Training for module swap-out, required in case of module replacement or calibration, was also provided by the manufacturer via telephone or internet (web-based).

An anonymous questionnaire survey during the initial FIND validation studies showed that users found the Xpert MTB/RIF assay very simple to operate. User satisfaction with the assay and user-independent read-out was very high.

Waste management
Cartridge disposal was done according to local guidelines for hazardous materials, identical to sputum containers. Of relevance was the relatively large volume of additional waste generated compared to smear microscopy (cartridges, buffer bottles and single-use pipettes).

Storage of cartridges
Several of the participating sites could not ensure that room temperature remained below 28˚C or did not have adequate storage space. Cartridges were therefore stored at supervisory sites with air conditioning or a cool room) and were distributed approximately every two weeks.

Electrical supply and back-up power
All participating sites reported frequent power outage (seconds to minutes in duration) and small UPS of 400 VA were therefore installed at all sites, sufficient to run for 15-20 minutes during power outages. One site suffering from longer power cuts (lasting hours) used serially connected car batteries to run the GeneXpert instrument and computer in case of power failures, without any apparent adverse effect on instrument or test performance.

Biosafety requirements
In a series of studies carried out at UMDNJ, the relative biosafety of the Xpert MTB/RIF procedure was compared to routine preparation of slides for direct acid-fast bacilli (AFB) microscopy. Treatment of sputum with a 3:1 ratio of the sample reagent (SR) eliminated *M. tuberculosis* growth in all subsequent cultures while a 2:1 ratio achieved >6 $\log_{10}$ cfu/ml killing of *M. tuberculosis* with 15 minutes of exposure.
Aerosolisation studies indicated that no detectable infectious aerosols were generated within the instrument during operation, and inoculating cultures with fluid from inside the cartridges showed 100% negative cultures.

4.6   Cost-effectiveness modeling

Data from three different epidemiological settings (Azerbaijan, India, South Africa) indicated that the use of Xpert MTB-RIF significantly increased TB case-finding (by roughly 30%) when used as a replacement or add-on test to microscopy. Use of Xpert MTB/RIF as replacement for conventional culture and DST also significantly increased MDR case-finding (roughly three-fold). The capital and running costs of Xpert MTB/RIF were substantially greater than those of microscopy, though similar to the cost for performing culture and drug susceptibility testing. The total cost per case detected varied considerably by country, but on average the replacement scenario had between 8% and 11% higher cost per DALY gained, still within acceptable cost-effectiveness ratios according to WHO criteria.

5.   Policy recommendations

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

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

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

Remarks:

These recommendations apply to the use of Xpert MTB/RIF in sputum specimens (including pellets from decontaminated specimens). Data on the utility of Xpert MTB/RIF in extra-pulmonary specimens are still limited;

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

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

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

6.   Implementation considerations

As with any new technology, a range of implementation issues was identified, without which Xpert MTB/RIF use would not be optimal. These include:

- Positioning: Xpert MTB/RIF is suitable for use at **district and sub-district level**. Although testing with Xpert MTB/RIF does not require additional laboratory equipment, the sophisticated nature of the device requires care of handling, i.e. stable and uninterrupted electrical supply to avoid interruption of the procedure and subsequent loss of results, security against theft, adequate storage space for the cartridges, dedicated staff to perform testing, and biosafety procedures similar to microscopy;

- Adoption of Xpert MTB/RIF does not eliminate the need for conventional TB microscopy, culture and DST capacity. Microscopy and/or culture remains necessary for monitoring of treatment, as it is unlikely that a test based on DNA detection would be suitable. **Xpert MTB/RIF should therefore not be used for monitoring of treatment**. In addition, conventional culture and DST are still required to detect resistance to anti-TB drugs other than rifampicin;

- The settings and algorithms for using Xpert MTB/RIF should be guided by **country-specific epidemiology** (TB, HIV and MDR-TB), **available resources**, and anticipated **cost-effectiveness**. Interim diagnostic algorithms are included in the *WHO Rapid Implementation of Xpert MTB/RIF, 2011* document.

- Testing costs should also be measured against public health and patient benefits, including direct financial savings associated with decreased diagnostic delays and medical and social benefits and reduced transmission associated with early and appropriate treatment;

- Countries already using molecular line probe assay (LPA) for rapid diagnosis of rifampicin resistance may introduce Xpert MTB/RIF at lower levels of the laboratory service (typically at district- or sub-district level) given that LPA is suitable for high-through-put testing at central/regional laboratory level (http://www.who.int/tb/laboratory/en/);

- The negative predictive value (NPV[iv]) for rifampicin resistance of the Xpert MTB/RIF assay is over 99% in settings with both low and high prevalence of rifampicin resistance, i.e. a negative result accurately excludes the possibility for rifampicin resistance and no further testing to confirm negative results is required.

- The positive predictive value (PPV[v]) for rifampicin resistance using Xpert MTB/RIF exceeds 90% in settings or patient groups where the underlying prevalence of rifampicin resistance is greater than 15%. The PPV ranges between 71% and 84% where prevalence of rifampicin resistance is between 5% and 10%, and diminishes further to less than 70% when the prevalence of underlying rifampicin resistance falls below 5%. In such circumstances, positive Xpert MTB/RIF results should be confirmed by conventional DST or LPA. The PPV of Xpert MTB/RIF can be greatly improved by careful risk assessment in individual patients and targeted Xpert MTB/RIF testing, as outlined in the *WHO Rapid Implementation of Xpert MTB/RIF, 2011* document;

- Adoption of Xpert MTB/RIF should be decided by Ministries of Health **within the context of national plans for management of TB, MDR-TB and HIV-associated TB**, including the development of country-specific screening strategies, timely access to quality-assured first- and second-line anti-tuberculosis drugs, and appropriate care delivery mechanisms;

[iv] NPV for rifampicin resistance: the proportion of diagnosed rifampicin-susceptible cases that are truly susceptible
[v] PPV for rifampicin resistance: the proportion of diagnosed rifampicin-resistant cases that are truly resistant

- Adoption of Xpert MTB/RIF should be **phased in** within the context of national laboratory strengthening plans, considering that the GeneXpert system may also provide a technology platform for other diagnostic services and reducing costs involved in providing integrated laboratory services;

- As Xpert MTB/RIF only detects resistance to rifampicin, countries with documented or suspected cases of **XDR-TB** should establish or expand conventional culture and **second-line DST capacity** for quality-assured testing of second-line drugs, based on current WHO policy guidance;

- Xpert MTB/RIF cartridges and the specimen reagent should be stored at 2-28˚C as per manufacturer recommendations. The cartridges are quite bulky when packed and require **substantial storage space**.

- The maximum capacity of a single, four-module GeneXpert instrument is **20 specimens per day**. Busier sites will therefore either need several four-module instruments or larger instruments (16 modules or more), with associated cost and storage implications.

- The manufacturer recommended **ambient operating temperature** for the GeneXpert instrument is currently limited to a maximum of 30°C. In settings where the ambient temperature regularly exceeds 30˚C, air conditioning of the room where the assay is being done should be considered;

- The Xpert MTB/RIF cartridges have a shelf life of 18 months, posing a challenge in relatively inaccessible areas with complex customs clearance procedures. Xpert MTB/RIF cartridges are usually shipped with a manufacturer-guaranteed minimum shelf-life of 6-9 months from the date of shipment. **Management of inventory** based on usage, shelf-life and lead time for delivery of orders is therefore needed;

- The GeneXpert modules require **annual calibration** which must be performed by a commercial distributor or carried out by swapping old modules for calibrated modules. A detailed commercial sales contract and customer support plan should be negotiated with the supplier, guaranteeing ample and continuous supply of cartridges, customs clearance, maintenance and calibration, repair and replacement;

- Mechanisms for **rapid reporting** of Xpert MTB/RIF results to clinicians and timely access to appropriate treatment must be established to provide patients with the benefit of an early diagnosis;

- The capital and running costs of Xpert MTB/RIF are substantially greater than those of microscopy, and currently similar to the cost for performing conventional culture and DST. Costs of manufacture (and therefore purchase) are expected to decrease substantially as demand and manufacturing volume increases.   FIND has negotiated preferentially pricing of Xpert MTB/RIF for the public health sector in developing countries; more information can be found on http://finddiagnostics.org.

7.   Research implications

A series of operational research questions have been identified related to the introduction of Xpert MTB/RIF and its impact on the diagnosis of TB and MDR-TB and patient management While these should not prevent or delay the implementation of Xpert MTB/RIF, priorities for research include:

- Evaluation of the interim diagnostic algorithms in different epidemiological and geographical settings and patient populations;

- Cost-effectiveness and cost-benefit of Xpert MTB/RIF in different programmatic settings;

- Evaluation of Xpert MTB/RIF in extra-pulmonary and paediatric TB;

- Evaluation of the impact on Xpert MTB/RIF in reducing the diagnostic delay and the time for initiation of appropriate treatment;

- Evaluation of the impact of Xpert MTB/RIF on treatment access and treatment outcomes, especially among hard-to-reach populations.

Key operational research questions for Xpert MTB/RIF are outlined in the *WHO Rapid Implementation of Xpert MTB/RIF, 2011* document.

8.   Plans for supporting roll-out of Xpert MTB/RIF

A Global Consultation was called by WHO on 30 November - 2 December 2010 where implementation considerations for roll-out of Xpert MTB/RIF were discussed and broad consensus on the way forward was achieved. Key outcomes of the consultation were agreement on interim diagnostic algorithms, the positioning of Xpert MTB/RIF in risk groups at different levels of health services, and implementation considerations for programmatic roll-out of Xpert MTB/RIF to maximise use and benefits of the technology. Interim diagnostic algorithms initially developed in consultation with the respective Working Groups of the Stop TB Partnership (GLI, MDR-TB, DOTS Expansion and TB/HIV), were discussed in depth and revised during the Global Consultation meeting.

In March 2010, the Rapid Implementation document developed by the WHO Stop TB Department was disseminated widely through e-mail distribution and placed on various websites of stakeholders. The Rapid Implementation document outlines the requirements for systematic roll-out of Xpert MTB/RIF in varying epidemiological and resource settings, with a view towards future large-scale implementation based on programmatic data collected during the roll-out phase. The document contains the interim diagnostic algorithms, recommended patient management approaches, and operational/logistical aspects which need to addressed during Xpert MTB/RIF implementation.

With the cooperation of partners, WHO will provide global coordination of Xpert roll-out to avoid duplication and overlap of efforts. During the roll-out phase a dedicated website will be established to map uptake of Xpert MTB/RIF, communicate operational problems reported from the field and corrective measures taken. Countries and partners embarking on Xpert MTB/RIF roll-out are encouraged to join this effort, use the interim diagnostic algorithms, and contribute to the standardised collection of data. A meeting of Early Implementers will be called by WHO at the end of 2011 to share and review findings. Results and subsequent refinement of testing strategies from the roll-out phase will be used to inform future scale-up of Xpert MTB/RIF at country level.

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10. GRADE tables

Table 1. GRADE evidence profile: Xpert MTB/RIF as a replacement test for conventional culture and DST in FIND- coordinated multi-centre evaluation and demonstration studies

No of participants (studies)	Study design	Limitations	Indirectness	Inconsistency	Imprecision	Publication bias	Quality of evidence (GRADE)	Importance
A. Outcome: Diagnostic accuracy for detection of pulmonary TB								
True Positives								
1,275 (10) [A1]	Cross-sectional	Serious (-1) [A2]	No serious indirectness [A3]	No serious inconsistency [A4]	No serious imprecision [A5]	Not likely [A6]	Moderate ⊕⊕⊕◯	Critical (7-9)
True Negatives								
2,447 (10) [A1]	Cross-sectional	Serious (-1) [A2]	No serious indirectness [A3]	No serious inconsistency [A4]	No serious imprecision [A5]	Not likely [A6]	Moderate ⊕⊕⊕◯	Critical (7-9)
False Positives								
31 (10) [A1]	Cross-sectional	Serious (-1) [A2]	No serious indirectness [A3]	No serious inconsistency [A4]	No serious imprecision [A5]	Not likely [A6]	Moderate ⊕⊕⊕◯	Critical (7-9)
False Negatives								
118(10) [A1]	Cross-sectional	Serious (-1) [A2]	No serious indirectness [A3]	No serious inconsistency [A4]	No serious imprecision [A5]	Not likely [A6]	Moderate ⊕⊕⊕◯	Critical (7-9)
B. Outcome: Diagnostic accuracy for rifampicin resistance detection								
True Positives								
376 (10) [A1]	Cross-sectional	Serious (-1) [A2]	No serious indirectness [A3]	No serious inconsistency [A4]	No serious imprecision [A5]	Not likely [A6]	Moderate ⊕⊕⊕◯	Critical (7-9)
True Negatives								
1,171(10) [A1]	Cross-sectional	Serious (-1) [A2]	No serious indirectness [A3]	No serious inconsistency [A4]	No serious imprecision [A5]	Not likely [A6]	Moderate ⊕⊕⊕◯	Critical (7-9)
False Positives								
21(10) [A1]	Cross-sectional	Serious (-1) [A2]	No serious indirectness [A3]	No serious inconsistency [A4]	No serious imprecision [A5]	Not likely [A6]	Moderate ⊕⊕⊕◯	Critical (7-9)
False Negatives								
14(10) [A1]	Cross-sectional	Serious (-1) [A2]	No serious indirectness [A3]	No serious inconsistency [A4]	No serious imprecision [A5]	Not likely [A6]	Moderate ⊕⊕⊕◯	Critical (7-9)

Footnotes:

[1] Quality of evidence was rated as high (no points subtracted), moderate (1 point subtracted), low (2 points subtracted), or very low (>2 points subtracted) based on five criteria: study limitations, indirectness of evidence, inconsistency in results across studies, imprecision in summary estimates, and likelihood of publication bias. For each outcome, the quality of evidence was regarded as high since all studies were cross-sectional or cohort studies, prospectively enrolling patients suspected with TB and/or MDR-TB. The evidence rankings were considered to be the same for consideration of true positives, false positives, false negatives, and true negatives.

[A1] Sensitivity and specificity of the Xpert MTB/RIF assay for detection of TB were determined using conventional culture as index test in 10 studies coordinated by FIND. These studies also included an assessment of the sensitivity and specificity of the Xpert MTB/RIF system for the detection of rifampicin resistance using conventional DST as the index test. Discordant rifampicin results were resolved by *rpo*B sequencing.

[A2] Study limitations were assessed using the QUADAS tool. It was noted that the majority of studies did not enrol children and that the studies were conducted in large urban settings due to the need for quality-assured culture and DST as reference methods. As the intended use of the test is in district and sub-district levels, the quality of evidence was downgraded.

[A3] Diagnostic accuracy was considered as a surrogate for patient-important outcomes.

[A4] No significant heterogeneity among studies was observed, with consistency in sensitivity and specificity estimates reported in different epidemiological settings.

[A5] Pooled sensitivity derived from the studies had narrow confidence intervals.

[A6] Publication bias was considered highly unlikely as the Xpert MTB/RIF is a new assay, produced by a single manufacturer and only available to a small number of investigators.

Table 2.   GRADE summary of findings – Diagnostic accuracy of the Xpert MTB/RIF assay in multi-centre clinical validation studies

Review question: What is the diagnostic accuracy of Xpert MTB/RIF for i) detection of pulmonary tuberculosis; and ii) detection of resistance to rifampicin?
Patients/population: Adult pulmonary TB suspects (for TB detection); Confirmed TB cases (for rifampicin resistance detection)
Setting: Multi-centre clinical validation studies
Index test: Conventional culture and DST
Importance: A rapid, accurate, simple test could replace conventional culture and DST and expand testing to lower levels of the health service
Reference standard: Conventional microscopy, culture, drug susceptibility testing, clinical diagnosis of pulmonary TB
Studies: Cross-sectional or cohort

Outcomes: TP, TN, FP, FN Diagnostic accuracy for *M. tuberculosis*	Effect % (95% CI)	No. of participants (studies)	What do these results mean given 10% prevalence among suspects being screened for TB?	What do these results mean given 30% prevalence among suspects being screened for TB?	Quality of evidence
All specimens **AFB smear positive/culture positive** **AFB smear negative /culture positive**	Sensitivity 92% (90, 94) Specificity 99% (98, 100) Sensitivity 98% (97, 99) Sensitivity 72% (65, 79)	1,341 (5)	With a prevalence of 10%, 100/1000 will have TB. Of these, 92 (TP) will be identified; 8 (FN) will be missed by Xpert MTB/RIF. Of the 900 patients without TB, 891 (TN) will not be treated; 9 (FP) may be unnecessarily treated.	With a prevalence of 30%, 300/1000 will have TB. Of these, 276 (TP) will be identified; 24 (FN) will be missed by the Xpert MTB/RIF. Of the 700 patients without TB, 693 (TN) will not be treated; 7 (FP) may be unnecessarily treated.	Moderate ⊕⊕⊕◯
Diagnostic accuracy for rifampicin resistance	**Effect % (95% CI)**	**No. of participants (studies)**	**What do these results mean given 10% prevalence of rifampicin resistance among persons with TB?**	**What do these results mean given 30% prevalence of rifampicin resistance among persons with TB?**	**Quality of Evidence**
All specimens	Sensitivity 98% (94, 99) Specificity 98% (96, 99)	720 (5)	With a prevalence of 10%, 100/1000 will have rifampicin resistance. Of these, 98 (TP) will be identified; 2 (FN) will be missed by Xpert MTB/RIF. Of the 900 patients with TB susceptible to rifampicin, 891 (TN) will not be treated for MDR; 9 (FP) may be unnecessarily treated.	With a prevalence of 30%, 300/1000 will have rifampicin resistance. Of these, 294 (TP) will be identified; 6 (FN) will be missed by Xpert MTB/RIF. Of the 700 patients with TB susceptible to rifampicin, 693 (TN) will not be treated for MDR; 7 (FP) may be unnecessarily treated for MDR.	Moderate ⊕⊕⊕◯

Table 3.   GRADE summary of findings – Diagnostic accuracy of the Xpert MTB/RIF assay in multi-centre demonstration studies

Review question: What is the diagnostic accuracy of Xpert MTB/RIF for i) detection of pulmonary tuberculosis; and ii) detection of resistance to rifampicin?
Patients/population: Adult pulmonary TB suspects (for TB detection); Confirmed TB cases (for rifampicin resistance detection)
Setting: Multi-centre clinical validation studies
Index test: Conventional culture and DST
Importance: A rapid, accurate, simple test could replace conventional culture and DST and expand testing to lower levels of the health service
Reference standard: Conventional microscopy, culture, drug susceptibility testing, clinical diagnosis of pulmonary TB
Studies: Cross-sectional or cohort

Outcomes: TP, TN, FP, FN Diagnostic accuracy for MTB	Effect % (95% CI)	No. of participants (studies)	What do these results mean given 10% prevalence among suspects being screened for TB?	What do these results mean given 30% prevalence among suspects being screened for TB?	Quality of Evidence
All specimens AFB smear positive/culture positive AFB smear negative/culture positive	Sensitivity 91% (88, 93) Specificity 99% (98, 99) Sensitivity 99% (97-100) Sensitivity 80% (75, 84)	2,530 (6)	With a prevalence of 10%, 100/1000 will have TB. Of these, 91 (TP) will be identified; 9 (FN) will be missed by Xpert MTB/RIF. Of the 900 patients without TB, 891 (TN) will not be treated; 9 (FP) may be unnecessarily treated.	With a prevalence of 30%, 300/1000 will have TB. Of these, 273 (TP) will be identified; 27 (FN) will be missed by the Xpert MTB/RIF. Of the 700 patients without TB, 693 (TN) will not be treated; 7 (FP) may be unnecessarily treated.	Moderate ⊕⊕⊕◯
Diagnostic accuracy for Rifampicin resistance	**Effect %** **(95% CI)**	**No. of participants (studies)**	**What do these results mean given 10% prevalence of rifampicin resistance among persons with TB?**	**What do these results mean given 30% prevalence of rifampicin resistance among persons with TB?**	**Quality of Evidence**
All specimens	Sensitivity 95% (91, 97) Specificity 98% (97, 99)	2,530(6)	With a prevalence of 10%, 100/1000 will have rifampicin resistance. Of these, 95 (TP) will be identified; 5 (FN) will be missed by Xpert MTB/RIF. Of the 900 patients with TB susceptible to rifampicin, 882 (TN) will not be treated for MDR; 12 (FP) may be unnecessarily treated for MDR.	With a prevalence of 30%, 300/1000 will have Rifampicin resistance. Of these, 285 (TP) will be identified; 15(FN) will be missed by Xpert MTB-RIF. Of the 700 patients with TB susceptible to rifampicin, 686 (TN) will not be treated for MDR; 14 (FP) may be unnecessarily treated for MDR.	Moderate ⊕⊕⊕◯

Table 4. GRADE summary of findings – Diagnostic accuracy of the Xpert MTB/RIF assay in single-centre unpublished studies[1]

Review question: What is the diagnostic accuracy of Xpert MTB/RIF for i) detection of pulmonary tuberculosis; and ii) detection of resistance to rifampicin?
Patients/population: Adult pulmonary TB suspects (for TB detection); confirmed TB cases (for rifampicin resistance detection)
Setting: Multi-centre clinical validation studies
Index test: Conventional culture and DST
Importance: A rapid, accurate, simple test could replace conventional culture and DST and expand testing to lower levels of the health service
Reference standard: Composite reference standards (LJ culture, histology/cytology, ADA for CSF and fluids, CT for CSF, follow-up at 3 months)
Studies: Cross-sectional or cohort

Outcomes: TP, TN, FP, FN Diagnostic accuracy for MTB	Crude pooled effect %[1]	No. of participants (studies)	What do these results mean given 10% prevalence among suspects being screened for TB?	What do these results mean given 30% prevalence among suspects being screened for TB?	Quality of Evidence
All specimens pulmonary and extrapulmonary	Sensitivity 92.5% Specificity 98.0%	4,373 (10)	With a prevalence of 10%, 100/1000 will have TB. Of these, 92 (TP) will be identified; 8 (FN) will be missed by Xpert MTB/RIF. Of the 900 patients without TB, 882 (TN) will not be treated; 18 (FP) may be unnecessarily treated.	With a prevalence of 30%, 300/1000 will have TB. Of these, 278 (TP) will be identified; 12(FN) will be missed by the Xpert MTB/RIF. Of the 700 patients without TB, 686(TN) will not be treated; 14 (FP) may be unnecessarily treated.	Moderate ⊕⊕⊕◯
Diagnostic accuracy for rifampicin resistance	Pooled effect %	No. of participants (studies)	What do these results mean given 10% prevalence of rifampicin resistance among persons with TB?	What do these results mean given 30% prevalence of rifampicin resistance among persons with TB?	Quality of Evidence
All specimens (pulmonary and extrapulmonary)	Sensitivity 98.6% Specificity 98.8%	917(3)	With a prevalence of 10%, 100/1000 will have rifampicin resistance. Of these, 98 (TP) will be identified; 2 (FN) will be missed by Xpert MTB/RIF. Of the 900 patients with TB susceptible to rifampicin, 889 (TN) will not be treated for MDR; 11 (FP) may be unnecessarily treated for MDR.	With a prevalence of 30%, 300/1000 will have rifampicin resistance. Of these, 296 (TP) will be identified; 4(FN) will be missed by Xpert MTB/RIF. Of the 700 patients with TB susceptible to rifampicin, 692(TN) will not be treated; 8 (FP) may be unnecessarily treated for MDR.	Moderate ⊕⊕⊕◯

[1] These studies were evaluated individually and crude pooled sensitivity and specificity estimates calculated since meta-analyses was not possible given variability in study design, use of various reference standards and availability of preliminary data only. The quality of evidence was consequently downgraded (on directness) by 1 point.

11. Annexes

Annex 1: Xpert MTB/RIF Expert Group members

Annex 2: WHO staff members

Annex 3: STAG-TB members

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