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Rapid Implementation of the Xpert MTB/RIF diagnostic test

Technical and Operational 'How-to' Practical considerations

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This document is a product of the discussions and consensus reached during the Global Consultation on implementation and scale-up of the Xpert MTB/RIF system, convened by WHO-STB in early December 2010, and subsequent active interaction by the various Working Groups and their secretariats.

Data underlying the evidence base for Xpert MTB/RIF assay were kindly provided by FIND (Foundation for Innovative New Diagnostics) to WHO. FIND will continue to play a key role in Xpert MTB/RIF implementation and roll-out, and will assist with monitoring of global sales, post-marketing surveillance, and technical assistance to countries.

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1. Background

Earlier and improved tuberculosis (TB) case detection - including smear-negative disease often associated with HIV - 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 diagnostic methods.

No single diagnostic test currently satisfies all the demands of 'quick', 'cheap', and 'easy'. Commercially available liquid culture systems and molecular line probe assays for rapid detection of MDR-TB have been endorsed by the World Health Organization (WHO); however, due to their complexity and cost, as well as the need for sophisticated laboratory infrastructure, uptake has been limited in many resource-constrained settings.

Genotypic (molecular) methods have considerable advantages for scaling up programmatic management and surveillance of drug-resistant TB, offering speed of diagnosis, standardized testing, potential for high throughput, and fewer requirements for laboratory biosafety. 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 *Mycobacterium 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 (DST), while scale-up of conventional culture and DST services remained slow and expensive, compounded by huge demands on laboratory infrastructure and human resources.

Over the past five years, and with support from the US National Institutes of Health (NIH), the Foundation for Innovative New Diagnostics (FIND) has partnered with Cepheid, Inc. (Sunnyvale, USA) and the University of Medicine and Dentistry of New Jersey (UMDNJ, Newark, USA) 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 system consists of an instrument, personal computer, barcode scanner, and preloaded software, and uses single-use disposable cartridges containing lyophilized reagents, buffers and washes. Target detection and characterization is performed in real time using a six-color laser detection device.

The 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 outside conventional laboratory settings. Xpert MTB/RIF detects M. tuberculosis as well as rifampicin resistance-conferring mutations 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. It is important to stress that 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.

2. Evidence base

Data from published papers, large multi-centre laboratory validation and demonstration studies coordinated by FIND, and unpublished data from investigator-driven, single-centre studies were reviewed in late 2010 by WHO (see references and scientific literature at the end of this document).

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 **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. 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 was 59.5%. HIV co-infection substantially decreased the sensitivity of microscopy (to 47%), 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 drug susceptibility testing (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 considerably more than for microscopy. Xpert MTB/RIF requires uninterrupted and stable electrical power supply and annual calibration of the modules, which may pose a problem in rural/remote settings.

Results from 12 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%.

3. Policy development and Global Consultation

In order to facilitate rapid policy guidance on the use of new diagnostic tools, new methods, and/or novel approaches using existing tools, WHO Stop TB Department has developed a systematic, structured, evidence-based process.

- The first step involves a systematic review and meta-analysis of available data (where feasible), using standard methods appropriate for diagnostic accuracy studies.

- The second step involves the convening of an Expert Group to evaluate the strength of the evidence base and recommend operational and logistical considerations for mainstreaming such tools/approaches into national TB control programmes, and/or identify gaps to be addressed in future research.

- The third step involves WHO policy guidance on the use of these tools/approaches, presented to the WHO Strategic and Technical Advisory Group for TB (STAG-TB) for endorsement and subsequent dissemination to Member States for implementation.

An **Expert Group** meeting convened by WHO in September 2010 reviewed data from four published papers on Xpert MTB/RIF, large multi-centre laboratory validation and demonstration studies coordinated by FIND, and unpublished data from 12 investigator-driven, single-centre studies, using the GRADE[1] process. The evidence synthesis process confirmed a solid evidence base to support widespread use of Xpert MTB/RIF for detection of TB and rifampicin resistance.

STAG-TB endorsed the Expert Group recommendations and advised that implementation of Xpert MTB/RIF technology be phased in within the context of comprehensive national TB and MDR-TB strategic plans. STAG-TB therefore recommended that WHO:

- 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 response scale-up and cost-effectiveness.

- 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).

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

A **Global Consultation** called by WHO on 30 November - 2 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 implementation considerations for systematic roll-out of Xpert MTB/RIF to optimize use and benefits of the technology. The interim diagnostic algorithms were initially developed in consultation with the respective Working Groups of the Stop TB Partnership (GLI, MDR-TB, DOTS Expansion and TB/HIV), discussed in depth and revised during the Global Consultation meeting.

[1] Schünemann H.J., Oxman A.D., Brozek J., Glaziou P., Jaeschke R., Vist G.E., Williams J.W., Jr., Kunz R., Craig J., Montori V.M., Bossuyt P., Guyatt G.H. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. BMJ, 2008;336:1106-1110. PMID: 18483053

4. Summary of recommendations

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 and resulted in the following main recommendations[2]:

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).

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