

## Frequently asked questions about the WHO Technical Expert Consultation findings on Xpert<sup>®</sup> MTB/RIF Ultra

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The development of the Xpert<sup>®</sup> MTB/RIF assay (Cepheid, Sunnyvale, USA) was a major step forward for improving the diagnosis of tuberculosis (TB) and rifampicin resistance detection globally. While Xpert MTB/RIF performs better than many other TB diagnostics, its sensitivity is imperfect, particularly in smear-negative and HIV-associated TB, and some limitations also remain in its determination of rifampicin resistance. The Xpert MTB/RIF Ultra assay (Ultra) has been developed by Cepheid as the next-generation assay to overcome these limitations, and uses the same GeneXpert<sup>®</sup> platform as Xpert MTB/RIF. The Global TB Programme of WHO convened a Technical Expert Consultation via webinar on 20 January 2017 to assess the findings from a multi-centre non-inferiority diagnostic accuracy study, coordinated by FIND, of the Ultra assay compared with the Xpert MTB/RIF assay for the detection of *Mycobacterium tuberculosis* complex (MTB) and the detection of rifampicin resistance. These frequently asked questions are intended to clarify the major questions that are described in detail in a [WHO Meeting Report of a Technical Expert Consultation: Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF](#).

### **How was the performance of the new Ultra cartridge assessed?**

FIND, in collaboration with the Tuberculosis Clinical Diagnostics Research Consortium (CDRC) conducted a multi-centre, non-inferiority diagnostic accuracy study of the Ultra assay compared with the Xpert MTB/RIF assay for the diagnosis of TB and the detection of rifampicin resistance. The study involved ten study sites in eight countries and enrolled 1,520 patients with signs and symptoms of TB for a direct comparison of the performance of Ultra compared with Xpert MTB/RIF on the same specimens. The results from the study were presented to a Technical Expert Group (TEG) convened by the WHO Global TB Programme on 20 January 2017. The TEG agreed that the Ultra assay is non-inferior to the Xpert MTB/RIF assay for the detection of MTB and for the detection of rifampicin resistance. This means that the new Ultra cartridge is at least as good for the detection of MTB and rifampicin resistance as Xpert MTB/RIF. In certain populations, the Ultra assay may perform better for MTB detection, especially for individuals whose specimens are frequently paucibacillary. The Ultra cartridge showed better performance for the detection of MTB in smear-negative, culture-positive specimens, paediatric specimens, extra-pulmonary specimens (notably cerebrospinal fluid) and in testing smear-negative culture-positive specimens from HIV positive individuals. The Ultra cartridge can replace the Xpert MTB/RIF cartridge in all settings.

### **Is the diagnostic accuracy of Ultra similar to that of Xpert MTB/RIF?**

Ultra has a higher sensitivity than Xpert MTB/RIF for the detection of MTB, particularly in smear-negative culture-positive specimens and in testing specimens from HIV-infected patients and children, and in testing extrapulmonary specimens, with at least as good accuracy for rifampicin resistance detection. However, Ultra may also be more prone to detecting small numbers of non-replicating or non-viable bacilli present, particularly in patients with recent history of TB, reducing the specificity of Ultra as a test for active TB.

## **What is considered a recent history of TB?**

A patient with a recent history of TB may have been treated for TB in the past two years. However, the exclusion of prior TB treatment may not always be reliable. In some instances, patients may hide their prior TB status due to fear of stigma and discrimination or have concerns regarding legal status for migrants, or the prior history is not adequately ascertained by the health care workers.

## **What are the reasons for the increased sensitivity of Ultra?**

For the detection of MTB, Ultra incorporates two new multi-copy amplification targets (*IS6110* and *IS1081*) and a larger DNA amplification reaction chamber than Xpert MTB/RIF that contributes to the increase in sensitivity. Ultra uses the same semi-quantitative categories used in the Xpert MTB/RIF assay (high, medium, low and very low) as well as the addition of a new semi-quantitative category “trace” that corresponds to the lowest bacillary burden for MTB detection. The “MTB Detected, trace”, henceforth referred to as “trace call” indicates that only the multi-copy targets were detected, and not the TB specific regions in the *rpoB* gene.

## **What are the consequences for the reduced specificity of Ultra?**

The consequences of reduced specificity of Ultra compared to Xpert MTB/RIF are an increase in the number of false-positive results for MTB detection. The adverse consequence of this is that patients without TB may be misclassified as having TB. The reduced specificity is considered likely to be due to imperfect confirmation of TB history and possibly self-cured TB (i.e. incipient TB that resolved without treatment) and probably also a result of well-described limitations of the reference standard (culture) used to compare the two versions of the molecular assays. The reduced specificity of the Ultra is a trade-off between false-positive results and the increase in detection of more cases, especially in patients with paucibacillary TB (such as persons living with HIV, children and in testing specimens from persons with extrapulmonary TB).

## **Which patient groups will potentially benefit most from Ultra?**

Because of increased sensitivity of Ultra, in particular because of the “trace call” results, the assay will potentially benefit most patients with signs and symptoms of TB who also have either HIV infection or an age less than 15 years or extrapulmonary TB. The “trace call” results may also contribute to increased case detection in systematic screening of low prevalence populations where persons are less likely to have had a recent TB infection.

## **How should “trace call” results be interpreted?**

- Among persons with HIV, children and extrapulmonary specimens, “trace calls” should be considered to be true positive results for use in clinical decisions;
- Among persons without HIV infection with an initial “trace call” positive result, a fresh specimen from the patient should undergo repeat testing and the result of the second Ultra test be used for clinical decisions. While clinical and available radiological information should always be considered in the diagnosis of tuberculosis, a second “trace call” positive should be sufficient to make a diagnosis of pulmonary TB;
- Among all persons that test “trace call” positive, rifampicin susceptibility results are not interpretable and additional investigations are needed to confirm or exclude resistance to rifampicin.

- For recording and reporting purposes, any notified TB case with at least one “trace call” positive result should be considered bacteriologically positive providing the above criteria are met.

## **How frequent are “trace call” results?**

In the MTB case detection group of the FIND study, 398/1243 (32%) results were positive for MTB detection across all quantitation categories (high, moderate low, very low, trace). Among the positive results only 30/398 (7.5%) were trace calls, and all were from sputum smear-negative specimens. Assuming a prevalence of TB of 10% in a general population, less than 1% of all tested individuals would be expected to have a trace call result, but this could be closer to 2% among HIV-infected individuals.

## **Do the current WHO policy recommendations for the use of Xpert MTB/RIF apply to Ultra?**

Yes. The Ultra assay is non-inferior to the current Xpert MTB/RIF assay for the detection of MTB and the detection of rifampicin resistance and can be procured as an alternative to the latter in all settings. Cepheid will have both products available for the foreseeable future to allow countries with long regulatory cycles to continue to use their current Xpert® MTB/RIF stocks while gradually switching over to the Ultra cartridge. The current WHO recommendations for the use of Xpert MTB/RIF<sup>1</sup> also apply to the use of Ultra as the initial diagnostic test for all adults and children with signs and symptoms of TB and in the testing of selected extrapulmonary specimens (CSF, lymph nodes and tissue specimens). An update of the current guidelines for the use of Xpert MTB/RIF is planned for 2018, when more information on use of the Ultra assay will be available.

## **Can the equipment to perform the Xpert MTB/RIF test be used for Ultra?**

Yes, the same set of equipment (GeneXpert platform, computer, monitor, keypad, barcode reader) can be used for Ultra. A specific Assay Definition File (ADF) will be included with the package of Ultra cartridges by the manufacturer (Cepheid). Once this ADF is installed, the GeneXpert instrument can be used to perform Ultra assay.

## **After I add Assay Definition File for Ultra, can I still use Xpert MTB/RIF cartridges?**

Yes, if GeneXpert instrument was already used to run Xpert MTB/RIF assay with relevant ADF installed, you can continue using Xpert MTB/RIF cartridges. You could even run an Xpert MTB/RIF cartridge on one module while running an Xpert Ultra cartridge simultaneously on a different module of the same multi-module GeneXpert instrument.

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<sup>1</sup> *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 in adults and children. Policy update.* Geneva, World Health Organization, 2013 (WHO/HTM/TB/2013.16). <http://www.who.int/tb/publications/xpert-mtb-rif-assay-diagnosis-policy-update>

## **Can I use Ultra cartridges in a prevalence survey, given the low underlying prevalence of TB in the tested population?**

Yes, in principle, as in prevalence surveys, it is perfectly acceptable to use a sensitive test with less than optimal specificity, and adjust post-hoc to calculate the survey prevalence. However, additional studies are needed to assess the use of the “trace call” result when Ultra is used for systematic screening of persons. Confirmation should be sought for managing adult survey cases with isolated “trace calls” and no evidence of HIV infection or extrapulmonary disease.

## **With the expected improved performance of Ultra for detecting rifampicin resistance, should I still do a second test when rifampicin resistance is detected in someone not from a high MDR-TB risk group?**

Yes. Until further evidence is available a repeat test should still be performed on a fresh specimen from persons who had a specimen initially rifampicin-resistant (RR) that are at low risk for RR-TB (e.g. new TB cases with no prior history of TB treatment, in countries where less than 5% of new cases have RR-TB). The use of melting temperature-based analysis with Ultra instead of real-time PCR analysis with Xpert MTB/RIF allows Ultra to better differentiate silent mutations (such as Q513Q or F514F) from resistance conferring mutations. The number of patients with rifampicin resistance enrolled in the non-inferiority study was, however, insufficient to confirm the initial analytical results that suggested a superior performance of Ultra for rifampicin resistance detection.

## **Can I use Ultra cartridges in a drug resistance survey?**

Yes, the use of melting temperature-based analysis with Ultra instead of real-time PCR analysis with Xpert MTB/RIF seems to have improved the performance for detecting rifampicin resistance. Although more data on patients with rifampicin resistance should be gathered to confirm the superior performance of Ultra for detection of rifampicin resistance, the Ultra cartridges are at least as good as the earlier cartridges and therefore can be used in drug resistance surveys. Xpert MTB/RIF is currently being used in most drug resistance surveys either as a follow-on test on patients with sputum smear-positive TB or as the entry point test to the survey on people with suspected TB. The use of Xpert MTB/RIF has shown to dramatically reduce the laboratory workload in drug resistance surveys as well as to decrease the proportion of specimens that are lost due to no growth or contamination.

## **How much does an Ultra test cartridge cost?**

The cost per cartridge stays the same USD 9.98 for the same list of countries that currently has access to concessional prices for Xpert MTB/RIF. For more information on concessional prices, see the FIND site: <http://www.finddx.org/find-negotiated-product-pricing/>

## **Can Ultra procurement be supported by the Global Fund grant?**

Yes, a Global Fund grant may be used to support procurement of Ultra and any other WHO-recommended diagnostic.<sup>2</sup> Please contact your Fund Portfolio Manager to discuss the ways to do this.

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<sup>2</sup> For all WHO-recommended TB diagnostics, see [http://www.who.int/tb/areas-of-work/laboratory/policy\\_statements/en/](http://www.who.int/tb/areas-of-work/laboratory/policy_statements/en/)