Planning for country transition to Xpert® MTB/RIF Ultra Cartridges

global laboratory initiative
advancing TB diagnosis

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Contents

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About this guide

This guide provides practical guidance to plan and implement a smooth transition from use of Xpert MTB/RIF to Xpert MTB/RIF Ultra cartridges, ensuring uninterrupted service and avoiding cartridge wastage. It includes advice on how to translate findings from the *WHO Meeting Report of a Technical Expert Consultation: Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF*[1] into an actionable implementation plan, from country-level to site-level, for adoption of the Xpert MTB/RIF Ultra cartridge. Topics include:

- Adapting national guidelines and diagnostic algorithms
- Managing existing cartridge supply, forecasting, procurement and distribution
- Planning site-level computer software upgrades and trainings of laboratory personnel and clinicians
- Ensuring coordination among donors and partners supporting Xpert implementation in countries
- Monitoring impact of the roll-out of Xpert MTB/RIF Ultra

Target audience

This guide is intended to inform Ministry of Health officials, donors, implementing partners, quality assurance unit personnel, programme managers, testing site managers, supervisory staff and GeneXpert users at national, regional and testing site level.

[1] http://www.who.int/tb/publications/2017/XpertUltra

Introduction

The Xpert® MTB/RIF assay (Cepheid, Sunnyvale, USA) is a cartridge-based, automated diagnostic test that can simultaneously identify *Mycobacterium tuberculosis* complex bacteria (MTB) and resistance to rifampicin (RIF) in less than two hours, using the GeneXpert® platform. Despite substantial increased sensitivity for MTB detection compared with smear microscopy, Xpert MTB/RIF sensitivity is nevertheless suboptimal, particularly with smear-negative and HIV-associated tuberculosis (TB). The assay also has some limitations in the determination of RIF resistance.

The Xpert® MTB/RIF Ultra assay (Ultra) has been developed as the next-generation assay to overcome these limitations. To address sensitivity, Ultra uses two different multi-copy amplification targets (IS*6110* and IS*1081*) and has a larger PCR reaction chamber (50 µl in Ultra compared with 25 µl in Xpert MTB/RIF). This has led to a lower limit of detection for Ultra compared with Xpert MTB/RIF (16 colony forming units per milliliter (cfu/ml) and 131 cfu/ml, respectively). Furthermore, the use of melting temperature-based analysis with Ultra instead of real-time PCR analysis used with Xpert MTB/RIF allows Ultra to better differentiate silent mutations from resistance-conferring mutations, hence improving the accuracy of RIF resistance determination. In samples characterized by a very low bacterial load, only the IS elements can be detected by the Ultra assay (due to their presence in multiple copies in the bacterial genome), and the new semi-quantitative category, 'MTB detected trace' is used to report these results.

Ultra runs on the same GeneXpert platform as Xpert MTB/RIF (using software version 4.7b or later; see Section 4) and will also run on the GeneXpert Omni platform, which is currently under development and planned for release in Q3 2017.

Evidence base

To determine if the Ultra assay could be used as a replacement for the Xpert MTB/RIF assay, FIND designed and conducted a multicentre, non-inferiority diagnostic accuracy study to compare the performance of the two assays in settings of varying prevalence of TB/HIV and MDR-TB. The main study involved 1520 adults with signs or symptoms of pulmonary TB. Companion studies included children being evaluated for TB and persons being evaluated for extrapulmonary TB. Samples from each patient were tested using Ultra, Xpert MTB/RIF and culture.

The study revealed that compared to culture the sensitivity of Ultra was 5% higher than that of Xpert MTB/RIF (87.8% vs 82.9%) but specificity was 3.2% lower (94.8% vs 98%). Sensitivity increases were highest among smear-negative patients (+17%; 95% CI: +10%, +25%) and among HIV-infected patients (+12%; 95% CI: +4.9%, +21%). Specificity decreases were higher in patients with a history of TB (-5.4%; 95% CI: -9.1%, -3.1%) than in patients with no history of TB (-2.4%; 95% CI: - 4.0%, -1.3%). The samples that produced 'MTB detected trace' results accounted for much of the increased sensitivity of the Ultra assay, particularly among smear-negative, culture-positive patients. Unfortunately, the

'MTB detected trace' results also accounted for much of the decrease in specificity of the Ultra assay.

WHO Technical Expert Consultation

The WHO Global TB Programme convened a Technical Expert Consultation in January 2017 to assess the results of the FIND study and provide guidance to WHO regarding the use of the Ultra assay.

In addition, data from several additional studies were reviewed to assess the performance of Ultra in extrapulmonary TB, paediatric TB and in low TB burden settings. Modelling was used to explore trade-offs based on the performance of the Ultra assay in different epidemiological settings.

The *WHO Meeting Report of a Technical Expert Consultation: Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF* (WHO Report) concluded the following:

- The Ultra assay can be used as an alternative to the Xpert MTB/RIF assay in all settings.
- The current WHO recommendations for the use of the Xpert MTB/RIF assay also apply to the use of the Ultra assay as the initial diagnostic test for all adults and children with signs and symptoms of TB and to the testing of selected extrapulmonary specimens (cerebrospinal fluid [CSF], lymph nodes and tissue specimens).
- The interpretation of results of the Ultra assay for MTB detection are the same as for the Xpert MTB/RIF assay, with the exception of 'MTB detected trace' results.
  - Criteria for interpreting the 'MTB detected trace' results were developed to balance the harms of potential overtreatment of patients with a false-positive result and the potential benefits of increased numbers of correctly diagnosed TB patients and decreased mortality associated with TB.
  - Because the 'MTB detected trace' result provides no information on rifampicin resistance, additional investigations (e.g., culture and phenotypic drug-susceptibility testing or molecular testing) are needed to confirm or exclude resistance to rifampicin.

Much of the increase in the number of TB patients correctly identified by the Ultra test compared to the Xpert MTB/RIF test is due to the ability to detect very small numbers of bacilli in a sample (i.e., 'MTB detected trace').

However, the higher sensitivity of Ultra as compared to Xpert MTB/RIF is accompanied by a loss of specificity (i.e., an increase in the number of incorrectly identified patients that do not have active TB). This loss of specificity may be due to the presence of very small numbers of non-viable or non-replicating bacilli in samples from persons who have been recently treated for TB or in patients that have recently been exposed or colonized with tubercle bacilli (incipient TB). Very small numbers of bacilli may also be present because of laboratory cross-contamination. Note that the Xpert MTB/RIF assay also has a similarly reduced specificity in previously treated persons than in previously untreated persons, but the decrease in specificity is less than that with Ultra because the Ultra assay is more likely to detect the very small numbers of bacilli in such samples.

1. Policy and planning

1.1 Define composition, roles and responsibilities of a transition team

The Ministry of Health (MOH), National TB Programme (NTP) and/or National TB Reference Laboratory (NTRL) should delegate the responsibility for planning and implementing transitional activities to a team comprised of representatives from all key stakeholders. This team should be mandated to do the following:

- Advise MOH/NTP/NTRL on strategies to transition to Ultra
- Develop action plans that ensure all aspects of the transition are included or covered
- Oversee implementation of transitional activities
- Assess the impact and success of the transition

Representatives from the following key stakeholders should be invited to participate in the transition team (to be modified according to the local situation):

- MOH
- NTP
- NTRL
- Clinical programmes
- Data management or Information Technology services
- Other programmes using GeneXpert, such as the HIV programme
- Regulatory bodies
- Central stores
- Procurement office
- Cepheid authorized service providers
- GeneXpert implementing partners, including those outside of TB
- Selected representatives from regional and site level

The team should be led by a suitably qualified individual, e.g., a national TB laboratory quality assurance officer or GeneXpert focal person (from NTP or NTRL).

Defining roles and responsibilities of members of the transition team as well as external partners and donors should be an integral component of the planning process. Suggested roles and responsibilities of entities involved in the implementation of Ultra transitional activities are provided in **Table 1**.

TABLE 1. **Roles and responsibilities for implementation of Ultra transitional activities**

ENTITY	ROLES & RESPONSIBILITIES
EXTERNAL BODIES	
Cepheid and authorized service providers	• Provide timely advice and technical support (in-country and/or remotely) to countries requesting transition to Ultra • Communicate all relevant information on availability of Xpert MTB/RIF and Ultra cartridges, ordering information and expiration dates to countries in a timely manner • Provide customers with latest GeneXpert software version, Ultra Assay Definition File (ADF) and Ultra product insert • Provide centralized training of trainers and remote training via webinar
NATIONAL / REGIONAL LEVEL	
MOH/NTP	• Update policies and guidelines relating to Ultra testing • Engage with Cepheid or authorized service provider in advance regarding intention to transition, to enable planning; provide information on instrument serial numbers • Provide assistance to streamline regulatory requirements • Appoint staff to carry out situational analysis on clinical, laboratory and procurement activities (see **Section 1.4**) • With NTRL and clinical programme managers, train and supervise laboratory and clinical staff at testing sites on diagnostic algorithm, interpretation of results • Establish indicators to monitor implementation and impact of Ultra
NTRL	• Train and supervise laboratory staff at testing sites (with NTP) • Provide technical assistance to sites • Establish a hotline for installers to contact if experiencing any problems and provide troubleshooting • Update laboratory SOPs for Ultra (including quality assurance) • Update test requisition (examination request) and results reporting forms • Provide assistance for updating of laboratory information systems and electronic reporting systems, if needed
Clinical programme managers	• In cooperation with NTRL, train regional clinical focal points on Ultra and develop printed sensitization materials for distribution to clinicians on Ultra, including how to interpret results • In cooperation with NTRL, monitor and evaluate implementation of the transitional activities

Regulatory bodies	• Define and communicate regulatory requirements to transition team • Work with MOH to enforce requirements
Government procurement bodies and medical stores	• Work with the MOH to forecast stock needs for Ultra • Integrate new stock into national systems • Ensure subsidiary store departments are informed on steps for transition • Monitor stock usage and provide timely feedback
Implementing partners	• Support development and implementation of country policies and plans, under NTP leadership
TESTING SITE LEVEL	
Testing site managers	• Provide information during situational analysis (see **Section 1.4**) • Perform site-level transition activities • Inform site clinical managers when Ultra testing has started • Monitor and evaluate implementation of activities
GeneXpert instrument users	• Provide information during situational analysis • Perform on-site verification with Ultra cartridges and report any troubleshooting issues to NTP and NTRL

1.2 Review WHO statements and implementation considerations for implementation at the country level

The WHO Report provides evidence-based statements on the performance of Ultra in low and middle-income countries (LMICs) and implementation considerations on its use. However, several statements and implementation considerations are context-specific, and countries should consider how to implement them in their own setting to maximize the benefit.

" The Ultra assay is non-inferior to the current Xpert MTB/RIF assay for the diagnosis of MTB and the detection of rifampicin resistance and can be used as an alternative to the latter in all settings."

" The current WHO recommendations for the use of Xpert MTB/RIF 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)."

This statement means that countries may transition all their current testing with the Xpert MTB/RIF assay to the Ultra assay, i.e., there is no need to maintain use of the Xpert MTB/RIF beyond the transition period.

Note: The option to delay transition to the Ultra cartridge is at the discretion of countries. Currently, the manufacturer has no plans for phase out of the Xpert MTB/RIF cartridge and will reassess this position based on customer preferences and ordering. Therefore, countries would be able to use both assays in parallel if desired.

" Ultra has a higher sensitivity than Xpert MTB/RIF particularly in smear-negative culture-positive specimens."

" 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."

" Much of the increase in sensitivity for MTB detection with the Ultra assay was attributed to the 'trace calls'."

" Among persons with HIV, children and extrapulmonary specimens 'trace calls' should be considered to be true positive results for use in clinical decisions and patient follow-up."

The greatest benefit of the transition to the Ultra assay will be the higher sensitivity of detection of MTB, particularly in smear-negative culture-positive specimens, paediatric specimens, extrapulmonary specimens (notably CSF) and those from people living with HIV (PLHIV).

Some low- and middle-income countries (LMICs) have focused Xpert MTB/RIF testing so far on targeting certain risk groups (such as MDR-TB risk), with limited testing being conducted in PLHIV and children and for detection of extrapulmonary TB, despite long-standing WHO recommendations on these indications. Countries should review the current coverage of Xpert MTB/RIF testing and how to scale-up testing to take advantage of the improved sensitivity of the Ultra assay in these patient groups.

In PLHIV, children and those being evaluated for extrapulmonary TB, trace calls should be considered as true positive results for use in clinical decisions and patient follow-up (see Algorithm in **Section 1.3**), given that a minor loss of specificity has been observed in these groups and the fact that in many high TB burden settings these patients are diagnosed on clinical grounds and undergo empiric treatment, an approach that has considerable limitations and can lead to significant over- and under-diagnosis.

Practical implementation considerations related to the trace results are discussed below, including recording and reporting and interpretation of results.

" As a result of the increased sensitivity, Ultra also detects non-replicating or non-viable bacilli present particularly in patients with recent history of TB, reducing the overall specificity of Ultra in high-burden TB settings."

" The impact of increased sensitivity results in decreased specificity for TB detection…and becomes a trade-off between increased diagnosis and overtreatment."

" The trade-off between potential overtreatment and increased diagnosis of TB and decreased mortality associated with TB treatment varies substantially between different settings with variable populations determined by HIV, prior TB history, and prevalence."

Due to the increased sensitivity of Ultra, the assay also detects non-viable or non-replicating bacilli, particularly in patients with a history of TB treatment, more frequently than the Xpert MTB/RIF test. This leads to reduced specificity of the test compared to the Xpert MTB/RIF assay in high TB burden settings, where there are a high number of previously treated patients. In low TB burden settings, and when testing extrapulmonary specimens, specimens from children and specimens from PLHIV, the reduction in specificity reported in studies was relatively minor.

The trade-off between increased sensitivity and reduced specificity should be considered by countries based on the prevalence of TB and the ability to reliably exclude history of TB treatment during evaluation of patients for testing. The impact of false-positive results is that over-treatment of patients without TB may result.

The following tables provide examples of population-level projections of the results of testing with Ultra compared with Xpert MTB/RIF in settings with different prevalence of TB (based on the *FIND Report for WHO. A Multicentre Non-inferiority Diagnostic Accuracy Study of the Ultra Assay Compared to the Xpert MTB/RIF Assay)*. These examples are intended to assist countries in examining the trade-offs in their own setting. For additional examples, refer to the WHO Report.

Countries may conduct additional modelling work to support decisions on implementation strategies based on the trade-offs between increased sensitivity and reduced specificity in their settings.

TABLE 2a **Population-level projection using TB prevalence of 20%**

OUTCOME	NUMBER OF RESULTS PER 1,000 INDIVIDUALS TESTED (200 WITH TB, 800 WITHOUT TB)		
	XPERT MTB/RIF SENS = 83% SPEC = 98%	ULTRA SENS = 88% SPEC = 95%	ULTRA WITHOUT TRACE[b] SENS=85% SPEC=97%
True positives (TPs) (individuals with TB)	166	176	170
False negatives (FNs) (individuals incorrectly classified as not having TB)	34	24	30
False positives (FPs) (individuals incorrectly classified as having TB)	16	42	24
True negatives (TNs) (individuals without TB)	784	758	776
FPs per 10 TPs	1.0	2.4	1.4
Incremental FP/TP ratio[a]	—	2.6	1.8

Note: Accuracy estimates are based on a 30% proportion of smear-/culture+ among TB cases, and a 21% proportion of having a prior TB episode (as in FIND study)

[a] Computed as (# Ultra FPs - # Xpert FPs)/(# Ultra TPs - # Xpert TPs). Can be interpreted as "How many additional FPs do I get per additional TP detected with Ultra **over and above** Xpert MTB/RIF?"

[b] For the Ultra without trace analysis, 'MTB detected trace' results were considered as negative results

TABLE 2b **Population-level projection using TB prevalence of 15%**

OUTCOME	NUMBER OF RESULTS PER 1,000 INDIVIDUALS TESTED (150 WITH TB, 850 WITHOUT TB)		
	XPERT MTB/RIF SENS = 83% SPEC = 98%	ULTRA SENS = 88% SPEC = 95%	ULTRA WITHOUT TRACE[b] SENS=85% SPEC=97%
TPs (individuals with TB)	124	132	128
FNs (individuals incorrectly classified as not having TB)	26	18	22
FPs (individuals incorrectly classified as having TB)	17	44	26
TNs (individuals without TB)	833	806	825
FPs per 10 TPs	1.4	3.4	2.0
Incremental FP/TP ratio[a]	—	3.7	2.6

Note: Accuracy estimates are based on a 30% proportion of smear-/culture+ among TB cases, and a 21% proportion of having a prior TB episode (as in FIND study)

[a] Computed as (# Ultra FPs – # Xpert FPs)/(# Ultra TPs – # Xpert TPs). Can be interpreted as "How many additional FPs do I get per additional TP detected with Ultra **over and above** Xpert MTB/RIF?"

[b] For the Ultra without trace analysis, 'MTB detected trace' results were considered as negative results

TABLE 2c **Population-level projection using TB prevalence of 10%**

OUTCOME	NUMBER OF RESULTS PER 1,000 INDIVIDUALS TESTED (100 WITH TB, 900 WITHOUT TB)		
	XPERT MTB/RIF SENS = 83% SPEC = 98%	ULTRA SENS = 88% SPEC = 95%	ULTRA WITHOUT TRACE[b] SENS=85% SPEC=97%
TPs (individuals with TB)	83	88	85
FNs (individuals incorrectly classified as not having TB)	17	12	15
FPs (individuals incorrectly classified as having TB)	18	47	27
TNs (individuals without TB)	882	853	873
FPs per 10 TPs	2.2	5.3	3.2
Incremental FP/TP ratio[a]	—	5.9	4.1

Note: Accuracy estimates are based on a 30% proportion of smear-/culture+ among TB cases, and a 21% proportion of having a prior TB episode (as in FIND study)

[a] Computed as (# Ultra FPs – # Xpert FPs)/(# Ultra TPs – # Xpert TPs). Can be interpreted as "How many additional FPs do I get per additional TP detected with Ultra **over and above** Xpert MTB/RIF?"

[b] For the Ultra without trace analysis, 'MTB detected trace' results were considered as negative results

In some settings, it may not be possible to reliably exclude a history of TB treatment in persons being evaluated for TB, due to patients hiding their status owing to fear of stigma, concern over legal status for migrants, or inadequate history taking by health care workers.

In patients with a recent history of TB treatment, false positive results may arise due to the persistence of non-viable bacilli that may be detected by Ultra. In order to avoid overtreatment, an algorithm that differentiates the interpretation, subsequent confirmatory testing, and patient management, depending on whether the patient has recent history of treatment, is advised.

Furthermore, Ultra will produce RIF indeterminate results related to detection of paucibacillary TB because trace calls do not provide any information on rifampicin resistance. This will require collection of a second specimen and retesting by Ultra for all patients whose specimens produce trace calls. In cases of a repeat trace call, the patient should be requested to submit an additional specimen that will need to be referred for culture/DST to determine RIF resistance.

Countries need to ensure that appropriate resources and mechanisms to enable repeat testing and referral of samples for confirmatory testing are in place. Routine monitoring of quality indicators which include proportion and results of repeat testing, as well as proportion of patients referred for DST where needed, should be established and reviewed on a regular basis.

Note: in line with the End TB Strategy, countries should work towards providing universal DST for all persons being evaluated for TB, meaning testing at least for resistance to RIF for all persons with TB; and in those found to be RIF resistant, testing for resistance to fluoroquinolones and second-line injectable drugs based on national guidelines.

1.3 Update national diagnostic algorithm and guidelines

All national guidelines that refer to screening and diagnosis of TB should be reviewed and updated, e.g., diagnosis of TB in PLHIV is often included in national HIV guidelines as well as TB guidelines.

Based on country review of the WHO Report, as well as information gathered during the situational analysis, a Technical Working Group should prepare recommendations on the revisions to the national guidelines and algorithms. The report statements should be reviewed and approved by the MOH according to country procedures, ensuring that all affected disease programmes are engaged in the process.

The *GLI Model TB Diagnostic Algorithms* provides examples of algorithms that are in line with the goals of the End TB Strategy and incorporate WHO recommendations for tests to detect MTB and resistance to first- and second-line drugs.

The following model algorithm has been developed by GLI based on the conclusions of the WHO Report and should be considered in conjunction with guidance provided in the report.

The interpretation of 'MTB detected trace' results and necessary follow-up testing are described in this algorithm. The reader is referred to Algorithm 1 in the *GLI Model TB Diagnostic Algorithms* for the interpretation and follow-up testing for other Ultra results. Algorithm 1 is reprinted in **Annex 3** of this document for reference.

The algorithm incorporates two features to decrease the potential overtreatment related to the increased sensitivity of the Ultra assay:

1. The diagnosis of TB based on clinical criteria and empiric therapy are common among PLHIV and children being evaluated for pulmonary TB, and among persons being evaluated for extrapulmonary TB. As such, the potential harm from the 'MTB detected trace' result is likely small because many of the incorrectly identified patients would be placed on empiric therapy based on clinical criteria. In addition, the increase in correctly identified cases (i.e., potential benefit) is greatest in these patient groups (e.g. an increase of 12% in the sensitivity for detecting TB in samples from PLHIV). Thus, in these patient populations, a single result of 'MTB detected trace' (or any other MTB detected result) should be considered bacteriological confirmation of TB, and the Ultra result and any other available clinical and radiological information should be used in making clinical decisions.
2. For adults who are not at risk of HIV and are being evaluated for pulmonary TB, empiric therapy is not common and the potential for overtreatment is greater than for PLHIV or children. Two important findings from the FIND multicentre evaluation were that many of the false-positive Ultra results were in samples from patients with a history of recent treatment for TB, and that for correctly identified TB patients (i.e., MTB detected, culture-positive), a second Ultra test was usually positive. Because of these findings, a second Ultra test and additional clinical investigations are recommended to confirm TB in these persons.

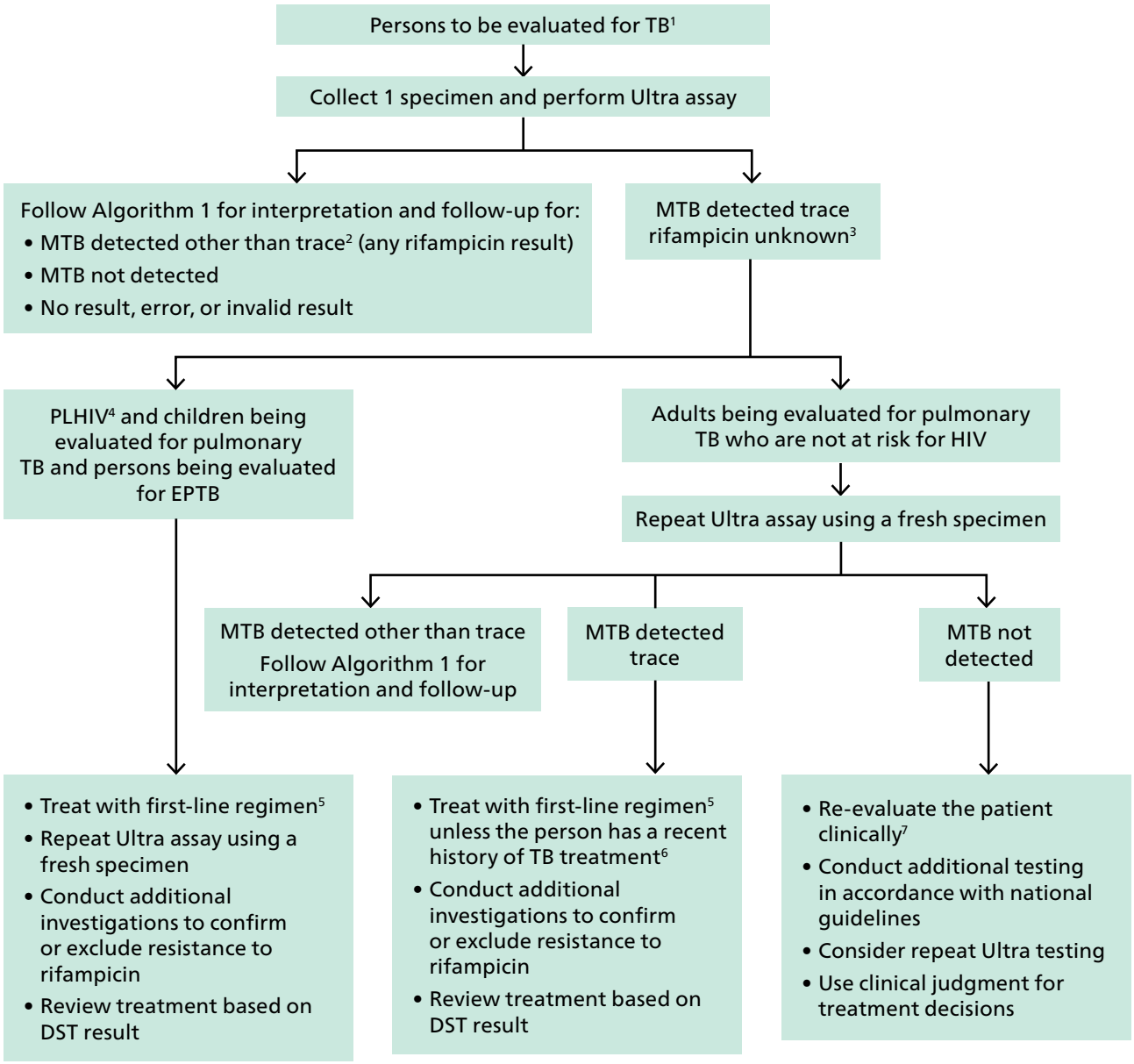
Decision tree for Algorithm 1a

Ultra test is used as the initial diagnostic test for all adults and children, regardless of HIV status, with signs or symptoms consistent with pulmonary TB or with a chest X-ray with abnormalities suggestive of TB.

- This algorithm is to be used along with the decision tree for Algorithm 1 (**Annex 3**).
- The considerations for the use of the Ultra assay are the same as for the Xpert MTB/RIF assay and are described in the decision tree for Algorithm 1. Only aspects of the testing that are unique to the Ultra assay are described here.
- The Ultra test is also recommended for use in persons being evaluated for extra-pulmonary TB when testing selected extrapulmonary specimens (CSF, lymph nodes and tissue specimens). Data are not available on the performance of Ultra with other types of extrapulmonary specimens.
- The evaluation should include determining the person's age, HIV-infection status, and possibility of a history of TB treatment.
- Note that in this algorithm, history of TB treatment is used as a risk for having a false-positive Ultra result. It is not used in the interpretation of 'MTB detected trace' results as a risk factor for rifampicin resistance.

1. Collect a good quality specimen and transport it to the testing laboratory. Conduct the Ultra test. For persons being evaluated for pulmonary TB, induced or expectorated sputum (preferred), bronchoalveolar lavage, gastric lavage, and gastric aspirate specimens may be used. Data are not available for the performance of the Ultra test

Algorithm 1a. Algorithm for universal patient access to rapid testing to detect MTB and rifampicin resistance incorporating Xpert MTB/RIF Ultra



[1] Persons to be evaluated for TB include adults and children with signs or symptoms suggestive of TB or with a chest X-ray with abnormalities suggestive of TB. This algorithm may also be followed for the diagnosis of extrapulmonary TB using CSF, lymph node and other tissue specimen. The evaluation should include determining the person's age, HIV-infection status, and possibility of a history of TB treatment.

[2] MTB detected (not trace) includes MTB detected high, moderate, low, or very low. Follow Algorithm 1 for interpretation and follow-up testing.

[3] MTB detected trace results do not provide any information regarding rifampicin susceptibility or resistance.

[4] PLHIV include persons who are HIV positive or 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. For all people with unknown HIV status, HIV testing should be performed according to national guidelines.

[5] Patients should be initiated on a first-line regimen according to national guidelines unless the patient is at very high risk of having MDR-TB or if a second Ultra assay indicates rifampicin resistance. Such patients should be initiated on an MDR-TB regimen.

[6] For adults who successfully completed a course of therapy within the past 2 years (i.e., recent TB treatment), the possibility of both Ultra trace results being false-positive results because of the presence of non-viable bacilli must be considered. Clinical decisions must be made on all available information and clinical judgment; further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, repeat Ultra testing, or culture.

[7] Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, repeat Ultra testing, or culture.

with other samples such as nasopharyngeal aspirates, string test samples or stool samples.

2. Follow Algorithm 1 and its decision tree if i) the Ultra test result is 'MTB detected high', 'moderate', 'low', or 'very low' with any rifampicin resistance result (detected, not detected, or indeterminate), ii) the Ultra test result is 'MTB not detected', or iii) the Ultra test does not give a result or gives a result of error or invalid.
3. If the Xpert Ultra test result is 'MTB detected trace', additional testing is needed.
    a. Review the clinical evaluation to determine the person's age, HIV-infection status, and history of TB treatment and determine if the samples are pulmonary or extrapulmonary samples.
        i. PLHIV include persons who are HIV positive or 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. For all persons with unknown HIV status, HIV testing should be performed according to national guidelines.
        ii. Children are defined as those less than 15 years of age.
        iii. Persons with a history of recent TB treatment include those who successfully completed a course of therapy within the past 2 years.[1] Persons who initiated therapy but did not complete the therapy and persons who failed therapy should be consider as being at high risk of having TB and require careful clinical evaluation.
        iv. Ultra is recommended for use with CSF, lymph nodes and tissue specimens. Data are not available for performance with other extrapulmonary samples.
        v. Health care workers must endeavour to obtain a reliable history of TB treatment, recognizing that some patients may not communicate their treatment history because of stigma or concern over legal status for migrants.
    b. For PLHIV and children who are being evaluated for pulmonary TB and for persons being evaluated for extrapulmonary TB using CSF, lymph nodes and tissue specimens:
        i. The MTB detected trace result obtained with the first specimen should be considered as bacteriological confirmation of TB (i.e., true positive results) and used for clinical decisions.
        ii. The patient should be initiated on an appropriate regimen using first-line TB drugs according to national guidelines unless the patient is at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen. Note that in most settings, a history of prior TB treatment is not sufficient to indicate that the patient is at very high risk of having MDR-TB for the purpose of making treatment decisions.

[1] Frequently asked questions about the WHO Technical Expert Consultation findings on Xpert® MTB/RIF Ultra. Geneva, World Health Organization, 24 March 2017. http://www.who.int/tb/areas-of-work/laboratory/diagnostics/XpertUltraFAQs.pdf

iii. A fresh specimen should be collected and a second Ultra test conducted (as the initial attempt to determine rifampicin susceptibility).

1. Interpret the result of the second Ultra test as described in Algorithm 1a.

2. An Ultra result of 'MTB detected trace' indicates that there are very few bacilli in the specimen. The number of bacteria present in a specimen from a TB patient does vary from specimen to specimen. The second specimen may contain a sufficient number of bacteria to provide a rifampicin result, or may contain few bacteria that generate a trace result, or may contain insufficient bacteria and therefore generate a result of 'MTB not detected'.

iv. Additional investigations such as culture and phenotypic DST are needed to confirm or exclude resistance to rifampicin if the second Ultra test provides no information on rifampicin resistance.

c. For adults being evaluated for pulmonary TB who are not at risk of HIV.

i. If the second Ultra test result is 'MTB not detected', consider the possibility that the first Ultra result was a false-positive result. Clinical decisions should be based on any available clinical and radiological information, and clinical judgement. Follow section 4 of Algorithm 1 for additional clinical assessments and investigations.

1. Consider the possibility of clinically defined TB (i.e., TB without bacteriological confirmation).

2. Consider additional testing with Ultra if there is a high clinical suspicion of TB. An Ultra result of 'MTB detected trace' indicates that there are very few bacilli in the specimen. Testing of a second sample, which also may contain very few bacilli may, in some cases, generate a result of 'MTB not detected'.

ii. If the second Ultra test result is 'MTB detected', including 'high', 'moderate', 'low', or 'very low', follow Algorithm 1.

iii. If the second Ultra test result is MTB detected trace:

1. For adults in whom a history of prior treatment for TB can be reliably excluded, this result should be considered as bacteriological confirmation of TB and used for clinical decisions.

2. For adults with a history of recent TB treatment, the possibility of both Ultra results being false-positive results because of the presence of non-viable bacilli must be considered. The patient should be re-evaluated clinically and additional testing conducted in accord with national guidelines and clinical judgment used for treatment decisions. See section 4 of Algorithm 1. Consider the possibility of TB caused by reactivation, relapse or reinfection.

3. Clinical decisions should be based on the Ultra results and any other available clinical and radiological information, and clinical judgement.

4. The patient should be initiated on an appropriate regimen using first-line TB drugs according to national guidelines unless the patient is at very high risk of having MDR-TB, in which case the patient should be initiated on an MDR-TB regimen. Note that in most settings a history of prior TB treatment is not

sufficient to indicate that the patient is at very high risk of having MDR-TB for the purpose of making treatment decisions.

5. Additional investigations should be conducted to confirm or exclude resistance to rifampicin because the trace result provides no information on rifampicin resistance.

This algorithm relies on testing of a sample with the Ultra test for the detection of MTB and assessment of susceptibility to rifampicin. On occasion follow-up testing is recommended to ensure that clinical decisions are well informed. However, discordant results may happen, usually when comparing culture-based results with molecular results. Each discordant result will need to be investigated on a case-by-case basis. Refer to Algorithm 1 for interpretation of Ultra tests that produce a result of 'MTB detected high', 'moderate', 'low', 'very low', or 'not detected'. Only Ultra results of 'MTB detected trace' are discussed here.

Ultra 'MTB detected trace', culture negative

The interpretation of this result must consider patient characteristics, specimen type, and whether the person had been previously treated for TB.

- Cultures may be negative for a variety of reasons including the patient being treated for TB or treated with fluoroquinolones, transport or processing problems that inactivated the tubercle bacilli, cultures lost to contamination, or inadequate testing volume, or the discrepancy may be due to laboratory or clerical error.
- The very small numbers of bacilli in a sample that generates an 'MTB detected trace' result may be due to active TB disease, laboratory cross-contamination, recent exposure to, or infection by, tubercle bacilli (insipient TB), and current or past treatment for TB.
- The FIND multicentre study revealed that many of the samples that generated results of 'MTB detected trace' and culture negative were from persons who had completed therapy within the past four to five years; presumably because of the presence of small numbers of non-viable or non-replicating bacilli. Thus, 'MTB detected trace' results must be interpreted in the context of prior treatment.
- Note that all initial 'MTB detected trace' results should be followed-up with a second Ultra test on a fresh specimen. An Ultra result of 'MTB detected trace' indicates that there are very few bacilli in the specimen. Testing of a second sample, which also may contain very few bacilli may, in some cases, generate a result of 'MTB not detected'.

    1. For PLHIV and children who are being evaluated for pulmonary TB, or when extrapulmonary specimens (CSF, lymph nodes and tissue specimens) are tested, the benefits of the increased sensitivity for the detection of MTB (i.e., true positives) outweighs the potential harm of decreased specificity (i.e., false positives).

        a. 'MTB detected trace' results in one or both samples should be considered as bacteriological confirmation of TB (i.e., true positive results) and used for clinical decisions if the samples were collected from a person who was not receiving treatment with anti-TB drugs or fluoroquinolones.

b. Follow-up actions may include: re-evaluate the patient for TB, assess the response to therapy (culture results may not be available until six to eight weeks after specimen collection), reassess the possibility of prior or current treatment with anti-TB drugs (including fluoroquinolone use), evaluate the possibility of laboratory or clerical error, and/or repeat culture.

2. For adults being evaluated for pulmonary TB who are not at risk of HIV, the balance of the benefit and potential harm varies based on whether the person had been previously treated for TB because of decreased specificity (i.e., false positives)

   a. For persons in whom a history of prior TB treatment can be reliably excluded:

      i. 'MTB detected trace' results in both Ultra tests should be considered as bacteriological confirmation of TB (i.e., true positive results) and used for clinical decisions if the samples were collected from a person who was not receiving treatment with anti-TB drugs.

      ii. If the second Ultra test result is 'MTB not detected', consider the possibility that the first Ultra test result was a false positive, recognizing that testing of a second sample, which also may contain very few bacilli may, in some cases, generate a result of 'MTB not detected'.

         - Clinical decisions should be based on the second Ultra result ('MTB not detected'), any other available clinical and radiological information, and clinical judgement.
         - Consider the possibility of clinically defined TB (i.e., no bacteriological confirmation).

      iii. Follow-up actions may include: re-evaluate the patient for TB, reassess possibility of prior or current treatment with anti-TB drugs (including fluoroquinolone use), repeat Ultra testing, evaluate the possibility of laboratory or clerical error, and/or repeat culture.

   b. For adults with a history of recent TB treatment:

      i. The possibility of the Ultra 'MTB detected trace' results being false-positive results because of the presence of non-viable bacilli must be considered.

      ii. Clinical decisions should be based on any available clinical and radiological information, and clinical judgement. Consider the possibility of TB caused by reactivation, relapse or reinfection.

      iii. Follow-up actions may include re-evaluate the patient for TB, conduct additional testing in accordance with national guidelines, repeat culture, and evaluate the possibility of laboratory or clerical error.

1.4 Perform situational analysis of network

A situational analysis of the existing GeneXpert network (as well as planned procurement of new instruments) should be conducted in order to inform the plans for transitioning to Ultra. The assessment should include the elements as per the checklist (**Annex 1**). Key elements to be assessed include: understanding regulatory requirements, checking software versions installed and database back-ups at sites, checking current stock levels

of Xpert MTB/RIF at sites and at regional and national stories and reviewing forecasts and ordering. The assessment will also determine needs for revision to training, recording and reporting forms and monitoring and evaluation (M&E) tools to inform the operational plan development.

The transition team should coordinate the assessment, delegate responsibility for conducting the assessment and establish a timeframe. All stakeholders involved in the assessment, including testing site management and staff, implementing partners and those responsible for data collection and reporting should be sensitized as to the purpose of the exercise. Where possible, and particularly where remote connectivity solutions are employed, much of this information may be gathered remotely, avoiding the need for visits to all sites. Furthermore, district or regional staff should incorporate gathering of site level information into routine monitoring visits.

1.5 Develop operational plan

A detailed operational plan should be developed to implement recommendations resulting from the situational analysis, as well as revisions to guidelines, in a phased manner.

Successful implementation of the transitional plan will require financial commitment from MOH/NTP and the testing sites, with possible support of implementing partners. A budget should be developed to address transitional activities in collaboration with key partners. **Annex 2** includes key budgetary considerations that may be used as a guide in budget development.

2. Regulatory

2.1 Determine importation requirements

Ultra is a new assay to be run on an existing diagnostic instrument. The Ultra assay is expected to achieve CE-IVD certification in April 2017. In addition, the *WHO Meeting Report of a Technical Expert Consultation: Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF* provides evidence-based statements regarding use of the assay in low- and middle-income countries. The manufacturer will prioritize initial introduction in countries in which the above is sufficient to fulfil regulatory requirements. Regulatory submissions in countries requiring more stringent procedures will follow, with the manufacturer being proactive in initiating regulatory submissions in those countries. However, countries should contact the manufacturer on their intention to transition to ensure that appropriate regulatory procedures are underway.

The transition team should work closely with relevant national authorities, the manufacturer and its authorized service providers in-country to determine importation requirements for the assay and to enable initiation of country verifications to be conducted, if required.

2.2 Conduct country verification as required

Verification of standard diagnostic methods (those used in accordance with manufacturer's instructions) is intended to provide confirmation of published performance characteristics.[1] Verification is a process that provides evidence that a laboratory can achieve the performance characteristics obtained during the manufacturer's method validation and that the method is suitable for its intended use in the population of patients being tested.

More extensive method **validation** (which includes establishing performance characteristics, limitations and acceptance criteria) is not required for standard methods with published performance parameters, and is only needed if an assay is being used outside of the manufacturer's claims.

When planning a verification study, the following should be considered:

- A detailed protocol outlining the number and nature of samples to be tested, as well as acceptance criteria should be prepared.

[1] ISO 15189:2012. Medical laboratories – requirements for quality and competence.

- For cost saving and efficiency, relatively extensive verification (e.g., 50-60 samples) may be done at the NTRL, with a limited verification study done at individual laboratories.
- According to international standards, some form of verification should be done at individual laboratories, although sample size could be reduced (e.g., 4 samples per site) following national level verification. However, countries should assess, based on cost, whether individual verification at every site is warranted. Verification at each site with a limited number of samples (e.g., proficiency testing samples) could serve a dual purpose and be part of competency assessment of users after on-site training.
- For verification at the NTRL, a mix of samples should be selected that will give results at test thresholds, e.g., a mix of positive and negative results, as well as samples giving a variety of semi-quantitative results. Samples for NTRL verification could be leftover sputum or frozen sputum samples with known results. Countries should select a variety of strains of rifampicin susceptible and resistant strains based on their local epidemiology for verification at the national level.
- A verification report should be compiled, and the observed performance parameters compared with the published performance, and a determination of acceptance made.
- Where countries choose to conduct additional verification at other laboratories in addition to NTRL, use of inactivated samples or proficiency testing samples is highly recommended to avoid the costs and biosafety hazard of shipment of samples containing live bacilli. Proficiency testing samples may be prepared by the NTRL or procured from commercial suppliers.
- The manufacturer can make available a limited number of cartridges to countries that require in-country verification studies to be conducted (100–200 cartridges per country). The availability of cartridges for verification studies will be prioritized for public programmes and should be negotiated with the manufacturer on a case-by-case basis.

The above are general considerations and countries must make their own determination on the needs for verification based on national guidelines and accreditation requirements.

2.3 Complete national regulatory processes

The transition team should work closely with the relevant government authorities, manufacturer and/or authorized service provider in order to meet the requirements of the national regulatory authority. An appropriate time period must be allowed to submit the application and provide any required supplementary evidence. Adequate planning is essential, particularly given the shelf-life of the Ultra assay at the time of wider roll-out by Cepheid (Q3 2017) is expected to be no more than 12 months (see **Section 5.2**)

3. Procedures

Table 3 provides a comparison of the Ultra assay compared with Xpert MTB/RIF.

TABLE 3. **Comparison of Ultra assay procedures with Xpert MTB/RIF**

PROCEDURE	ULTRA
Sample collection	Same sample types and procedures as for Xpert MTB/RIF
Testing procedure	Same as Xpert MTB/RIF
Assay time	Shorter than Xpert MTB/RIF: 65–87 minutes for Ultra (negative or positive results) versus 112 minutes for Xpert MTB/RIF
Results reporting	Same reporting categories as Xpert MTB/RIF, plus additional trace category
Results interpretation	Interpretation of Ultra trace calls differs based on history of TB treatment, HIV co-infection, age and whether persons are being evaluated for extrapulmonary TB (see Algorithm in **Section 1.3**)

3.1 Update laboratory SOPs and job aids

The procedure for the Ultra test remains the same as for the Xpert MTB/RIF assay. However, Ultra has a reduced assay time compared with Xpert MTB/RIF (65-87 minutes compared with 112 minutes for Xpert MTB/RIF; see **Table 3**). This means that laboratories may be able to perform one additional run of assays per working day, if compatible with the current laboratory workflow, staffing and working hours. In any case, the actual test consumption must be carefully reviewed at sites to avoid under-utilization of the instruments due to lack of demand for testing.

In addition, the results reporting and interpretation are different, due to the additional trace calls. Therefore, laboratory SOPs and job aids should be updated accordingly. Updated procedures must be reviewed and approved according to established mechanisms, and be readily available at all sites. All laboratory staff involved in testing must be trained on updated procedures. Existing quality assurance procedures, including PT, may be used for Ultra testing. See Section 8 for recommended updated laboratory indicators.

Ultra, due to its increased sensitivity, is more likely to pick up paucibacillary contamination than the Xpert MTB/RIF assay. Cross contamination was found to be a probable cause of some false-positive Ultra results in the FIND study. Cross-contamination events may be

more likely to occur in busy reference laboratories that also process other TB molecular or culture/DST tests. Most Xpert testing is performed in laboratories that do not have culture or other molecular test facilities, and therefore the likely impact of cross-contamination may be limited in these settings. However, the following precautionary measures should be taken in order to minimize the risk of cross-contamination:

- Thorough cleaning of working surfaces and instruments;
- Move the GeneXpert instrument or change the workflow such that samples for Ultra testing are not processed where culture samples (especially positive cultures) are processed.. This is particularly significant in busy reference laboratories conducting culture and DST;
- Accredited laboratories introducing a new test such as Ultra should consult their accreditation bodies as to the requirements.

3.2 Update clinical procedures

Changes in the reporting of Ultra results compared with Xpert MTB/RIF, i.e., addition of the trace call result and its implications on result interpretation, and the need for confirmatory testing and patient management, must be integrated into updated SOPs and job aids for clinical staff. All staff involved in the diagnosis and management of patients must be sensitized on updated procedures prior to use of Ultra at the site. Clinical staff from referral sites must also be sensitized using staff trainings in combination with standardized printed materials developed by the national programme.

4. Software upgrade

4.1 Plan for software upgrade requirements

All existing equipment needs to be upgraded to 4.7b (or higher) software in order to run the Ultra assay. This upgrade will also enable all other tests, including HIV, HCV and Ebola to be run on the GeneXpert instrument, and countries are encouraged to cooperate across disease programmes to integrate software upgrades and assay definition file (ADF) installation for various tests.

Xpert MTB/RIF tests are compatible with 4.7b or higher software versions, meaning that the upgrade can take place any time before procurement and implementation of Ultra assays. A GeneXpert with 4.7b or higher software can run Xpert MTB/RIF and/or Ultra, even at the same time in different modules.

4.2 Update software and install new assay definition file (ADF)

Based on the needs assessment and availability of personnel to conduct the upgrades, the country should develop an operational plan, including budget and human resource (HR) allocation to carry out the upgrade in an efficient manner. In some countries, Cepheid's authorized service provider may directly conduct the upgrade. Alternatively, local information technology (IT) personnel (e.g., laboratory information system [LIS] specialists, hospital IT staff or other in-country IT specialists) may conduct the upgrade after receiving in-country/remote training for data back-up, software upgrade and ADF installation. In addition, the training could also include updates to LIS or a remote connectivity solution by the manufacturer as well as LIS/connectivity providers. It is critical to engage competent personnel in the upgrade process. Remote phone, email or computer support will be provided by the manufacturer.

Sites with version 4.4 or higher software will be able to upgrade to the 4.7b/4.8 version directly. Any sites still running lower versions than 4.4 may need an initial upgrade to version 4.4 prior to upgrade to 4.7/4.8. Software upgrades should be done in advance, where possible, and countries should request the files from the manufacturer when ready to initiate the upgrade process, or they will be shipped automatically with the first order for Ultra cartridges. In some countries implementing HIV assays using the GeneXpert platform, software upgrade has already been initiated. Countries are therefore advised to check with both TB and HIV programmes to ascertain the status of software on instruments.

Upgrade procedures require different protocols depending on the current Windows software and testing software versions.

Programmes should have documented confirmation that software has been updated at a GeneXpert testing site in advance of distributing Ultra cartridges to that site. Sites can confirm that a successful upgrade was accomplished by sending an installation qualification report to the head of the transition team (and Cepheid) after upgrade is completed: see *GLI Training Package on Xpert MTB/RIF. Module 5. Installation*. Alternatively, sites with remote connectivity solutions may access this information remotely.

There is a risk of loss of testing data, so back-up of data must be done prior to installation of new software. Back up of data onto an external CD is recommended. The time required for back-up of data prior to initiating software updates will vary depending on the number of tests to be backed up. This may take several hours prior to starting the software upgrade.

Users must ensure that all software user settings, LIS settings and connection to third party connectivity solutions remain unchanged after upgrade of the instrument software. It may be necessary to update LIS and remote connectivity solutions to accommodate the new test. Countries should consult LIS and remote connectivity solution service providers for specific requirements.

In addition to the upgrade of the instrument software, a new ADF will be required to run the Ultra assay. The ADF will be provided in each kit; it may also be requested in advance.

Where possible, countries should perform the software upgrade, the ADF installation, and updates to the LIS or remote connectivity solution at the same time. The time required for software upgrade and ADF installation procedure may be up to 2 hours per laboratory. Therefore, timing of upgrades should be carefully managed to minimize interruption of routine services.

After upgrade and ADF installation, and while the personnel responsible for installation is still on-site, laboratory staff should run one Xpert MTB/RIF cartridge to confirm the following: (a) software update was successful, and (b) LIS and/or remote connectivity solution is still connected and sending test results. If the software upgrade fails, there is a risk that the settings for connection to third party connectivity solutions may be altered; thus programmes should make plans for this situation (including cost for additional site visits where needed, and access to technical support from third party connectivity solution providers).

Full functionality of the updated system to perform the Ultra assay and send results will be assessed during the verification process.

4.3 Provide technical support

The central Cepheid training team will be informed whenever an initial order for Ultra cartridges is received from a country and will be able to provide remote support to end-users during the upgrade process. Any support required during the upgrade process should be communicated to Cepheid technical support team according to the usual procedures.

Support from the authorized service provider for the software upgrade and ADF installation process is included in the service and maintenance contract, and will be

provided free of charge according to the terms of the agreement. Additional charges may apply for travel to remote facilities. For countries or programmes without a service and maintenance contract in place, authorized service providers will be able to provide support, but costs for travel and accommodation to visit sites to affect the upgrade will be charged to the customer at rates quoted by the relevant authorized service provider.

Note: the GeneXpert extended warranty only includes costs of spare parts and does not include any authorized service provider costs. For more information, see the FIND website: *Tools & Support, Negotiated Product Pricing*,

https://www.finddx.org/find-negotiated-product-pricing/

Programmes are strongly advised to discuss details of support to be provided with the authorized service provider in advance to ensure clear understanding of coverage.

5. Supply chain

5.1 Responsive forecasting, ordering and distribution

The quality of TB diagnostic testing services depends on the uninterrupted availability of reagents at testing sites. During a transitional period, there may be a phased approach where some sites are using Xpert MTB/RIF test cartridges and others are using Ultra cartridges.

The transition team should engage all stakeholders involved in procurement of GeneXpert reagents to have a complete picture of current orders and planned procurement. Some customers place large orders and receive delivery in phases. In such cases, it may be possible to convert a portion of such orders to Ultra. This will need to be discussed with Cepheid on a case-by-case basis well in advance.

The cost of the Ultra cartridge will be the same as the Xpert MTB/RIF cartridge, i.e., 9.98 US dollars, ex works price.

Countries must carefully plan for how distribution will happen while two types of cartridges are available in the country. The MOH should coordinate this process in close consultation with Cepheid's authorized service provider, together with stakeholders.

The following measures will be required to ensure uninterrupted supply of reagents during transitioning:

- Streamlining of importation and in-country distribution procedures to ensure sufficient shelf-life once Ultra cartridges reach testing sites;
- Careful forecasting to avoid expirations or stock outs;
- Careful planning to ensure sites have received training, updated documentation, software upgrade and an updated ADF ahead of Ultra cartridge shipment;
- Engagement of partners who provide stock outside of the usual MOH supply chain;
- Monitoring all steps of procurement and supply chain to ensure delays are minimalized;
- Monitoring to ensure correct sites receive correct reagents as per planned schedule;
- Monitoring test consumption and be prepared to re-allocate stocks.

Purchasing and distribution strategies should be reassessed at regular intervals to ensure they are responsive to the needs and current situation.

5.2 Shelf-life considerations

As of March 2017, Ultra cartridges have a limited shelf life (8 months). This shelf life will be extended incrementally over the coming year as real time stability data are accumulated. At the time of wider distribution of Ultra cartridges to countries in Q3 2017, the shelf life is expected to be 12 months. The targeted shelf life for Ultra is 24 months, as is the case for the current Xpert MTB/RIF assay. The Xpert MTB/RIF and Ultra cartridges are almost visually identical, except for the labelling and the reaction 'fin'. Therefore extreme care should be taken to segregate stocks and ensure the correct cartridges are used. In terms of packaging, in contrast to the Xpert MTB/RIF 10-test kit, the Ultra 10-test kit does not include a cartridge pouch to enclose each cartridge. Both 50-test kits have five pouches, each enclosing 10 cartridges.

Ultra has been validated for storage between 2–28 °C. Current stability data do not support storage at higher temperatures. The regular TB supply chain should be used for distribution of reagents; however, due to the limited shelf life and temperature requirements, it may be necessary to consider more frequent or temperature-controlled distribution to sites in certain settings/seasons, or establishing a district-level buffer stock for more rapid distribution to sites. Additional transportation costs should be considered.

6. Recording and reporting

6.1 Request for examination and reporting forms

Depending on the current format of the country's requisition (specimen examination request) form, it may or may not be necessary to make any revisions to incorporate transition to Ultra. Many countries use the terminology Xpert MTB/RIF, and this may equally apply to the Ultra assay.

Countries should determine if an update of the examination forms is necessary, considering the cost and time taken for such a revision. An important aspect to consider is that due to the different algorithms for interpretation of results based on patient categories, it is critical that programmes capture information on request forms related to the patient category, to be able to track the impact of trace results.

Where information on patient categories is not present on request forms, or is not routinely captured and entered into the test information on the GeneXpert, it is recommended that during the process of preparation for Ultra introduction countries introduce patient categories onto the examination form and recording and reporting systems, and provide refresher training to clinical staff to ensure the data are entered. This will be important to enable a baseline from which to measure the impact of Ultra once introduced.

Similarly, if not already in place, countries should establish a numbering system to identify repeat samples from the same patient, in order to monitor the proportion and performance of repeat tests.

Given that information in the patient category is critical for the correct interpretation of the Ultra result, especially in the case of "trace calls", programmes should make sure to capture such information on the test request form. In many countries, patient categories are already included on request forms but are incompletely or inconsistently completed. Refresher training to clinical and laboratory staff should be conducted to ensure that forms are filled out correctly and completely.

In addition, such information, even if collected, may often not be included in the test information entered into the GeneXpert software and therefore is unavailable for easy monitoring via remote connectivity solutions. This information should always be entered in the GeneXpert software as well as in the laboratory recording and reporting system. It is important that countries establish a numbering system to easily identify samples from the same patient to monitor the proportion of test repeats, which is vital for impact measurement and supply forecasting.

With regard to report forms, it would be essential to include the "MTB detected trace" category as a possible result, and hence there is a need for revision of reporting forms. It is also critical that clinicians are aware of which assay (i.e., Xpert MTB/RIF vs Ultra) was used for testing; during the transition period; this may be included manually on the report. A standard remark should also be added to the report form that indicates the need to refer a fresh specimen when the Ultra result is "MTB detected trace".

Countries should determine the most efficient way to implement updates based on their own situation.

6.2 Laboratory and clinical registers

The WHO *Definitions and reporting framework for tuberculosis – 2013 revision* provides the following categories for Xpert MTB/RIF results:

T = MTB detected, RIF resistance not detected

RR = MTB detected, RIF resistance detected

TI = MTB detected, RIF resistance indeterminate

N = MTB not detected;

I = invalid / no result / error

An additional result category for the Ultra trace call should be added to ensure differentiation between the TI category and the new trace call.

The TI category should still be used for "MTB detected, RIF resistance indeterminate" results other than the trace calls (i.e., where the semi-quantitative result is very low or higher).

For Ultra, the following abbreviation may be used:

TT = MTB detected (trace), RIF resistance indeterminate

Countries should implement a standardized approach to record trace results and use it consistently across all testing sites. Depending on the format of laboratory and clinical registers, existing registers could still be used provided that an addendum, including the new reporting code for trace calls, is published and disseminated to all testing sites.

Similar to when Xpert MTB/RIF tests are repeated for other purposes (e.g., RIF resistance indeterminate), when trace results are repeated on a new sample from the same patient, laboratories should record both the first and second test results to allow for monitoring and evaluation (M&E). Countries should track the proportion of patients with an initial trace result for whom a second specimen produces an interpretable result (as well as RIF susceptibility result). This is critical to assess the impact of repeat testing and determine the performance of the Ultra assay in countries.

7. Training

7.1 Update national training curricula

National approved training curricula (basic user training, advanced training and training for clinicians) for GeneXpert testing should be updated to include new national guidelines and algorithms incorporating Ultra, as well as technical aspects of the test for laboratory users.

7.2 Conduct training of trainers and cascade training to sites

Cepheid or its authorized service provider will provide either centralized training of trainers or remote training via webinar, depending on the country and the specific needs. In addition, remote training via webinar may be requested for end-users. However, this training will focus on the technology and not include other key aspects, such as country algorithm, recording and reporting of results, etc., which remain the country's responsibility.

Training of laboratory and clinical staff at health facilities should be scheduled to ensure that all staff have received training prior to first introduction of Ultra at that site, as well as staff from all facilities that refer specimens for testing. Since laboratory procedures are essentially the same as the Xpert MTB/RIF assay, training of laboratory staff should focus on changes in the national diagnostic algorithm, interpretation of results (i.e., trace calls), test requests and the recording and reporting system.

Clinical training/sensitization must be done in conjunction with training of laboratory staff to ensure all clinicians involved in screening and management of TB patients are sensitized to the new algorithm and interpretation of results. Given the large number of GeneXpert testing sites in many low- and middle-income countries, most countries should consider conducting a Training-of-trainers workshop at a national level with NTRL staff or other advanced users (preferably those who already provide support to sites). A schedule of on-site or regional level trainings should be prepared to ensure alignment with country introduction of Ultra, and conducted by the national cadre of trainers. All training should include determination of competency (both laboratory and clinical training).

GLI is updating its Xpert training package to include information related to the Ultra assay. This will be available at: **http://www.stoptb.org/wg/gli/**

8. Monitoring the transition

Monitoring of key indicators is essential to inform decision-making. Performance indicators should include testing site performance indicators, clinical indicators and programmatic indicators, including those that measure test results, supplies, test performance, linkage to care and the progress of Ultra scale-up.

Most countries will have an existing M&E framework developed to monitor Xpert MTB/RIF testing, which can be utilized to monitor the success of the transition to Ultra. Programmes that have installed remote connectivity solutions will be at an advantage in terms of ease of monitoring the transition to Ultra and its impact on laboratory indicators (for more information, see the *GLI Quick Guide to TB Diagnostics Connectivity Solutions*).

Countries should establish a set of key indicators that can be used to monitor the transition process during the initial planning phase. The following process indicators are recommended:

- Site readiness (software upgrade, training and documentation) as per schedule
- Stock levels of Xpert MTB/RIF and Ultra
- Availability of Ultra in regions or at sites, as per schedule
- Number of Xpert MTB/RIF and Ultra tests performed
- Number of cartridges expiring before use

Laboratory indicators

In addition to the laboratory quality indicators recommended in the *GLI Practical Guide to TB Laboratory Strengthening*,[1] indicators for trace results and repeat testing should be monitored by laboratories, and should be compiled at a regional and national level to determine overall trends.

Recommended additional laboratory indicators include:

- Number and proportion of trace calls, disaggregated by patient group
- Number and proportion of patients whose first sample produces a trace result and who have a repeat test conducted, disaggregated by patient group
- Number and proportion of patients who have a repeat test conducted whose second sample gives a result for MTB detection and rifampicin resistance, disaggregated by patient group

Note: as mentioned in **Section 6**, countries should ensure that systems are in place to capture disaggregated data on patient groups prior to Ultra introduction at sites.

[1] http://stoptb.org/wg/gli/assets/documents/GLI_practical_guide.pdf

Clinical impact indicators

- Total number of TB cases diagnosed, disaggregated by patient group (e.g., PLHIV, children, extrapulmonary TB)
- Number and proportion of bacteriologically confirmed TB cases, disaggregated by patient group
- Number and proportion of bacteriologically confirmed TB cases with DST results for rifampicin
- Number and proportion of patients who are initiated on treatment according to the national algorithm

For M&E purposes, any notified TB case in the register that is HIV positive, < 15 years old or with extrapulmonary disease and with at least one "trace call" positive result from Xpert Ultra testing should be considered bacteriologically positive. For all other notified TB cases, at least two "trace call" positive results would be required.

Responsibilities for data collection, a system for sending data to regional and/or national level, and responsibility for data analysis and reporting should be clearly defined. Mechanisms for feedback of data and reports, including recommendations for action items, should be established and clearly communicated to all involved. Where possible, countries should use existing cadres, e.g., advanced users, and incorporate data collection for M&E into existing supervision and technical support activities.

Monitoring the cartridge transitions is the overall responsibility of the national TB programme, led by the programme manager, with other programmes and institutions critically involved, including the national TB reference laboratory, department of laboratory services, Ministry of Health, national AIDS programme and health facilities. All M&E reports should be prepared and provided to the national TB programme manager for review, finalization and approval, prior to being disseminated to stakeholders.

When site visits for transitioning purposes are being planned, countries should consider other interventions that could be done during the same visits. This may include review of quality indicators at sites to identify challenges and implement corrective actions, review procedures for linkage of diagnosed patients to care, assess the efficiency of referral network and utilization capacity of instruments, as well as planning for integration of testing for other diseases on the GeneXpert platform (including HIV and HCV).

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GLI Quick Guide to TB Diagnostics Connectivity Solutions. Geneva, Global Laboratory Initiative, 2016.
http://www.stoptb.org/wg/gli/assets/documents/gli_connectivity_guide.pdf

GLI Training Package on Xpert MTB/RIF. Geneva, Global Laboratory Initiative.
http://www.stoptb.org/wg/gli/TrainingPackage_Xpert_MTB_RIF.asp

GLI Practical Guide to TB Laboratory Strengthening. Geneva, Global Laboratory Initiative, 2017.
http://stoptb.org/wg/gli/assets/documents/GLI_practical_guide.pdf

Annex 1.

Ultra transition situational analysis checklist

1. Policy and planning

- Have roles and responsibilities for coordinating the transition process been clearly defined?
- Which national guidelines, policies and other materials will need to be updated to include Ultra (consider NTP policies and guidelines, diagnostic algorithm, TB/HIV policies and guidelines, etc.)?
- Has a stakeholder mapping process been conducted, including all key internal (within government) and external stakeholders (local and international)?
- What support can partners provide for the transition process?

2. Regulatory

- What is the regulatory process required for importation of Ultra cartridges?
- Is country verification of Ultra needed?
- If so, what type of protocol and number of samples are required? Timeline? Where will verification studies be conducted?
- Is the designated authority (NTP and/or procurement agency) engaged with Cepheid or its authorized service provider to support regulatory processes?

3. Procedures

- Which SOPs and forms will need to be updated to include Ultra (provide list)?

4. Software upgrade

- List computer and GeneXpert software version installed at each GeneXpert testing site and any new instruments awaiting installation
- Which staff are competent and available to conduct site visits for software upgrades?
- What support are partners able to provide for software upgrades?

5. Procurement and supply chain

- Which partners support Xpert implementation in the country, and what is their scope of activities (how can they contribute to the transition)?
- Which partners procure instruments and cartridges?
- What is the current stock of Xpert MTB/RIF cartridges in-country?
- What stock is at national and/or regional stores?

- What stock is at sites (conduct stock on hand counts at sites and national/regional stores)?
- What orders have already been placed with Cepheid for Xpert MTB/RIF?
- What is the planned procurement by MOH and partners for 2017?

6. Recording and reporting

- Is a national request form in use? If no, review all request forms being used to request Xpert MTB/RIF testing.
- Is revision of the current request for examination form required for introduction of the Ultra test?
- Is the WHO recommended reporting format for Xpert MTB/RIF in use at all sites?
- Can the need to record the additional "trace" result be fitted into the current format, and is there space to record the "trace" result as well as repeat tests?
- If an electronic LIS system is in use, what updates will be required?
- If an electronic recording and reporting system is in place, what updates will be required?

7. Training

- Is a national approved training curriculum available?
- Who is responsible and what is the process for update of training materials for laboratory staff, advanced users and clinical staff?
- Is the approved curriculum used for all trainings, including those delivered by partners?

8. Monitoring the transition

- What changes to M&E tools and processes would be required to enable monitoring of additional indicators (i.e., progress indicators, laboratory indicators and clinical impact indicators)?
- What support can partners provide in monitoring of new algorithms and adherence to guidelines at sites?
- What support can partners provide for operational research to monitor the impact of Ultra?

Annex 2.

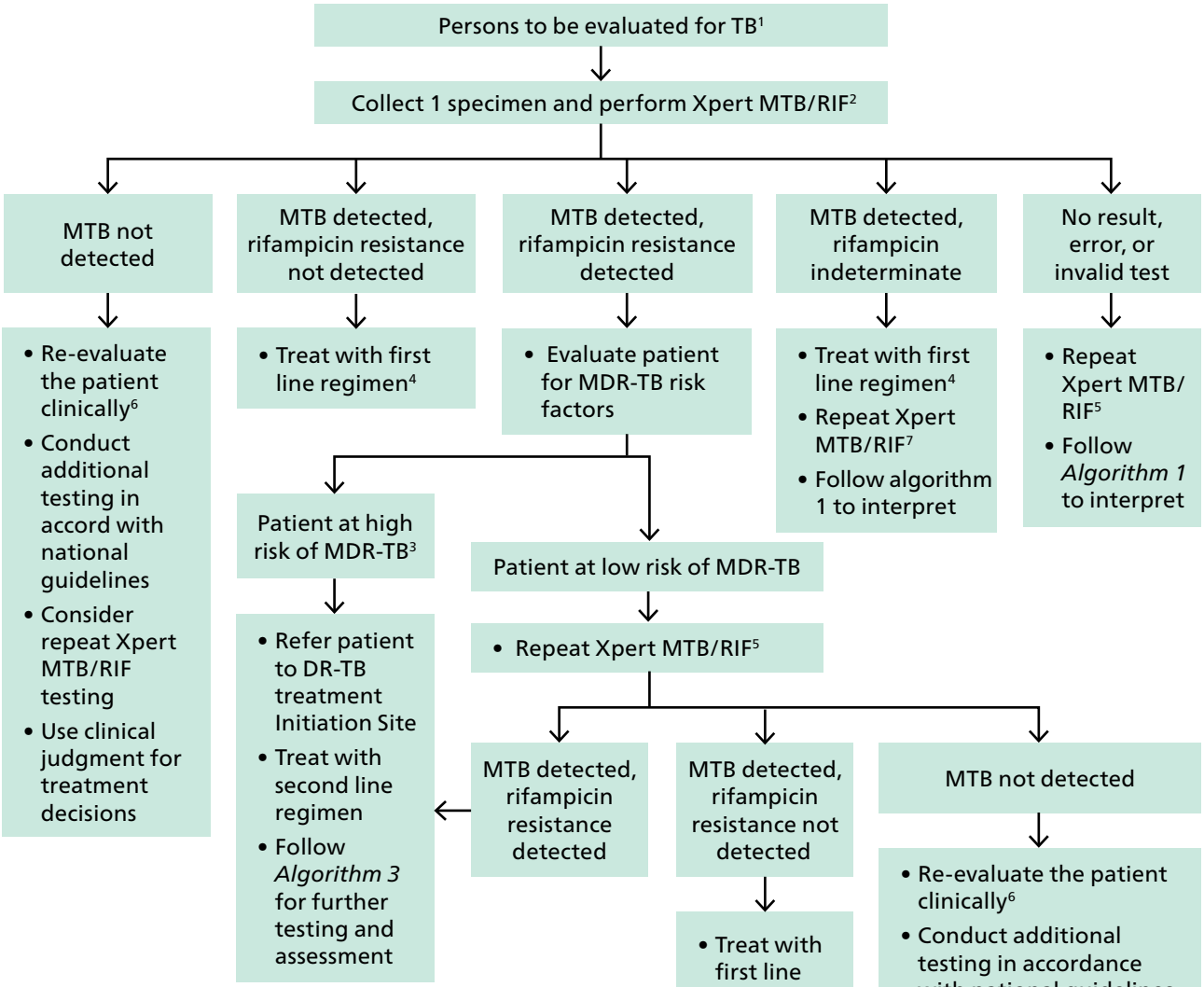
Budgetary considerations for Xpert MTB/RIF to Ultra transition

	BUDGETARY CONSIDERATIONS
Policy and Planning	• Workshop for stakeholder engagement and planning • Technical workshop for guideline and algorithm update • Situational analysis cost – HR, travel and report writing • Printing and distribution costs for revised guidelines and algorithms
Regulatory	• Regulatory submission costs, if applicable (borne by manufacturer) • Local travel costs to regulatory authority • Verification study – samples, reagents, HR
Procedures	• Printing and dissemination of revised procedures
Software upgrade	• Travel and per diems for site visits for installation and troubleshooting • Cost of technical assistance from authorized service provider or national team
Procurement and supply chain	• Workshop for stakeholders involved in procurement • Cost of more frequent distribution schedule, if applicable
Recording and reporting	• Workshop and HR to update recording and reporting forms, registers • Printing and distribution of updated materials
Training	• Workshop and HR to update training packages • Training of trainers workshop, on-site trainings/sensitization meetings • Printing and distribution of updated training manuals
Partner coordination	• Meetings for stakeholder engagement and planning (see Policy and Planning)
Monitoring	• Meetings to update M&E system and regular meetings to review impact of transition and re-plan • M&E refresher training • Operational research study to measure clinical impact

Annex 3.

Preferred algorithm for universal patient access to rapid testing to detect MTB and rifampicin resistance

(Algorithm 1 in the 2017 *GLI Model TB Diagnostic Algorithms*)



[1] Persons to be evaluated for TB include adults and children with signs or symptoms suggestive of TB or with a chest X-ray with abnormalities suggestive of TB. This algorithm may also be followed for the detection of MTB using CSF, lymph node and other tissue specimen from persons being evaluated for extrapulmonary TB. For persons being evaluated for TB who are HIV positive and have CD4 counts ≤100 cells/µl or are seriously ill, see Algorithm 4.

[2] Programmes may consider collecting two specimens upfront. The first specimen should be promptly tested using the Xpert MTB/RIF test. The second specimen may be used for the additional testing described in this algorithm. For persons being evaluated for pulmonary TB, sputum is the preferred specimen.

[3] Patients at high risk for multidrug-resistant TB (MDR-TB) include previously treated patients including those who had been lost to follow-up, relapsed, and failed a treatment regimen; non-converters (smear positive at end of intensive phase); MDR-TB contacts; and any other MDR-TB risk groups identified in the country.

[4] Patients should be initiated on a first-line regimen according to national guidelines. A sample may be sent for molecular or phenotypic DST for isoniazid if the patient has been previously treated with isoniazid or if there is a high prevalence of isoniazid resistance not associated with rifampicin resistance (i.e., isoniazid mono- or poly-resistance) in this setting or for DST for rifampicin if rifampicin resistance is still suspected.

[5] Repeat Xpert MTB/RIF test at the same testing site with a fresh specimen. Interpret the result of the repeat test as shown in this algorithm. Use the result of the second Xpert MTB/RIF test for clinical decisions.

[6] Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, repeat Xpert MTB/RIF testing, or culture.

[7] Repeat Xpert MTB/RIF test at the same testing site with a fresh specimen. Use the rifampicin result of the second Xpert MTB/RIF test in this algorithm for a decision(s) regarding choice of regimen (first line or second line regimen).

www.stoptb.org/wg/gli