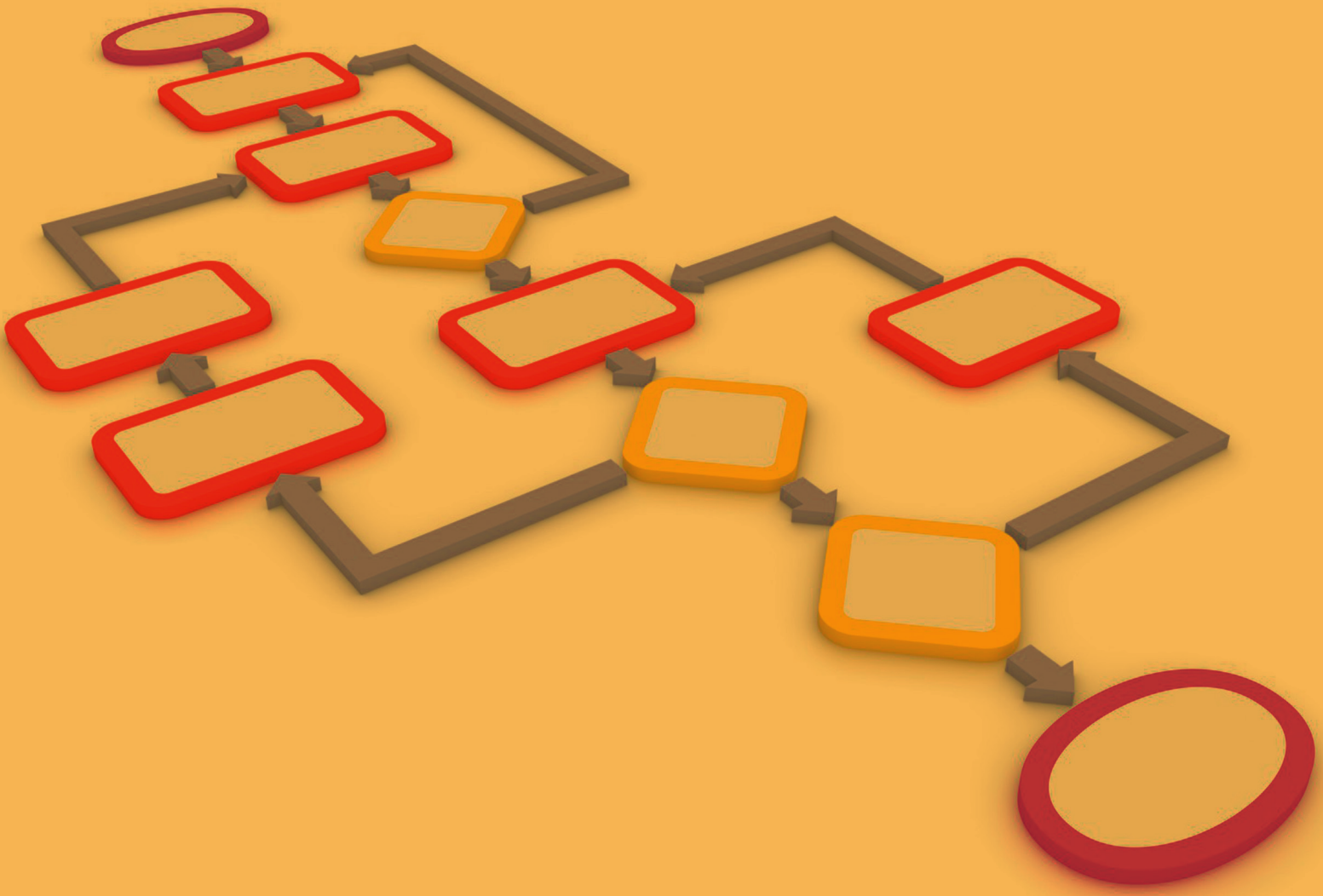
ORGANISMO ANDINO DE SALUD - CONVENIO HIPÓLITO UNANUE

PROGRAMA "FORTALECIMIENTO DE LA RED DE LABORATORIOS DE TUBERCULOSIS EN LA REGIÓN DE LAS AMÉRICAS"

ALGORITHMS

FOR THE DIAGNOSIS OF TUBERCULOSIS



Organización Panamericana de la Salud

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OFICINA REGIONAL PARA LAS Américas

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ALGORITHMS
FOR THE DIAGNOSIS OF TUBERCULOSIS

Catalogación realizada por el Organismo Andino de Salud – Convenio Hipólito Unanue

ALGORITHMS FOR THE DIAGNOSIS OF TUBERCULOSIS / Program "Strengthening of the Network of Tuberculosis Laboratories in the Region of the Americas" -- Lima: ORAS - CONHU; 2018.
xx p.; ilus, tab.
ALGORITHMS/ DIAGNOSIS/ TUBERCULOSIS/ LABORATORIES/SMEAR MICROSCOPY

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This document has been prepared in the framework of the execution of the Program "Strengthening of the Network of Tuberculosis Laboratories in the Region of the Americas" financed by the Global Fund, which has the Andean Health Organization –Hipolito Unanue Agreement as a Principal Recipient; and as Subrecipients to the Council of Ministers of Health of Central America and the Dominican Republic (COMISCA) and to the Pan American Health Organization (PAHO/WHO).

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ACRONYMS AND ABBREVIATIONS

CSF	Cerebrospinal fluid
CXR	Chest radiography
DR TB	Drug resistant tuberculosis
DST	Drug susceptibility testing
FLD	First-line antituberculosis drugs
FL	First-line
FQ	Fluoroquinolone
GLI	Global Laboratory Initiative
HIV	Human immunodeficiency virus
Hr TB	Confirmed rifampicin-susceptible and isoniazid-resistant TB
INH	Isoniazid
LAM	Lipoarabinomannan
LPA	Line probe assay
MDR	Multidrug resistant / Multidrug resistance
MGIT	Mycobacterial Growth Indicator Tube
MTB	*Mycobacterium tuberculosis* complex
NTP	National Tuberculosis Programme
PLHIV	People living with HIV
R	Rifampicin
RR	Rifampicin-resistant / Rifampicin resistance
SLD	Second -line antituberculosis drugs
SLID	Second line injectable drug (kanamycin, amikacin, capreomycin)
SL	Second-line
SM	Smear microscopy
TB	Tuberculosis
TB-LAMP	Loop mediated amplification of *M tuberculosis* complex DNA
WHO	World Health Organization
WRD	WHO-recommended rapid diagnostic
XDR-TB	Extensively drug-resistant tuberculosis
Xpert	GeneXpert® system employing MTB/RIF cartridges, last version recommended by WHO to detect *M. tuberculosis* complex and RR

Definitions

Algorithm	Set of rules to be followed to reach an objective. In this document the objective is to diagnose rapidly and accurately TB and DR-TB.
Cross- resistance	Resistance to two or more antibiotics that share at least one mechanism of activation or action
Diagnostic accuracy parameters for tests employed to detect TB	These parameters are generally defined with reference to the culture results. Sensitivity: the test ability to correctly identify people with TB (percentage of TB cases with positive result among all TB cases investigated by the test). Specificity: the ability to correctly distinguish people without TB disease (percentage of individuals with negative result among all those without TB investigated by the test). Positive predictive value: the probability that the individuals with a positive test truly have TB (percentage of TB cases among the individuals with positive result). Negative predictive value: the probability that subjects with a negative test result truly do not have TB (percentage of individuals without TB among those with negative result).
Diagnostic accuracy parameters for tests employed to detect anti-TB drug resistance	These parameters are generally defined with reference to the culture-based susceptibility tests results. Sensitivity: the test ability to correctly identify people with TB resistant to a drug (percentage of TB cases with test results that mean drug-resistance among the truly drug-resistant TB cases investigated by the test).

Specificity: the test ability to correctly distinguish TB cases without resistance to a particular drug (percentage of TB cases with test results that mean drug-susceptibility among all the truly drug-susceptible TB cases investigated by the test).

Positive predictive value: reliability of a test result that denotes resistance to a drug (percentage of drug-resistant TB cases among those TB cases with test results denoting drug resistance).

Negative predictive value: reliability of a test result that denotes susceptibility to a drug (percentage of drug-susceptible TB cases among those TB cases with test results denoting drug susceptibility).

Extensively drug-resistant tuberculosis

TB that is resistant to isoniazid and rifampin, plus any fluoroquinolone and at least one of the three injectable SLD (i.e., amikacin, kanamycin, or capreomycin).

Genotypic anti-TB drug susceptibility test

A laboratory assay developed to investigate the presence of mutations associated to anti-TB drug-resistance in relevant *M. tuberculosis* genes.

Hr TB

Confirmed rifampicin-susceptible and isoniazid-resistant TB.

Line probe assay

Molecular test based on DNA amplification and hybridization of the labeled PCR products with specific oligonucleotide probes immobilized on a strip. Captured labeled hybrids are detected by color development. In this document the term refers to the tests based on this principle and recommended by WHO to detect the presence of *M. tuberculosis* complex, drug susceptibility-

associated wild-type DNA segments and drug resistance-associated mutations (i.e. Genotype MTBDR plus and Genotype TBMDR sl HainLifescienceGmbH, Germany; NTM+MTBDR NIPRO, *Nipro* Corporation, Japan).

M. tuberculosis complex

It includes four *Mycobacterium* species that can cause TB in humans *(Mycobacterium tuberculosis, M. bovis, M. africanum, M. canetti)* and other species that infect animals *(M. caprae, M. pinnipedii)*. In America *M. tuberculosis* is the species commonly isolated from specimens of individuals with presumptive TB, while *M. bovis* is significantly less frequent and occurs typically among individuals that are exposed to bovine TB.

Multidrug-resistant TB

TB that is resistant to rifampicin and isoniazid with or without resistance to another FLD.

Mycobacterial growth indicator tube system

Culture system designed to detect mycobacteria early in culture performed with broth and a fluorescent compound embedded in silicone on the bottom of the tube. The fluorescent compound is sensitive to the consume of oxygen which, in turn, is used by the actively respiring microorganisms growing in the broth.

People living with HIV

Persons who are HIV positive or with unknown HIV status for whom there is strong clinical evidence of HIV infection in settings with a high HIV prevalence or in a risk group for HIV.

A laboratory assay developed to measure the ability of *M. tuberculosis* to grow in culture media with defined concentrations of anti-TB drugs.

Prevalence

The proportion of the population with a given disease at a given time. In this document the

	disease is TB /DR TB/Hr TB/RR TB/ MDR TB/ XDR TB/ HIV -TB.
Risk group	In this document the term refers to any group of people in which the prevalence or incidence of TB or DR-TB is significantly higher than in the general population.
RR TB	TB that is resistant to rifampicin as determined by using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. (it includes any resistance to rifampicin, i.e. mono-resistance, poly-resistance, MDR or XDR).
Screening	In this document the term refers to a test, examination or other procedure that distinguishes people with a high likelihood of having active TB from people who are highly unlikely to have active TB. The screening procedure is not intended to be diagnostic. People with positive results on a screening test/procedure should undergo diagnostic evaluation.
Bacteriologically confirmed TB	TB confirmed by SM, culture or a WRD
TB LAMP	Commercial assay designed to amplify *M. tuberculosis* complex DNA at a constant temperature (i.e. without the need of a thermal cycler) and to generate products that are visible to the naked eye.
XDR-TB	MDR-TB that is also resistant to a FQ and at least one of three SLID (i.e., amikacin, kanamycin, or capreomycin) as determined by using phenotypic methods or WRD

INTRODUCTION

This document presents models of algorithms that allow the bacteriological investigation of tuberculosis (TB), TB associated to the human immunodeficiency virus (HIV-TB) and drug-resistant TB (DR-TB).

Clear diagnostic algorithms, adapted by countries to the local situation, are necessary to organize the rapid and accurate diagnosis of TB, ensuring the highest attainable coverage and equity for the population.

The development and implementation of every diagnostic algorithm needs a process. The national epidemiological situation and that of the vulnerable groups should be considered while designing the algorithm. Consensus building and engaging of key stakeholders is important for its successful implementation. The National Reference Laboratory should be involved throughout the process. Finally, the algorithm should be approved by National Tuberculosis Programme (NTP). The algorithms should be revised when new evidence-based recommendations are issued and/or a significant change in the epidemiological situation is observed. The implementation and impact of any new algorithms should be strictly monitored.

Upon the modification of the national algorithms, the NTP should update all the definitions and guidelines that refer to the results of the TB diagnostics integrating the testing pathway that have been modified (e.g. those related to case detection, treatment and chemoprophylaxis, infection control, contact tracing).

Prerequisites

Health system organization and resources

Proper coverage and equity in the access to rapid and accurate diagnosis of TB cannot be achieved if the following minimum requirements are not previously ensured:

- a well-coordinated health system engaging all the relevant providers (i.e. public, private, social security, prison, military health services), trained personnel and tools to detect and register the individuals at risk of/ with presumptive TB or DR-TB such as those included in the following groups
    - homeless
    - residents and employees of high-risk congregate settings (e.g., correctional institutions, certain hospitals)
    - indigenous population
    - migrants
    - drug users
    - patients with diabetes/HIV infection/immunosuppression
    - contacts of TB/DR-TB cases, particularly children
    - symptomatics with history of anti-TB treatment

- permanent and efficient cross-cutting services that support the health system
  - specimen transportation
  - procurement and distribution of supplies
  - connectivity, telecommunication
  - information management

- laboratories organized in a network, operating in accordance with updated standards and adequately provided to meet the needs in:
  - human resources
  - quality management system
  - biosafety
  - equipment included in a preventive and corrective maintenance system
  - supplies

The NTP should also ensure the tools, supplies and training required for the administration, supervision and follow-up of treatment.

Screening

TB case finding is usually passive (i.e. it is performed among patients who seek care at the health centers). In certain circumstances and risk groups (e g. hard-to-reach population, TB contacts, prisoners) active case finding may be performed outside of the health facilities.

In some congregate settings where the prevalence and risk of transmission of TB or multi-drug resistant TB (MDR-TB) is very high and/or the number of undetected cases is estimated to be high (e.g. prisons), it is worthwhile to evaluate the feasibility and benefit of examining periodically all the individuals by using laboratory assays or chest X-ray (CXR). This intervention, followed by effective treatment, has proven to be cost-effective in such scenarios.

However, in general, it is not convenient nor feasible to investigate by laboratory methods all the individuals in a population to identify the TB cases. The number of tests and cost per TB case detected through this strategy are relatively high, especially when considering the use of the best rapid diagnostics. Thus it is necessary **to employ a test, examination or other procedure to distinguish people with a high likelihood of having active TB from people who are highly unlikely to have active TB.** This screening procedure is not intended to be diagnostic, people with a positive screen test should then undergo diagnostic evaluation. The screening tool should be highly sensitive to include most of the TB cases among those with positive results, even if some of them do not really have TB, in other words even if its specificity is not optimal. Unfortunately, there is no ideal screening test or procedure. Pulmonary TB can be presumed when some symptoms or CXR abnormalities are identified with a performance of these tools depends on how strict the definitions of TB symptoms or lung abnormalities are. The CXR is more sensitive than the symptom identification, whereas the detection of any symptom suggestive of TB (chronic cough, fever, night sweats) is more

sensitive than the recognition of chronic cough only. TB screening should be affordable and feasible in most scenarios and, particularly, at the primary health-care level. This is why the identification of respiratory symptoms (i.e. persistent or chronic cough) has been employed historically for screening. Anyway, CXR should be considered for certain population groups at very high risk of TB if high quality imaging and interpretation are available.

Systematic TB screening should be implemented among defined populations at high risk of developing TB disease such as TB contacts, especially children, people living with HIV (PLHIV) and diabetics.

The risk of progression to TB disease following exposure to infection is higher among children, particularly the younger ones, and once they are ill they may have non-specific symptoms more often than adults. This is the reason why the definition of pediatric presumptive TB is usually based on the history of exposure to infection, the results of clinical examinations and the tuberculin skin test.

In scenarios with low prevalence of TB, the symptom screening pre-selects many patients affected by respiratory diseases other than TB that, in this circumstance, are more prevalent than TB. So, a great number of laboratory tests are required to find a TB case and, at the same time, the positive predictive value of the diagnostics is poor. In such situation, screening for active TB using CXR can help to reduce the number of persons that should undergo laboratory examinations.

Pre-selected individuals with presumptive TB should then be investigated by one test or a sequence of tests. The CXR may be included in the diagnostic pathway if it has not been previously used for screening. The screening allows not only to diminish the number of tests to be performed and the consequent diagnostic cost but also to improve the positive predictive value of the diagnostic tests. In other words, it reduces the relevance of the false-positive or false-resistant results.

In relation to the use of laboratory tests to diagnose DR TB, TB resistant to isoniazid (Hr TB) and MDR TB, some risk factors must be identified for screening. They are those related to the resistance to antiTB drugs (i.e. exposure to DR/Hr/MDR TB or history of TB treatment especially when deficiencies in the prescription or adherence occurred). Regarding the exposition to infection, not only the contacts of DR/Hr/MDR TB cases should be considered but also other groups with high rates of DR/Hr/MDR TB identified in the country such as prisoners, residents of / immigrants from certain geographical areas , patients / workers of some hospitals, etc. Screening people that should undergo anti-TB drug susceptibility testing (DST) improves the predictive value of the results meaning resistance, in other words, it reduces the relevance of false resistant results.

The definitions of person with presumptive TB, person at high risk of DR/Hr/MDR TB, PLHIV should be clearly formulated by the NTP and other relevant programmes of each country, considering the national situation, the adults and the children. These definitions must be known by the health-care workers.

The health centers where the patients seek care are responsible of the screening procedures. They should adhere to the standards, instructions and definitions of the NTP related to the case-finding activity, and should be trained to interrogate patients, to identify symptoms and risk factors, to provide the instructions to collect good quality sputum specimens and to employ the tools and logistics implemented in the health system for investigating the individuals with presumptive TB. Thus, starting with the first-level of care, all the health centers must be furnished with the operational guidelines, the containers for sputum specimen collection, all the registers and forms standardized by the NTP, and should be able to use a system to transport specimens safely and regularly. The samples required for the investigation of extrapulmonary TB, which is less frequent, are usually collected in second or third level hospitals which usually operate their own laboratory services.

Laboratory diagnosis

Every patient should have **easy access to the laboratory tests** that are the most appropriate considering the individual clinical and epidemiological characteristics. The diagnostic pathway begins with a basic and rapid test to confirm the TB diagnosis and to identify the resistance to the drugs that are the key to the success of the first-line treatment. If drug resistance is detected, the sequence continues with the tests that support the selection of effective alternative treatment regimens. Usually, it is not necessary nor advisable to refer the patients to a higher level of care. Instead, the specimens should be transported from the health center where the patients are assisted to a nearest accessible laboratory. There, the biological material reaches the TB laboratory network which must be hierarchically structured to expedite all the examinations that are required for each case. The development of single platforms that allow multiple testing to investigate different diseases has brought the need of integrating diverse laboratory services in the network such as those involved in HIV and TB diagnosis. The awareness of the standards and procedures to be applied must be ensured at each level of the laboratory network as well as the identification of the cases for which the continuity of the investigation should be ensured by submitting the biological material to the linked reference laboratory.

WHO-recommended rapid diagnostic (WRD) to detect TB and rifampicin-resistant TB (RR TB) can be performed at the peripheral and/or intermediate level of the network. The health-care workers must clearly recognize the laboratory to which the specimens should be initially submitted. Subsequent tests for which higher biosafety and/or technical capacity are required can be implemented at the intermediate or national reference laboratories. All the tests required for the patients must be offered in-country, at least, at the national reference level since the submission of biological material to a supranational reference laboratory generates costs and delays that are incompatible with the need of rapid clinical responses. Reference laboratories must receive the results of the initial tests performed at local and/or intermediate levels to avoid overlapping tests, to complement the diagnostic sequence and to support the management of complex cases. The personnel of the reference laboratories, must be highly qualified to conduct the patient investigation following the corresponding algorithms, to elucidate the diagnosis of patients with discordant test results and to conduct special studies for exceptionally complicated cases interacting with physicians specialists.

To concatenate all these steps fluently and efficiently, the health system must ensure the resources and logistics needed to submit the clinical specimens and the Mycobacterium tuberculosis (MTB) isolates, to perform the laboratory tests and to record/transmit/analyze information easily and opportunely.

The **rapid communication of laboratory results** to the health-center that provide care to the patients must be ensured to allow the immediate administration of the appropriate treatment, among other interventions. This requires modern means of communication to transmit electronic data and instant messages and software to receive/ interpret the information required by the NTP and the TB laboratory network and to issue the reports needed for the clinical management and the epidemiological surveillance.

All processes must be under **a quality management program** to minimize the possible errors.

The health-care workers should know and understand the sequence of procedures that should be completed to use the services of the TB laboratory network, and the methods to be applied. This is possible through the **dissemination of flow charts that represent, as simply as possible, the algorithms to be followed.**

WHO-recommended rapid diagnostic (WRD)

The methods mentioned below are the WRD that have been endorsed at the time of writing of this document. The WHO recommendations are reviewed whenever technology is developed, and/or new scientific evidence is produced. In the future alternative substitute or complementary methods may be available as long as they prove to be equally or more accurate and/or convenient because of the cost, simplicity or flexibility to simultaneously investigate a high number of specimens. The information on this issue kept up to date.

Xpert MTB/RIF (Xpert)

The MTB/RIF cartridges used on GeneXpert instrument (Xpert, Cepheid, USA) have consistently shown to be more sensitive than smear microscopy (SM) and less sensitive than culture. The sensitivity of this test, like that of all available bacteriological assays, is directly related to the number of bacilli that are present in the clinical specimens. This is why the Xpert is relatively less sensitive in children than in adults. It detects virtually all smear-positive cases, and a little more than two-thirds of smear-negative and culture-positive cases and thus helps to expedite the diagnosis of the latter significantly. The Ultra version of Xpert cartridges is even more sensitive than the previous one but less specific, especially among patients with a history of anti-TB treatment.

The Xpert identify the tubercle bacillus and the RR simultaneously and accurately, by investigating specific DNA sequences and the most frequent rpo β gene mutations that confer this resistance which, in turn, is a good marker of MDR TB. The assay helps to to

improve the detection of RR/MDR TB and to reduce significantly the time to the initiation of the appropriate treatment.

The Xpert can be implemented in simple laboratory facilities that meet the requirements to perform the SM.

At the time of writing of this document, the Xpert is the most useful and accurate of the the rapid initial tests recommended by WHO to diagnose TB.

TB LAMP

The TB LAMP (Eiken Japan) is other molecular method recommended by WHO as a replacement for SM. It can also be implemented in simple laboratory facilities. The evidence analyzed by WHO showed that the TB LAMP is slightly less sensitive than Xpert to detect smear-negative and culture-positive cases and did not demonstrate advantages over SM to diagnose TB among HIV- positive patients. This assay was recommended only to examine sputum specimens and does not investigate RR. Thus, it should not be used for PLHIV or patients at high risk of MDR-TB. It has not yet been recommended for extrapulmonary TB, nor is it useful to reach the universal access to drug-susceptibility testing (DST).

The TB LAMP is not easier to use than Xpert, though it requires only one simple equipment which is less costly than the Xpert instrument which is advantageous.

At the end of 2016, based on the analysis of this information, the Regional TB Laboratory Work Group recommended for the Americas to focus the programmatic efforts on the implementation of Xpert rather than TB LAMP as the initial rapid diagnostic test, as indicated by the Algorithms presented in this document. The results of subsequent TB LAMP assessments that were published are controversial.

TB LAM

This assay permits to detect a mycobacterial polysaccharide antigen in urine by lateral flow chromatography (Determine TB-LAM, Alere Inc, Waltham, USA, then Abbott, California, USA). The test is so easy to perform that can be used at the point of patient care , without the need for a laboratory. It is less sensitive and specific than Xpert. The test was recommended to assist the diagnosis of TB only among HIV-positive patients with signs and symptoms of pulmonary or extrapulmonary TB and CD4 count < 100 cells /µL or severely ill. It may be particularly useful for patients with these characteristics who cannot produce sputum. Even with limited evidence, WHO recommendations were generalized to HIV-positive children with the same clinical characteristics but, at the same time , concern was raised on the low specificity evidenced by the initial assessments in pediatric population . WHO emphasized that this test does not eliminate the need for more accurate confirmatory diagnostics (Xpert, culture, LiPA and phenotypic DST).

After reviewing this background information, at the end of 2016 the Regional TB Laboratory Work Group recommended the Xpert as the rapid test of choice even for severely- ill HIV-positive patients, because it is more accurate than TB LAM and allows the detection of RR TB. However, the group admitted that the TB LAM could be used where Xpert is not yet accessible. Subsequent evaluations consolidated the information described below and showed that the results of TB LAM contribute to reduce the mortality when used as the only rapid tool to decide the initiation of antiTB treatment for seriously-ill hospitalized patients with advanced immunosuppression.

The algorithms proposed in this document indicate the employment of Xpert for all HIV-positive patients, with the vision of providing universal access to high quality diagnosis. The use of TB-LAM should be only a transitional aid to be used up to the capacity to perform Xpert is built.

LPA for the detection of resistance to first-line antituberculosis drugs

The LPA developed to simultaneously identify MTB complex and the resistance to first-line drugs (FL LPA) mentioned in this document are those recommended by WHO. They are Genotype MTBDR plus (Hain, Lifesciences, Germany) and NTM + MTBDR NIPRO (Nipro Corporation, Japan), both detect resistance to H and R. Since these assays are relatively complex, they can be implemented only in intermediate or national reference laboratories.

The sensitivity of this type of assay is very good to identify the tubercle bacilli in smear-positive sputum samples or isolates obtained by culture, but it is limited for smear-negative and culture-positive specimens. All of them accurately identify drug resistance by detecting the most frequent mutations in the rpoβ gene that confer RR, and in the katG gene and the *inh*A promoter that generate H resistance The resistance of about 5% and 15% of MTB isolates that are truly resistant to R and H, respectively, may not be detected because they carry mutations in DNA regions that are not investigated by these tests.

The laboratory can determine whether the resistance to H is due to a mutation in *kat*G or *inh*A. This information may be helpful for clinical purposes. As far as it is known, the mutations in *kat*G gene that are more frequent worldwide, and the simultaneous mutations in *kat*G and *inh*A genes, are associated to high-level H resistance. In these mutations are identified, it is presumed that the inclusion of H in the therapeutic regimen, even at high doses, does not increase its effectiveness. On the other hand, most of the relevant mutations that occur in the *inh*A gene are associated to low-level H resistance, though some rare mutations in this gene were related to moderate level of resistance. In general, and hypothetically, in this case the use of high- dose H could be useful. All these inferences are based on results obtained from in *vitro* experiments, though clinical research is still needed to demonstrate them.

LPA for the detection of resistance to second-line antituberculosis drugs

WHO endorsed the LPA Genotype TBMDR *sl* (Hain Lifescience GmbH, Germany) to investigate the resistance to second-line anti-TB drugs (SL LPA). This method identifies the most prevalent mutations in the *gyr*A and *gyr*B genes that are associated with fluoroquinolone (FQ) resistance, and in the *rrs* gene and the *eis* promoter causing resistance to injectable drugs. The presence of these mutations indicates drug resistance accurately, but it does not necessarily imply resistance to all drugs within the FQ or second line injectable (SLID) classes that are used for anti-TB treatment. The cross-resistance profile originated by each mutation is not completely known. As an exception, it has been recognized that certain mutations in the *eis* promoter generate resistance limited to kanamycin. Besides, the resistance of about 14% the MTB isolates that are truly resistant to these drugs may not be detected because they have genetic alterations in regions that are not investigated by this LPA. This molecular assay should be complemented with conventional phenotypic DST to further analyze the resistance pattern, however they may be used to make quick decisions about the treatment of Hr or RR/MDR TB. To this end, WHO recommends this LPA to investigate not only smear-positive but also smear-negative specimens, even acknowledging that the probability of not detecting the TB bacillus is higher in the latter.

At the time of writing of this document, the Global Laboratory Initiative (GLI) is preparing a guide to help to interpret the significance of the mutations that can be identified by LPA.

In general, the LPA cannot be used as an initial diagnostic test as replacement of SM because they have limited sensitivity and require laboratories with a certain level of complexity to be run.

Algorithms for the diagnosis of tuberculosis

Some models of algorithms that guide the microbiological investigation of TB, TB-HIV and TB DR are presented below. They are based on those published by the GLI in 2017, and were adapted considering the situation in Latin America and the most recent recommendations regarding the use of Xpert Ultra and a FQ for the treatment of Hr TB.

These algorithms are in line with the End TB strategy that requires ensuring access to the WRD for all people with signs or symptoms of TB, and DST for all bacteriologically-confirmed TB cases. The indicators and targets defined by WHO to assess the progress of countries in strengthening the capacity of laboratories to reach these goals are presented in the Annex.

Each algorithm was designed for a group of patients that is easily identifiable at the health centers. Taking into consideration the published evidence. the synergistic combination of the laboratory tests recommended by WHO until the time of writing of this document is proposed, with the objective of confirming the diagnosis of TB and supporting the choice of treatment regimens, as rapidly and accurately as possible.

Algorithm 1. Preferred algorithm for the universal access to the rapid investigation of TB, Hr TB, and RR/MDR TB

This algorithm helps to achieve the targets set for the indicators of the End TB strategy that depend on the TB laboratory services. It is feasible if the health system ensures rapid access to the results of WRD.

The Algorithm 1 indicates the initial investigation of all patients with presumptive pulmonary TB by Xpert as a replacement of SM, regardless of their age, HIV- infection status, risk factors for DR-TB and other circumstances.

When the Xpert is employed, the SM is not necessary in the initiation of the diagnostic process because the latter is less sensitive even if two samples are examined by microscopy and only one by Xpert. Nor is it indispensable for monitoring the treatment outcome. In effect, regardless of the result of the initial sputum SM, DST is recommended for all cases that are smear-positive after two months of treatment, to investigate at least RR if it had not been previously detected. However, the Xpert-positive cases might be examined by microscopy at the time of diagnosis when necessary to apply referral guidelines for infection control or treatment of latent infection that categorize the risk of TB transmission according to the result of the sputum SM, until the standards are updated.

For pulmonary TB, the sputum is the sample of choice for laboratory testing. The following recommendations apply to this type of specimen:

- At least two sputum samples should be collected and sent to the laboratory
- The morning sample should be preferred to be investigated by Xpert
- The second sample may be preserved for a second Xpert test, LPA and/or culture as appropriate.

It is possible to request a single sputum specimen initially and then a second one only if needed as shown in the algorithm. However, this option can be operationally challenging and usually results in delays and loss of opportunities to confirm the TB diagnosis. When the policy indicates to collect two specimens, a third one and even a fourth specimen may be required to complete DST for patients with inconclusive results, or to complete the diagnostic workup for patients that have been diagnosed with Hr/RR/MDR TB. Since these cases are generally rare, these additional samples may be opportunely requested only to the pertinent cases, when necessary.

The investigation of specimens obtained by bronchoalveolar lavage, induced sputum and gastric lavage or aspiration, especially from young children, should also follow this algorithm. If the Xpert result is negative, this type of specimens should always be cultured. Then, if the culture is positive, Xpert or LPA and, if necessary the phenotypic DST should be carried out from the isolate to reach the universal DST. As the probability of a positive Xpert test is

relatively low with these samples, it is worthwhile to process them routinely for culture and employ the sediments both to inoculate the culture media and to perform the Xpert assay.

This algorithm can also be applied to investigate extrapulmonary specimens for which the Xpert is recommended. At the time of writing this document they are the cerebrospinal fluid (CSF), lymph nodes and other tissue. All the extrapulmonary specimens that yield Xpert negative results and those that are not acceptable for Xpert testing should be cultured. Collecting abundant or more than one of this type of specimen is usually difficult, so it is worthwhile to process them for culture routinely, and employ the sediments both to inoculate the culture media and to perform the Xpert assay. If the volume is not sufficient for both, and especially in the case of CSF, Xpert testing should be preferred because the time required to produce culture results is too long to support the therapeutic decisions that must be taken immediately when meningeal TB is presumed.

The probability of pulmonary TB in adults with respiratory symptoms and negative Xpert result TB is very low. However, when the clinical presumption of TB is high after reevaluating studies and/or additional information (CXR, clinical response, etc.), an additional patient specimen may be examined by Xpert and by culture if the second Xpert result is also negative. With reference to clinical diagnosis, the Xpert has low sensitivity to identify the pediatric TB. So it is advisable to process the specimens obtained from children simultaneously by Xpert and culture. TB may be clinically diagnosed despite being negative all the laboratory tests.

When a disease caused by mycobacteria other than MTB is presumed, some genotypic assays may be used such as the LPA developed to identify the environmental mycobacteria, and/or culture followed by the identification of the isolate.

When the Xpert recognize the presence of MTB but does not detect RR, the algorithm directs to investigate Hr TB in scenarios with high prevalence of H resistance (e.g. higher than 10% among all TB cases) that may not be associated with RR (i.e. H mono-resistance or poly-resistance involving H but not R). The results collected by the surveillance system, including the surveys carried out in the country, should be considered to estimate the prevalence of resistance to H. A high risk of Hr TB may also be presumed among patients who have received chemoprophylaxis with this drug and among Hr TB contacts. Nowadays, the LPA is the only rapid methodology recommended by WHO to assess the susceptibility to H, and therefore the only one indicated in the algorithm to be used when the Xpert does not detect RR. When H resistance occurs, the identification of the mutation(s) conferring this resistance may be clinically relevant as diverse mutations may be associated with different levels of drug resistance. This may further be elucidated by determining the H minimum inhibitory concentration. When resistance to H is not detected in the context of high prevalence of H resistance, it may be convenient to confirm that the isolate is truly susceptible by phenotypic DST, since the LPA does not investigate all mutations associated with resistance to this drug.

If the LPA is negative and/or contradicts the result related to R susceptibility/resistance obtained with the Xpert, the decision on the chemotherapy depends on the clinical judgement

supported by the analysis of the epidemiological situation, until the laboratory can obtain a positive culture and characterize it by using the diagnostic tools that are available to clarify the event.

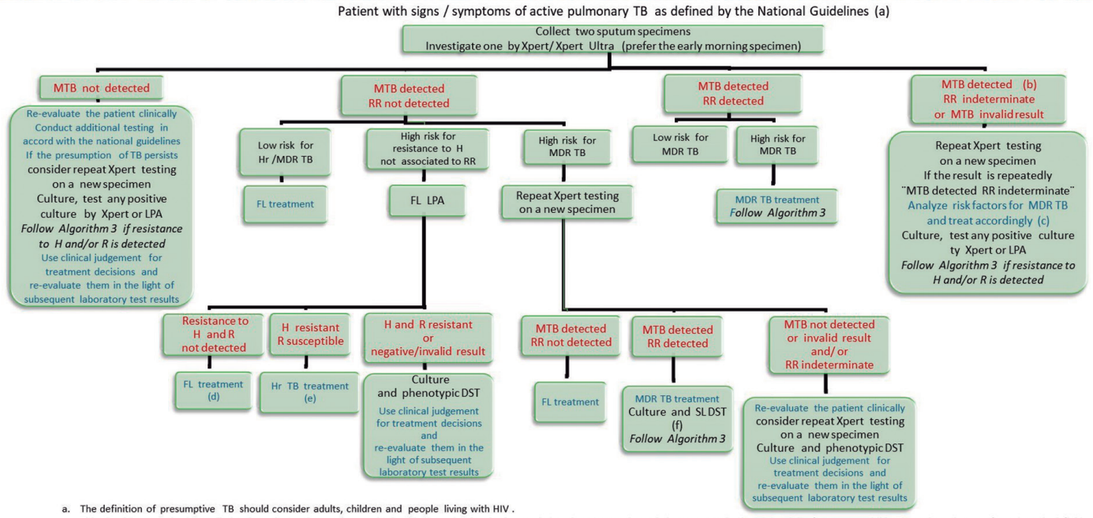
When a molecular test produce results in relation to R that are not completely expected (i.e. R-susceptible result in scenarios with high risk of RR/MDR TB, or R-resistant result among cases at very low risk for RR/MDR TB) it is advisable to repeat testing paying close attention to possible laboratory errors to ensure the accuracy. In this situation, a new patient specimen can be investigated by Xpert or FL LPA. If the second sample has few bacilli, the probability of a negative LPA is relatively high, so it may be preferable employ the Xpert.

When the results of the first and second molecular tests are discordant, the algorithm indicates to use the second result for treatment decisions assuming that the quality has been carefully guaranteed while repeating the assay. Notwithstanding, any discordance should be elucidated by analyzing the results of culture and phenotypic DST and, ideally, also DNA sequencing to identify mutations associated with resistance. The last tool is not widely available at the time of writing this document.

The result ¨MTB detected, RR indeterminate¨, including in this category the result ¨ trace¨ of Xpert Ultra, occurs frequently when the sample contains very few bacilli which hampers the characterization of the DNA region where RR-associated mutations occur. In patients with recent history of anti-TB treatment, the ¨trace¨ result must be interpreted with caution because the probability of false positive results is increased in this group. When this type of result is obtained, it advisable to repeat Xpert testing with a new specimen, however if the second sample also contains few bacilli the result ¨trace¨ and/or ¨RR indeterminate¨ may be obtained again. In this situation it is advisable to complete the investigation by culture and, if positive, to test the isolate by Xpert or LPA as rapidly as possible. Then the clinical decisions may be reconsidered in light of the subsequent test results.

The SLD susceptibility pattern is relevant for cases who were diagnosed with Hr/RR/MDR TB and can be determined by following the Algorithm 3.

Algorithm 1. Preferred algorithm for the universal access to the rapid investigation of TB, rH TB, and TB RR/MDR



a. The definition of presumptive TB should consider adults, children and people living with HIV . This algorithm can also be applied to investigate the specimens obtained by bronchoalveolar lavage, induced sputum and gastric lavage or aspiration, (especially from young children) and specimens of cerebrospinal fluid lymph nodes and other tissue.

b. This category includes the result "trace " of Xpert Ultra .

c. In patients with recent history of anti-TB treatment, the (repeated) result "trace" should be analyzed with caution because the probability of false positive results is increased in this group.

d. It may be convenient to confirm that the isolate is truly susceptible by phenotypic DST, since the LPA does not investigate all mutations associated with H resistance .

e. If the patient has history of treatment with pyrazinamide and/or fluoroquinolones , or is a contact of a Hr TB case, perform SL LPA and pyrazinamide susceptibility testing. *Follow Algorithm 3*.

f. Request an additional specimen, if necessary to complete the phenotypic and genotypic testing .

TB: tuberculosis MTB: *Mycobacterium tuberculosis* complex H: isoniazid RR: rifampicin resistant /resistance MDR: multidrug resistance Hr : resistant to H and susceptible to R SL : second line
Xpert: GeneXpert using MTB/RIF (Ultra) cartridges LPA : line probe assay DST: drug susceptibility test FL: first-line SL: second -ine
MTB detected : include the semi- quantitative categories "high", "medium", "low", " very low" and "trace". MTB invalid result : include the categories " no result", "error " and "invalid"
High risk for Hr TB not associated to RR: populations or scenarios with high prevalence of H resistance , patients with history of chemoprophylaxis with H , or contacts of Hr TB cases
High risk for MDR TB : retreatment after failure, lost to follow-up or relapse, contacts of MDR TB cases and any other risk group identified in the country

Algorithm 2. Interim algorithm for the rapid investigation of TB and Hr/ RR/MDR TB in priority populations while ensuring the resources needed to achieve universal access as presented the Algorithm 1

This algorithm proposes the prioritization of certain groups of patients to be investigated by Xpert and is applicable in countries where the access to the rapid results of Xpert is still limited. In this situation, the GeneXpert instruments must be strategically located in health centers where the priority populations concentrate and that are ready to receive the specimens submitted from other centers. This facilitates the nationwide coverage with rapid testing in the priority groups. Then, as the resources are mobilized and the specimen transport network and the telecommunication infrastructure are strengthened, it is possible to scale up the use of WRD in additional populations or geographical areas moving towards the universal access shown in the Algorithm 1.

Undoubtedly, the Xpert should be used primarily for patients with presumptive TB who are PLHIV, at high risk for DR/MDR TB and/or children. The countries may decide to include other vulnerable or risk groups among those prioritized according to the situation characterized by the NTP.

The Algorithm 2 conducts to investigate the priority groups following the Algorithm 1, as seen in the left branch of the flowchart. The Algorithm 1 should be followed not only to test sputum specimens but also the specimens obtained by bronchoalveolar lavage, induced sputum and gastric lavage or aspiration for the diagnosis of pulmonary TB, and the samples of CSF, nodules and other tissues for the diagnosis of extrapulmonary TB.

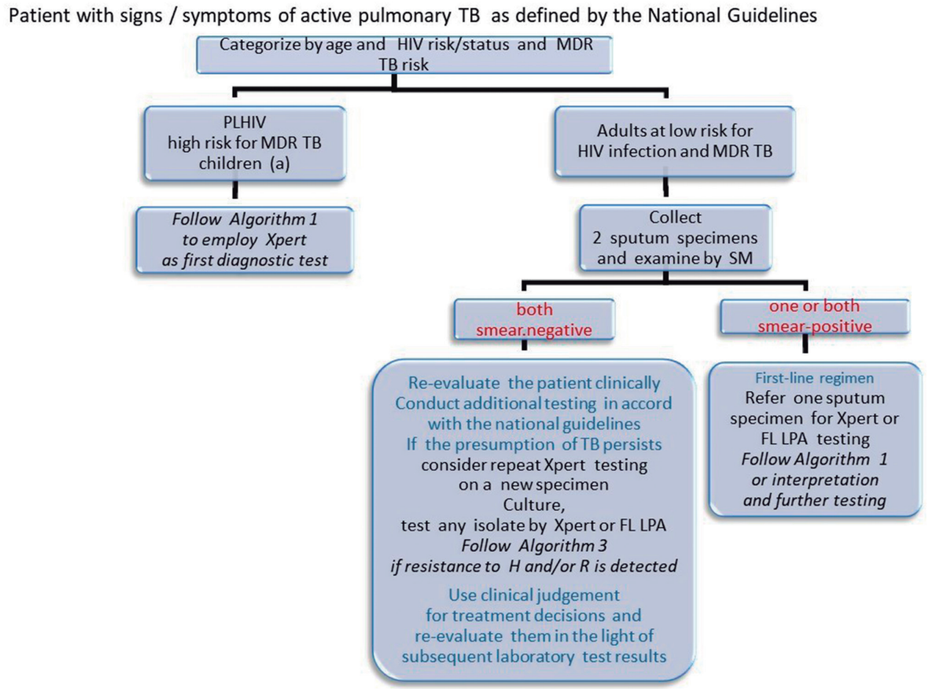
Other patients may be investigated by the traditional bacteriological tools, starting with the SM. For the smear-positive TB cases, the Xpert testing of at least one smear-positive specimen should be performed. For patients with two smear-negative specimens and persistent symptoms, culture should be performed. Then, if culture is positive, the isolate should be tested by Xpert or LPA to universalize the DST.

Some countries routinely collect three sputum specimens to be investigated by SM. It is worthwhile to assess the additional diagnostic value of the third specimen and the suitability of this policy for case-finding considering the workload in the TB laboratory network and the possible resultant delays.

When all the specimens examined by SM produce negative results, it is advisable to re-evaluate the patient in the light of additional studies and/or information (CXR, clinical response, etc.). If the presumption of TB persists, culture is indicated. TB may be clinically diagnosed despite being negative all the laboratory tests.

Algorithm 2. Interim algorithm for the rapid investigation of TB and Hr/RR/MDR TB in priority populations

while ensuring the resources needed to achieve universal access as presented in the Algorithm 1



(a) Other vulnerable or risk groups may be included among those prioritized according to the situation characterized by the NTP TB-LAM might be employed to assist the TB diagnosis among HIV –positive patients with CD4 count < 100 cells /µl or seriously ill only as a transitional aid, until the capacity to perform Xpert is built
This branch of the algorithm can also be applied to investigate the specimens obtained by bronchoalveolar lavage, induced sputum and gastric lavage or aspiration, and specimens of cerebrospinal fluid lymph nodes and other tissue

TB: tuberculosis HIV: human immunodeficiency virus MDR: multi-drug resistant PLHIV: people living with HIV
Xpert: GeneXpert using MTB/RIF (Ultra) cartridges SM: smear microscopy FL LiPA: *line probe assay for first –line drugs*

Algorithm 3. Second-line drug susceptibility testing for Hr/RR/MDR TB cases

The workup presented in this algorithm is relatively more important for patients with history of treatment with SLD or contacts of TB cases that are resistant to SLD and those who were exposed in scenarios with high prevalence of resistance to FQ or SLI.

Molecular DST may support the rapid selection of the short MDR TB treatment regimen that has been recommended for cases that are not resistant to the drugs that are part of this regimen, except for H, among other conditions. Then, ideally, the results of the SL LPA should be known prior to the initiation of the treatment. Hence it is recommended to perform SL LPA rapidly and directly on pulmonary specimens, even acknowledging that the probability of not detecting MTB is high when few bacilli are present in the sample.

So far, the evidence is not enough to recommend the use of the SL LPA to test extrapulmonary specimens and the short regimen was not recommended for extrapulmonary TB yet.

When the LPA detects MTB in the sputum sample, it is necessary to consider the limitations of the results in relation to the SLD resistance discussed earlier in this document. Phenotypic DST to at least FQ and SLI complement the determination of the susceptibility pattern corresponding to the strain isolated from each patient. The following are the main reasons for performing phenotypic DST:

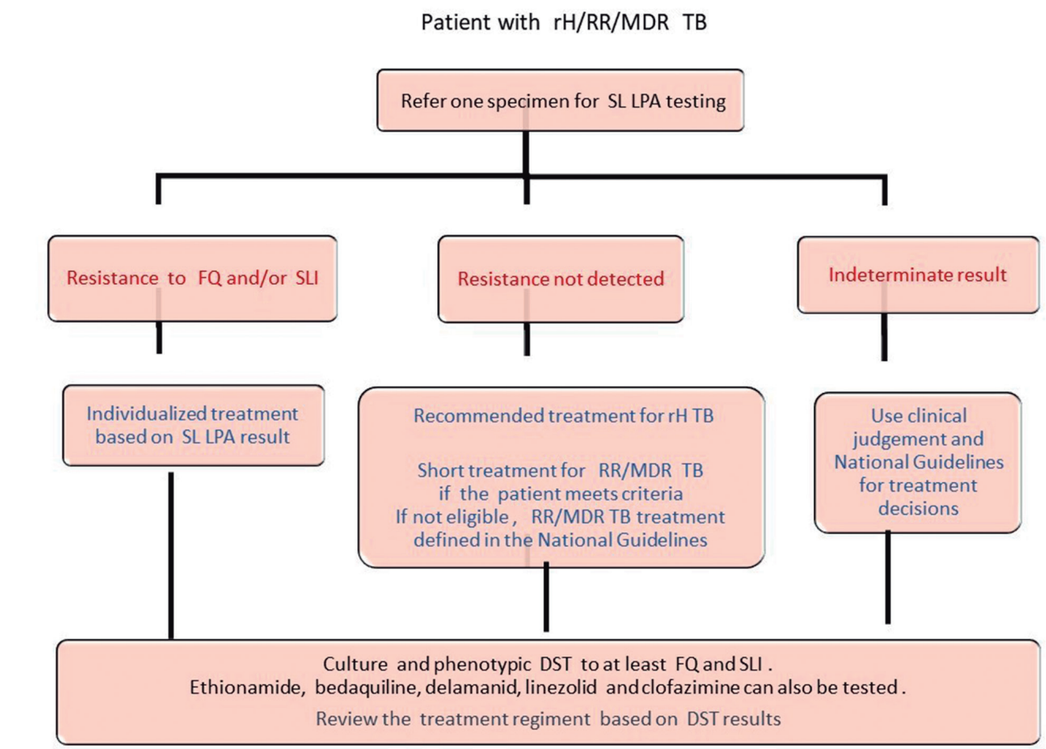
- to exclude the resistance to FQ and SLI with greater certainty in the event that the LPA does not detect it.
- to determine whether the FQ-resistance associated mutations detected by LPA confer resistance limited to levofloxacin or also implies resistance to moxifloxacin and/or gatifloxacin (cross resistance)
- to determine the cross-resistance pattern when the LPA identifies mutations associated to SLID resistance.

WHO has defined the critical concentrations to carry out phenotypic DST to assess the activity of other drugs (ethionamide, clofazimine, bedaquiline, delamanid and linezolid). The results of these tests should be interpreted with caution. Ethionamide susceptibility testing is not reliable enough, progress in the standardization of the DST to recently introduced drugs is still needed and, in general, more knowledge is required about the validity of the DST results to predict the clinical effectiveness of these drugs.

The investigation of resistance to pyrazinamide is relevant for cases with Hr/RR/MDR TB. Recommendations to asses the activity of this drug are controversial because none of the available methods (MGIT, Wayne, LPA, and even DNA sequencing) can be considered as a gold

standard. The phenotypic methods have technical limitations, and the knowledge about the mutations producing the resistance to this drug is still incomplete. No matter which method is employed, information on this limitation should be mentioned in the laboratory reports and discussed with the specialist involved in the treatment of the case.

Algorithm 3. Second-line drug susceptibility testing for Hr/RR/MDR TB cases



TB: tuberculosis Hr : resistant to isoniazid and susceptible to R RR: rifampin resistant MDR: multidrug-resistant
SL LPA : line probe assay for second-line drugs FQ: fluoroquinolone SLI: second-line injectable drugs

Bacteriological monitoring of antituberculosis treatment

Monitoring of the bacteriological confirmed TB cases under treatment should be performed according to the National Guidelines. The following general recommendations apply to the pulmonary TB:

- Cases on first-line treatment
  SM at the end of 2nd, 4th, and 6th month of treatment.
  If the sputum SM is positive at the end of the 2nd month of chemotherapy, test the sputum specimen by Xpert or FL LPA to investigate H and/or R resistance. If resistance is not detected, the continuation phase of the treatment can be given. If the sputum smear is positive subsequently, perform culture to determine if the expectorated bacilli are still viable which indicates treatment failure . Carry out DST on any positive culture. If RR is detected during treatment, follow Algorithm 3

- Cases on treatment for Hr TB
  SM at the end of 2nd and 4thh month of treatment. SM and culture at the end of treatment.
  If the sputum SM is positive at the end of the 2nd month of chemotherapy or afterwards, perform Xpert or FL LPA on the specimen to investigate RR, and culture to determine if the expectorated bacilli are viable. If RR is detected during treatment, follow Algorithm 3

- Cases on treatment for RR/MDRTB
  Monthly SM and culture of 1 or 2 sputum specimens. After 2 consecutive negative cultures from samples collected at least 30 days apart, follow-up can be carried out by monthly SM and culture every 3 months. If the SM becomes positive afterwards, culture and DST should be performed. The frequent repetition of DST either by genotypic or phenotypic methods is not necessary nor useful, unless poor response is evident and treatment failure is suspected. Even in these cases, generally, DST repetitions within three months are unnecessary

Practical Considerations for the laboratories

Processing of biological materials

The procedures for the SM, culture and DST are detailed in the following technical guidelines

Organización Panamericana de la Salud /Organización Mundial de la Salud. Manual para el Diagnóstico Bacteriológico de la Tuberculosis. Parte 1 Baciloscopia. 2008
http://www1.paho.org/Spanish/AD/DPC/CD/tb-labs-baciloscopia.pdf

Organización Panamericana de la Salud /Organización Mundial de la Salud. Manual para el Diagnóstico Bacteriológico de la Tuberculosis. Parte 2 Cultivo. 2008.
http://www1.paho.org/Spanish/AD/DPC/CD/tb-labs-cultivo.pdf

Guía Técnica para el diagnóstico Bacteriológico de la Tuberculosis. Parte 3. Prueba de sensibilidad. Programa de Fortalecimiento para las Redes de Laboratorios de Tuberculosis en la Región de las Américas. 2017.

Ideally the specimens should be processed the day they arrive in the laboratory to speed up the delivery of the results and to prevent the alteration of samples. However, when molecular tests are needed later in the diagnostic pathway (e.g. for cases with diagnosis of RR/MDR TB), it is possible to use frozen samples. Also culture can be performed on frozen samples but, if they contain few bacilli, the chances of obtaining a positive result are reduced due to loss of viability during freeze-thaw.

When Xpert and culture are indicated, it is advisable to process the specimen for culture following the standard procedure and to use the sediment both to perform Xpert and to inoculate the culture media.

The identification of MTB is always required prior to performing/ reporting DST as it may be confused with other mycobacteria. Any of the genotypic methods recommended by WHO for DST simultaneously identifies *M. tuberculosis* complex. In case that the phenotypic DST is made from a positive culture, it must be previously identified by some of the methods described in the above-mentioned guidelines.

Whenever possible, DST should be made directly from the specimens to accelerate the results. The accuracy of LPA and DST on solid media performed directly from the sputum samples is similar to that made from isolates (positive cultures). However, the smaller the number of bacilli in the specimen the higher is the probability that the LPA is negative, and that both tests have uninterpretable results. On the other hand, MGIT 320/960 DST is not standardized to be carried out directly on specimens.

The phenotypic first-line DST (at least with R e H) on solid media is standardized to be performed with the specimens according to the quantity of bacilli seen by the SM. Thus, when necessary, Xpert positive specimens may be examined by microscopy before proceeding to direct DST. As a rough estimate , it has been reported that about 95% and 74% of the samples which smears grade are 3+ and 2 + respectively produce the result of Xpert result ¨ MTB detected medium or high ¨ (Blakemore R et al, Am J Respir Crit Care Med. 1; 184:1076-84,2011).

The algorithms proposed here indicate to test by Xpert any positive culture obtained from particular patients whose specimens yielded negative or inconclusive results initially. At the time of writing this document, the Supranational Reference Laboratories situated in Latin America are developing a protocol to carry out this investigation which will be adequately disseminated.

Resolution of discrepancy in results

Discordant results obtained by repeating a laboratory test or by performing two o more different methods must be infrequent, otherwise they should trigger corrective actions. When a discrepancy occurs, possible errors should be investigated. Those related with specimen handling or processing may be identified by the internal quality control system and those related to the interpretation or transcription of the results may be evidenced by checking the records.

If no evidence of error is found, each unexpected discordance must be rigorously investigated considering the possible causes, with the assistance of the reference laboratory. The true result should be elucidated by all the available tools and the analysis of the clinical /epidemiological history of the pertinent patient. The final interpretation of the result should be reported to the physician who manages the care of the patient.

Some inconsistencies that may occur as well as the possible actions to be taken to resolve them are listed below.

Discrepancies in the detection of *Mycobacterium tuberculosis* in a clinical specimen

Result	Possible explanation	Recommendation
Molecular test negative and culture positive	Expected because the sensitivity of the methods developed to detect MTB decreases in the following order: culture, Xpert, SM - LPA.	Culture result should be considered to guide clinical decisions
Molecular test positive and culture negative	Unexpected at the time of the initial diagnosis. Possible causes are: • The specimen was not collected for diagnosis but for control of treatment, the drugs prevented the growth of the bacillus. • The specimen was stored and/or transported under improper conditions and therefore the bacilli viability was lost. • Insufficient volume of the specimen was processed for culture. • A laboratory error occurred while processing the specimen for molecular testing or culture or recording the results.	Treatment was very probably initiated based on the result of the molecular test which is the first available. Review the laboratory records and the internal quality controls. Check particularly the decontamination process prior to culture. Report any evidence of error. If no error is identified, check the result of SM performed during treatment. If positive, the result of the molecular test is confirmed. If negative, any specimen sent for treatment follow-up should be cultured. Re-evaluate the patient clinically

Discrepancies in the detection of drug-resistance

Result	Possible explanation	Recommendation
The results of two molecular assays carried out at the time of diagnosis are inconsistent	Unexpected, the commercial molecular methods target the same DNA segments to detect mutations. Error(s) may have occurred while specimen handling/testing or recording	Review the laboratory records and the internal quality controls Test the most recent specimen or isolate of the patient that is available by the methods previously employed. If the results match report the error correction. If the discordance persists, perform DNA sequencing when available, culture and phenotypic DST which will define the result.
Resistant by one or more molecular test and susceptible by phenotypic DST.	In general, unexpected. An error in phenotypic DST may have occurred. In relation to R, certain mutations generate this type of discordance, particularly when the phenotypic test is performed with the BACTEC MGIT system. The instrument automatically terminates the DST and interprets the results missing the detection of some bacilli carrying these mutations that may grow later. They have been associated with first-line treatment failure. Thus, the discordance may reflect a false susceptible result of the MGIT system. Some isolates with borderline resistance that may not grow at the drug concentration tested by the phenotypic method may be the cause of this discordance. More rarely silent mutations might occur. These mutations do not generate an observable effect (particularly they do not generate drug resistance) but they are detected by the molecular methods. In this case, the discordance reflects a false resistant result of the molecular test.	Review the laboratory records and the internal quality controls. Ideally, use DNA sequencing to elucidate the result. If sequencing is not available, repeat DST by both genotypic and phenotypic methods on the most recent specimen or isolate obtained from the patient. In case of discrepancy in R susceptibility, perform phenotypic DST on solid culture medium by the proportion method. The determination of the minimum inhibitory concentration can contribute to identify isolates with borderline resistance.

Result	Possible explanation	Recommendation
Drug resistance is not detected by one or more molecular tests, but it is detected by phenotypic DST	Expected at determined frequencies, as the commercial molecular assays do not investigate all the mutations that confer drug resistance. Lack of detection of H and SLI resistance is more frequent than R and FQ resistance.	Review the laboratory records and the internal quality controls. If DNA sequencing is not available to identify the pertinent mutations, make decisions based on the result of phenotypic DST.

Cases infected by two or more distinct MTB strains have been reported, this also may be the cause of discrepancies in the results of the DST. In general, the Xpert and MGIT systems evidence the susceptibility pattern of the predominant strain. The LPA may reveal the mixed infection if it shows the targeted DNA segments simultaneously unmutated and mutated. DST on solid medium allows the quantification of the proportion of bacilli that are resistant to each drug.

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ANNEX

Indicators and targets for laboratory strengthening under the End TB strategy (WHO/HTM/TB/2016.18. 2016)

Objective 1. Increase access to rapid and accurate detection of TB

Indicator 1.	**Does the national diagnostic algorithm indicate a WRD is the initial diagnostic test for all people with signs and symptoms of TB?**
Target 2020	Yes, for all countries *Note:* The target should be reached by 2018 for countries with high burdens of TB and HIV, and MDR-TB.

Indicator 2.	**Percentage of notified new and relapse TB cases tested with a WRD as the initial diagnostic test**
Target 2020	80% of cases
Target 2025	100% of cases
Numerator	Number of notified new and relapse TB cases tested with a WRD as the initial diagnostic test
Denominator	Number of notified new and relapse TB cases
Remarks	• WRD test results may be positive or negative. • WHO will monitor this indicator in low- and middle-income countries. • Where electronic registers or periodic surveys allow stratification, national-level monitoring of this indicator should be stratified by patient risk group: a target of 100% should be reached by the end of 2018 for people living with HIV and people at risk of DR-TB. • As additional proxy indicators for patients' access to WRD testing, some countries may wish to monitor the percentage of districts or basic management units with WRDs, or the percentage of eligible specimens being referred for testing at sites that have WRDs. • This indicator is also included as one of the top 10 priority indicators for monitoring the implementation of the End TB Strategya

Indicator 3.	Percentage of notified new and relapse TB cases with bacteriological confirmation
Target 2020	80% of cases (relapse cases: 90%)
Target 2025	90% of cases (relapse cases: 95%)
Numerator	Number of notified new and relapse TB cases with bacteriological confirmation
Denominator	Number of notified new and relapse TB cases
Remarks	• Where electronic registers or periodic surveys allow stratification, national-level monitoring of this indicator should be stratified by site of disease (pulmonary versus extrapulmonary), by age group (children versus adult), and by HIV status, given the challenges of obtaining bacteriological confirmation using available sputum-based tests among people living with HIV and children, and the challenges of collecting specimens for detecting extrapulmonary TB. • The indicator's targets will be reviewed and refined based on the performance characteristics of technologies that are available and in the pipeline.

Indicator 4.	Percentage of testing sites using a WRD at which a data connectivity system has been established that transmits results electronically to clinicians and to an information management system
Target 2020	100% of sites
Numerator	Number of testing sites using a WRD at which a data connectivity system has been established that transmits results electronically to clinicians and to an information management system
Denominator	Number of testing sites using a WRD
Remarks	• Electronic data connectivity solutions are able to rapidly make test results available to clinicians and information management systems (including a laboratory information management system or an electronic register, or both) via the Internet, mobile data networks or text messaging (SMS).

Indicator 5.	Does national policy indicate that TB diagnostic and follow-up tests provided through the national TB programme are free of charge or that fees can be fully reimbursed through health insurance, or both, for all people with signs and symptoms of TB?
Target 2020	Yes, for all countries *Note:* The target should be reached by 2018 for countries with a high burden of TB.
Remarks	• Monitoring of this indicator may be cross-checked by data captured in patient cost surveys, when data are not subject to significant patient recall bias.

Objective 2. Reach universal access to DST

Indicator 6.	Does national policy and the diagnostic algorithm indicate there is universal access to DST?
Target 2020	Yes, for all countries *Note:* The target should be reached by 2018 for countries with a high burden of MDR-TB.
Remarks	• In 2016, universal access to DST is defined as providing DST for at least rifampicin for all patients with bacteriologically confirmed TB and providing further DST for at least fluoroquinolones and second-line injectable agents for all TB patients with RR-TB. • DST methods include genotypic (molecular) and phenotypic methods.

Indicator 7.	Percentage of notified, bacteriologically confirmed TB cases with DST results for rifampicin
Target 2020	100% of cases *Note:* The target should be reached by 2018 for countries with a high burden of MDR-TB.
Numerator	Number of notified, bacteriologically confirmed TB cases with DST results for rifampicin
Denominator	Number of notified, bacteriologically confirmed TB cases
Remarks	• Monitoring of this indicator by WHO will be stratified by new versus history of previous treatment: a target of 100% should be reached in all countries by the end of 2018 for people with previous treatment. • Where electronic registers or periodic surveys allow stratification by method of DST testing, the percentage of bacteriologically confirmed TB cases with DST results for rifampicin using a molecular method as the initial drug-susceptibility test should be monitored at the national level. By 2020, the initial method should use a molecular (genotypic) technology (which currently includes the Xpert MTB/RIF assay, LPAs or sequencing) for all tested cases (Target 2020: 100% of cases). • In settings with a high frequency of isoniazid resistance, countries may also wish to monitor the percentage of notified, bacteriologically confirmed TB cases with DST results for isoniazid. • This indicator is also included as one of the top 10 priority indicators for monitoring implementation of the End TB Strategy.

Indicator 8.	Percentage of notified, rifampicin-resistant TB cases with DST results for fluoroquinolones and second-line injectable agents
Target 2020	100% of cases
Numerator	Number of notified RR-TB cases with DST results for fluoroquinolones and second-line injectable agents
Denominator	Number of notified RR-TB cases
Remarks	• Second-line anti-TB agents to be tested include fluoroquinolones and second-line injectable agents, as specified by national guidance. Anti-TB agents tested are subject to change in line with future WHO recommendations on DST and treatment regimens. DST results include those from molecular methods (for example, LPAs) or liquid culture methods.

Objective 3. Strengthen the quality of laboratory services

Indicator 9.	Percentage of diagnostic testing sites that monitor performance indicators and are enrolled in an EQA system for all diagnostic methods performed[a]
Target 2020	100% of sites
Numerator	Number of diagnostic testing sites (stratified by type of diagnostic testing) that monitor performance indicators and are enrolled in an External Quality Assurance system for all diagnostic methods performed, as defined in remarks below
Denominator	Number of testing sites (stratified by type of diagnostic testing)
Remarks	• Monitoring of this indicator should be stratified by the type of diagnostic testing: microscopy, WRD (including the Xpert MTB/RIF assay), LPA, culture or phenotypic DST. • For WRDs, key performance indicators should be monitored at least monthly and remote monitoring should be used via a data connectivity solution. • External Quality Assurance should include regular supervision visits and panel testing (or slide rechecking, in the case of microscopy), according to the national system.

Indicator 10.	Percentage of DST sites that have demonstrated proficiency by EQA panel testing for all DST methods performed
Target 2020	100%
Numerator	Number of DST sites that have demonstrated proficiency by EQA panel testing, as defined in remarks below
Denominator	Number of DST sites
Remarks	• Monitoring of this indicator should be stratified by first-line and second-line DST. • DST includes phenotypic and molecular methods. • Panel testing should be conducted at least annually. • Demonstrated proficiency is defined as a site achieving satisfactory results, per the External Quality Assurance programme's predetermined criteria, on the most recent panel tested.

Indicator 11.	Percentage of laboratories conducting culture, LPA or phenotypic DST, or a combination of these, in which a formal quality management system is being implemented that aims to achieve accreditation according to international standards
Target 2020	50% of laboratories
Target 2025	100% of laboratories
Numerator	Number of laboratories conducting culture, LPA or phenotypic DST, or a combination of these, in which a formal quality management system is being implemented that aims to achieve accreditation according to international standards
Denominator	Number of laboratories conducting culture, LPA or phenotypic DST, or a combination of these
Remarks	• Evidence that laboratories have established a formal quality management system include conducting a baseline assessment of the laboratory's quality management system using a recognized checklist based on ISO 15189:2012, developing an action plan for quality improvements and starting to implement recommendations. The checklist should specify requirements for quality and competency aimed at developing and improving TB laboratory services to raise the quality to ISO standard 15189:2012

Indicator 12.	Is the National Reference Laboratory accredited according to the ISO15189:2012 standard?
Target 2020	Yes, for all countries with a high TB burden
Target 2025	Yes, for all countries

ISO 15189:2012. Medical laboratories: requirements for quality and competence. Geneva: International Organization for Standardization; 2012
(https://www.iso.org/obp/ui/#iso:std:iso:15189:ed-3:v2:en, accessed 1 October 2016).

Accreditation should comply with the most recent version of the ISO15189 standard.

Organización Panamericana de la Salud

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