



# **Training on *Mycobacterium tuberculosis* drug susceptibility testing (first and second line LJ DST)**

## **Module 1: WHO recommended TB diagnostic assays & algorithms**

Venue

Presenter's name:

Date:

# Content Outline

- Introduction
- Objectives
- End TB strategy
- WHO endorsed TB diagnostic techniques
- Existing and recent WHO policies on TB diagnosis
- Summary
- References
- Acknowledgements



# Introduction

- There are a number of WHO recommended diagnostics to accelerate global efforts to end tuberculosis (TB), as outlined in the 2015-2035 End TB Strategy



# Objectives

By the end of this module participants should be able to:

- Understand the End tb Strategy , goals and indicators
- Know all WHO endorsed TB diagnostics currently recommended



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# The END TB strategy

- Is a strategy with a goal and vision to a world free of TB, zero death, no disease and suffering due to TB in order to end the global

Target	2020	2025	2030	2035
Reduction in the number of deaths	35%	75%	90%	95%
Reduction in TB incidence rate	20%	50%	80%	90%
TB affected families facing catastrophic costs	0%	0%	0%	0%

# The END TB strategy Cont.....

## The Strategy:

- Provides a unified response to ending TB deaths, disease, and suffering.
- Builds on three strategic pillars underpinned by four key principles.



# The END TB strategy: Pillars and Principles



# Diagnosis and treatment of TB and MDR-TB in the END TB Strategy?



IMPLEMENTING  
THE END TB  
STRATEGY:  
**THE ESSENTIALS**



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Early diagnosis of tuberculosis including universal access to drug susceptibility testing, and systematic screening of contacts and high-risk groups

Treatment of all people with tuberculosis including drug-resistant tuberculosis, and patient support



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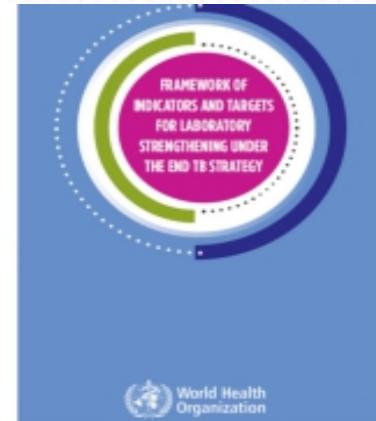
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# Achieving early diagnosis and universal access to DST

- Requires **rapid molecular diagnosis** at the first entry point to the health system
- All bacteriologically confirmed case require a **rapid DST** (at least rifampicin)
- All rifampicin-resistant TB or MDR-TB require rapid second-line DST
- Conventional microscopy and culture required for **monitoring TB patients response** to therapy
- This requires a functional laboratory network with strong **sample referral mechanism**



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# Lab indicators and targets under End TB Strategy

**Three (3) Laboratory objectives and Twelve (12) indicators**

**Objective 1: Increase access to rapid and accurate detection of TB**

**Objective 2: Reach universal access to Drug Susceptibility Testing (DST)**

**Objective 3: Strengthen quality of laboratory services**



# Objective 1: Increase access to rapid and accurate detection of TB

Indicator 1	Does the national diagnostic algorithm indicate a WRDs is the initial diagnostic test for all people with signs and symptoms of TB?
Indicator 2	Percentage of notified new and relapse TB cases tested with a WRD as the initial diagnostic test
Indicator 3	Percentage of notified new and relapse TB cases with bacteriological confirmation
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
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?

# Objective 2: Reach universal access to DST

Indicator 6	Does national policy and the diagnostic algorithm indicate there is universal access to DST?
Indicator 7	Percentage of notified, bacteriologically confirmed TB cases with DST results for rifampicin
Indicator 8	Percentage of notified, rifampicin-resistant TB cases with DST results for fluoroquinolones and second-line injectable agents



# Objective 3: Strengthen the quality of laboratory services

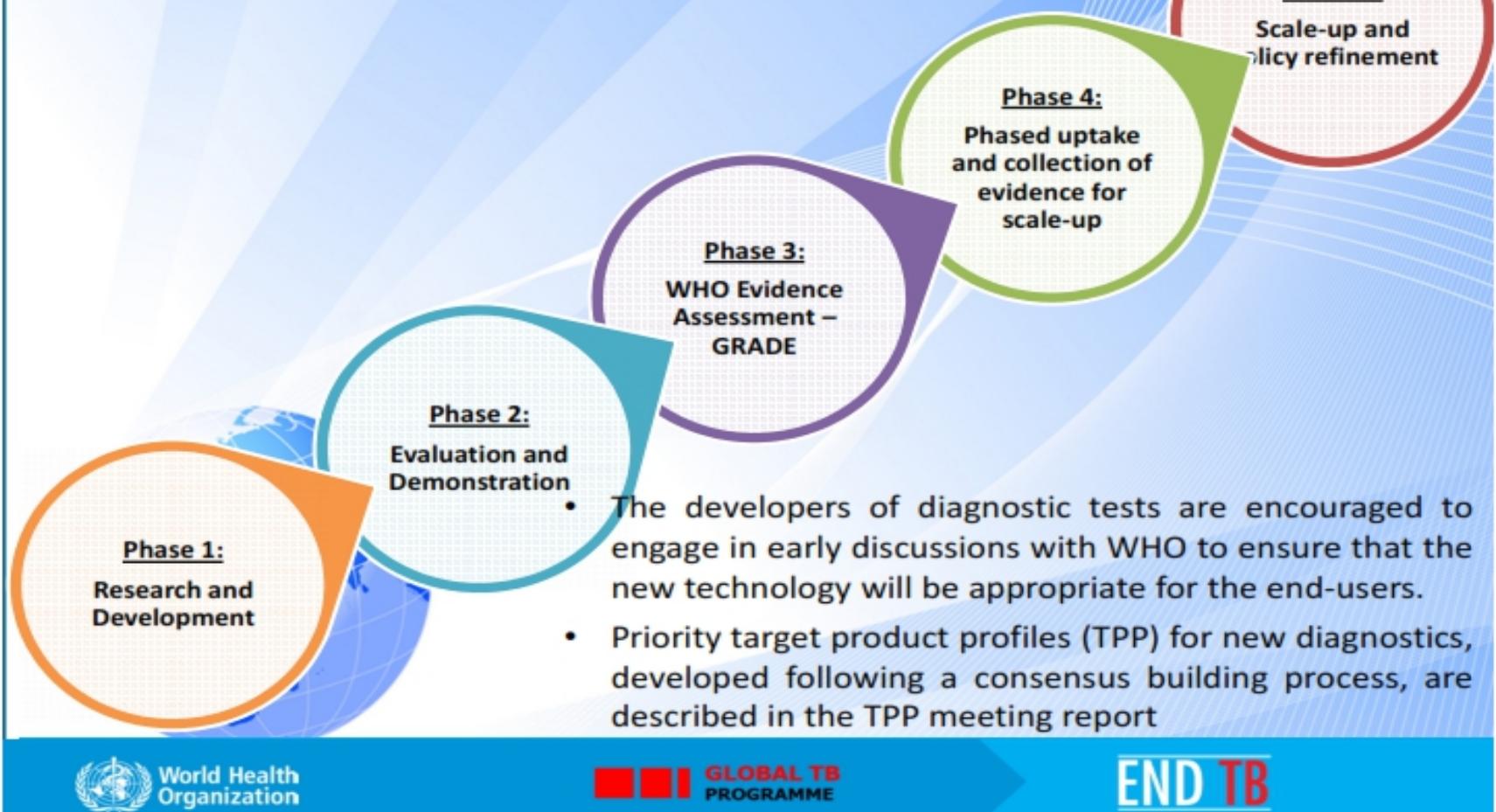
<b>Indicator 9</b>	Percentage of diagnostic testing sites that monitor performance indicators and are enrolled in an EQA system for all diagnostic methods performed
<b>Indicator 10</b>	Percentage of DST sites that have demonstrated proficiency by EQA panel testing for all DST methods performed
<b>Indicator 11</b>	Percentage of laboratories conducting culture, line probe assay 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
<b>Indicator 12</b>	Is the National Reference Laboratory accredited according to the ISO15189:2012d,e standard?



# Challenges to considerer under these 12 indicators:

- Approved diagnostic algorithm at country level
- Rapid test as initial diagnostic test for all the TB suspected cases
- Lab data transmitted electronically to clinicians and management
- All bacteriologically confirmed TB have DST at least for RIF
- All Rif resistant cases (by GeneXpert) have DST for FQ and SLI drugs
- Availability of Quality Assurance for all tests, QMS implementation towards accreditation

# Evidence required and stages of WHO review of diagnostics



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# WHO's recommended techniques for diagnosing TB: until 2022

- Microscopy
  - conventional, Light Microscopy
  - Light emitting diode fluorescent Microscopy
- Culture
  - Culture on solid media
  - Commercial liquid culture systems and Rapid speciation



# WHO's recommended techniques for diagnosing

## TB

- Drug susceptibility testing

- DST for 1<sup>st</sup> & 2<sup>nd</sup> line anti TB drugs( Rif.....)

- Non commercial methods

## Molecular testing

- LPA (1<sup>st</sup>&2<sup>nd</sup> Line)

- Expert MTB RIF( Ultra+ Others)



# WHO's recommended techniques for diagnosing TB.....

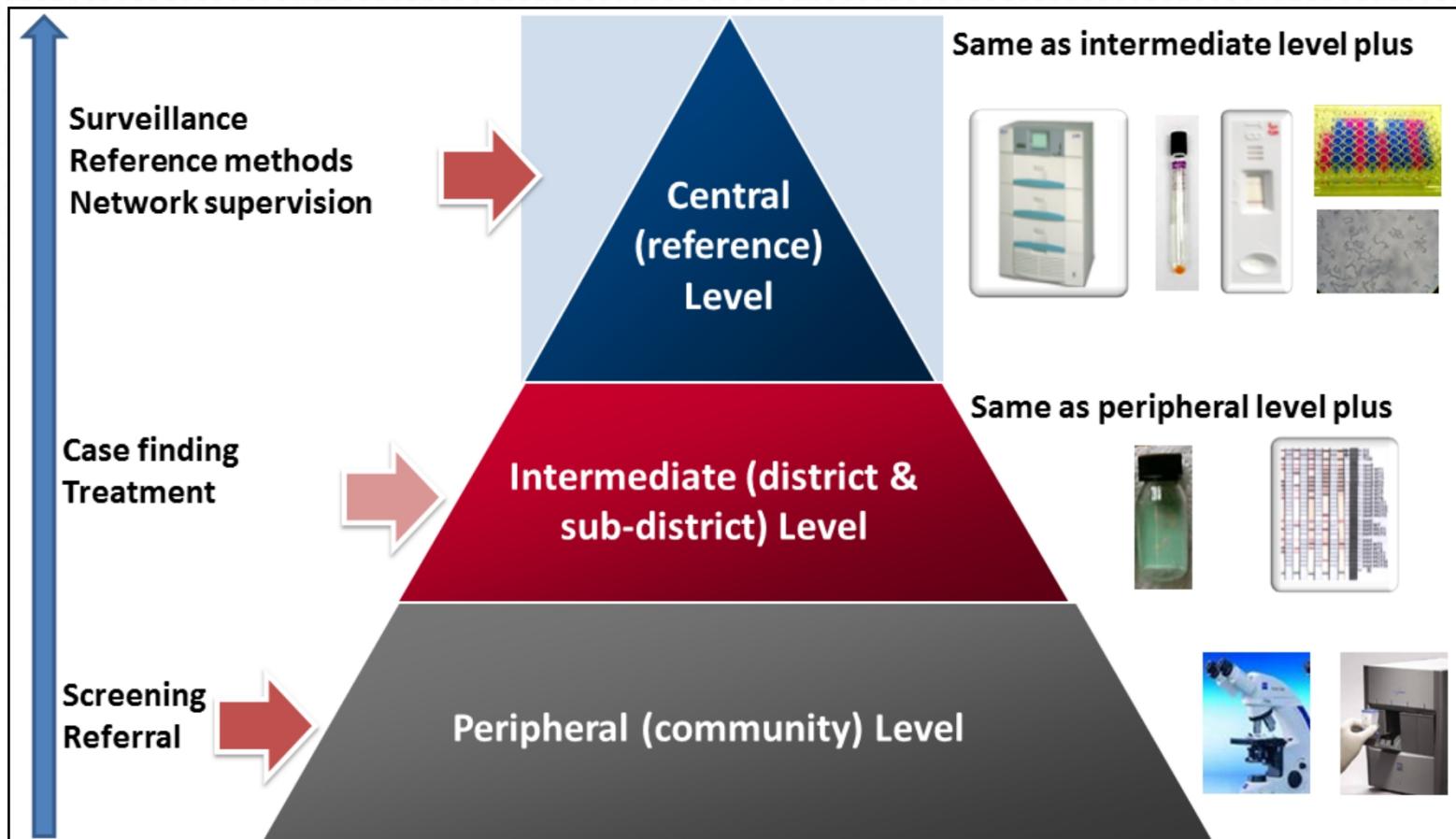
## Molecular testing

- TB LAMP
- Next Generation Sequencing (NGS)

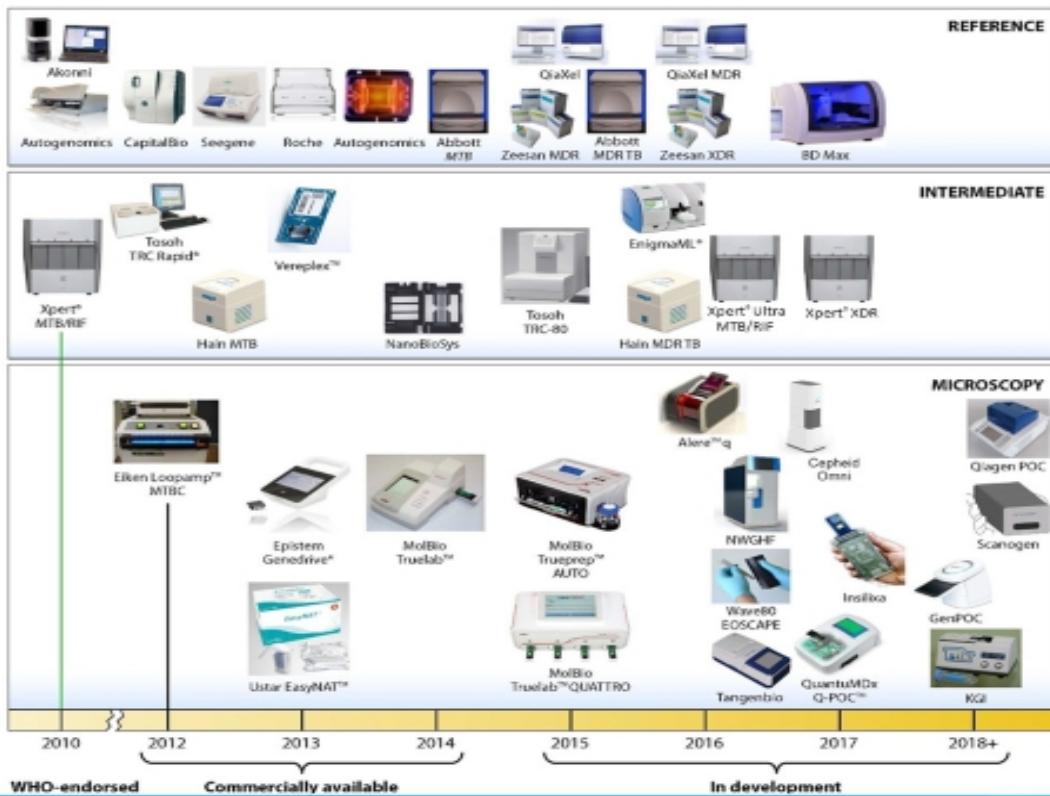
- Immuno Chromatography
- LF-LAM urine test for PLHIV



# Placement of different tests at the levels of laboratory sophistication

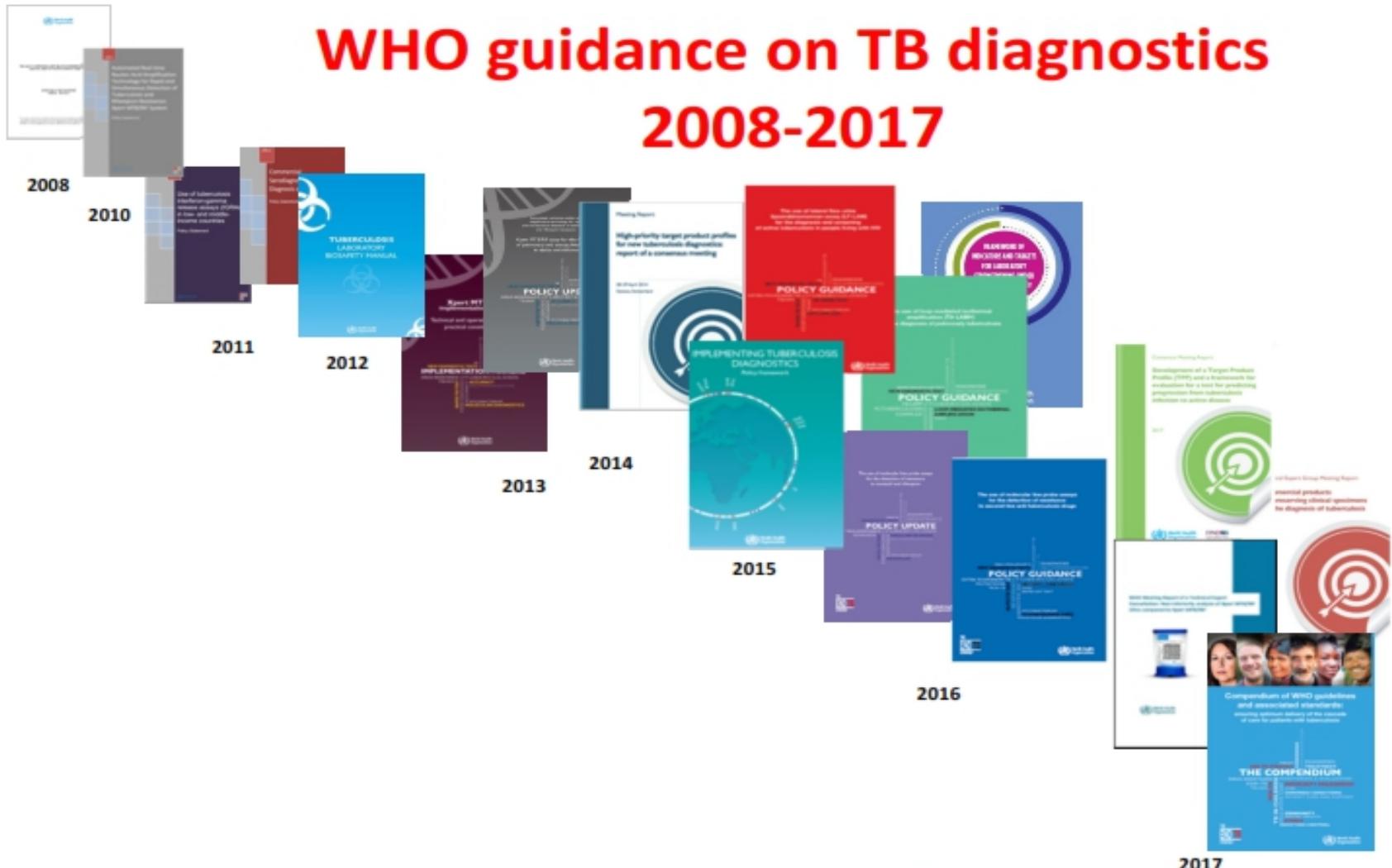


# Interest in TB is high and the pipeline of technologies is robust



- Majority of technologies developed for the intermediate and central level laboratories
- More technologies suitable for the peripheral level as are replacement for microscopy are needed
- Greater investment in conducting the field evaluation and demonstration studies in high burden setting is needed





# Phenotypic methods for the diagnosis of DR-TB

Phenotypic culture methods are based on assessment of the ability of *M. tuberculosis* to grow in culture media (solid or liquid) containing a critical concentration of specific anti-TB agents (which indicates **resistance**) or, conversely, its inability to grow in the same media (which indicates **susceptibility**).

The indirect proportion method is the most common method

Resistance is defined when at least 1% of growth is observed at the critical concentration of drug in the culture medium.

Commercial liquid culture systems for DST reduce the time to result to as little as 10 days, compared with the 28–42 days needed for DST using solid media



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# Comparison of solid and Liquid phenotypic DST

Phenotypic solid method (LJ DST)	Phenotypic LIQUID DST methods
Cost effective in low income countries	Expensive technique
Long turn around time(4 to 6 weeks)	Short turn around time(21 days)
Effective at determining borderline resistance in rifampicin and Ethambutol	Borderline resistances in rifampicin and Ethambutol can easily be missed out
Time consuming.	Less time consuming
Less prone to contamination	Prone to contamination

# Molecular methods for the diagnosis of DR-TB

Molecular (genotypic) methods detect specific DNA mutations in the genome of the *M. tuberculosis*, which are associated with resistance to specific anti-TB drugs.

Molecular methods have considerable advantages for programmatic management of drug-resistant TB, in particular with regard to their speed, the standardization of testing, their potentially high throughput and the reduced requirements for laboratory biosafety.

Molecular tests for detecting drug resistance to rifampicin alone or in combination with isoniazid have been recommended for use by WHO since 2008



# Xpert MTB/RIF

## 2010 Policy Recommendation

Xpert MTB/RIF is recommended rather than conventional microscopy, culture and DST as the **initial diagnostic test** in **adults** presumed to have MDR-TB or HIV-associated TB.

## 2013 Policy Update

Xpert MTB/RIF is recommended rather than conventional microscopy and culture as the initial diagnostic test in **all adults and children** with signs and symptoms of TB



Xpert MTB/RIF remains the **only WHO-recommended diagnostic test** that can simultaneously detect TB and rifampicin resistance that is suitable for **use at lower levels of the health system**



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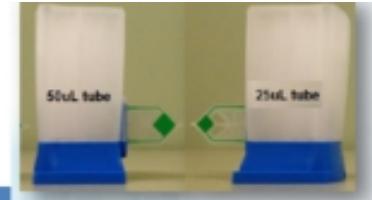


# The Xpert® MTB/RIF Ultra

- Ultra test cartridge (Ultra) was developed as a next-generation test, with higher sensitivity for MTB detection especially among smear-negative TB patients as well as more accurate determination of rifampicin resistance.
- Ultra runs on the same GeneXpert platform as Xpert MTB/RIF, using software version 4.7b or later.



# Xpert MTB/RIF and the new generation Xpert MTB/RIF Ultra



	Xpert	Ultra	Benefits
Target	Single copy <i>rpoB</i>	Multi-copy IS6110 & IS1081 + <i>rpoB</i>	<ul style="list-style-type: none"> <li>Increased sensitivity: 16 CFU/ml for Ultra vs 114 CFU/ml for Xpert</li> </ul>
Cartridge	25mcl tube	50 mcl tube	<ul style="list-style-type: none"> <li>Ultra has an additional semi-quantitative category, called "trace" that reflects MTB detected only based on multi-copy targets</li> </ul>
Enzyme		Enzyme optimization	
Probes		IS cooperation; probe optimization	<ul style="list-style-type: none"> <li>Improved specificity with NTMs (no cross reactivity in over 30NTM tested)</li> <li>Improved classification of silent mutations (e.g. Q513Q; F514F)</li> </ul>
Analysis	Real time PCR curves	Melt curve analysis	<ul style="list-style-type: none"> <li>Improved ability to detect mutations in mixtures (1% for S531L with 99% WT)</li> <li>Robust detection of all mutations associated to Rifampin resistance (i.e. <i>rpoB</i> C533G mutations).</li> <li>Avoid false + for Rifampin resistance in samples with low bacterial load</li> </ul>

Slide courtesy S. Schumacher, FIND

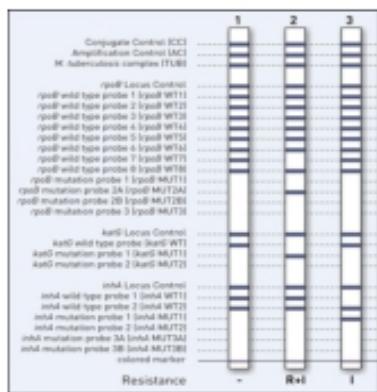
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# Xpert MTB/XDR test

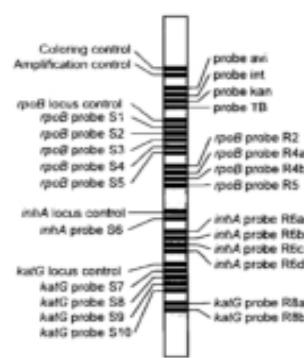
- detects resistance to amikacin, ethionamide, fluoroquinolones and isoniazid.
- However, it requires the latest instrument with 10-colour optics to support detection of additional molecular targets.



# First-line LPAs



Examples of different line probe assays strip readouts:  
a) Hain GenoType MTBDRplus V1 and V2 strip readout  
b) Nipro NTM+MDR Detection Kit 2 strip



>500 LPA laboratories had been established in low and middle-income countries



New version 2 of the Hain MTBDRplus assay available

New manufacturer of LPA Corporation, Tokyo

Both assays show equivalence to Hain version 1.

Guideline Development Group convened by WHO in March 2016

New guidance recommends the use of LPA as the initial test for the detection of resistance to rifampicin and isoniazid in sputum smear – positive specimens and cultures of MTBC

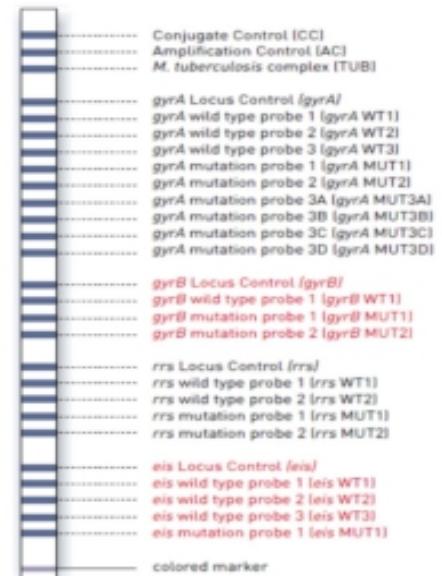
# Second-line LPAs



WHO recommends the use of the SL-LPA for patients with **confirmed rifampicin-resistant TB or MDR-TB** as the **initial test** to detect resistance to fluoroquinolones and the second-line **injectable drugs**, instead of phenotypic culture-based drug-susceptibility testing (DST).



**GenoType MTBDR<sub>sl</sub> VER 2.0**



**500 LPA laboratories had been established in low and middle-income countries**



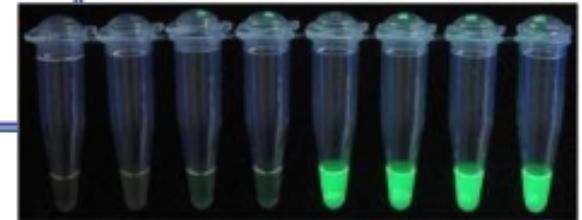
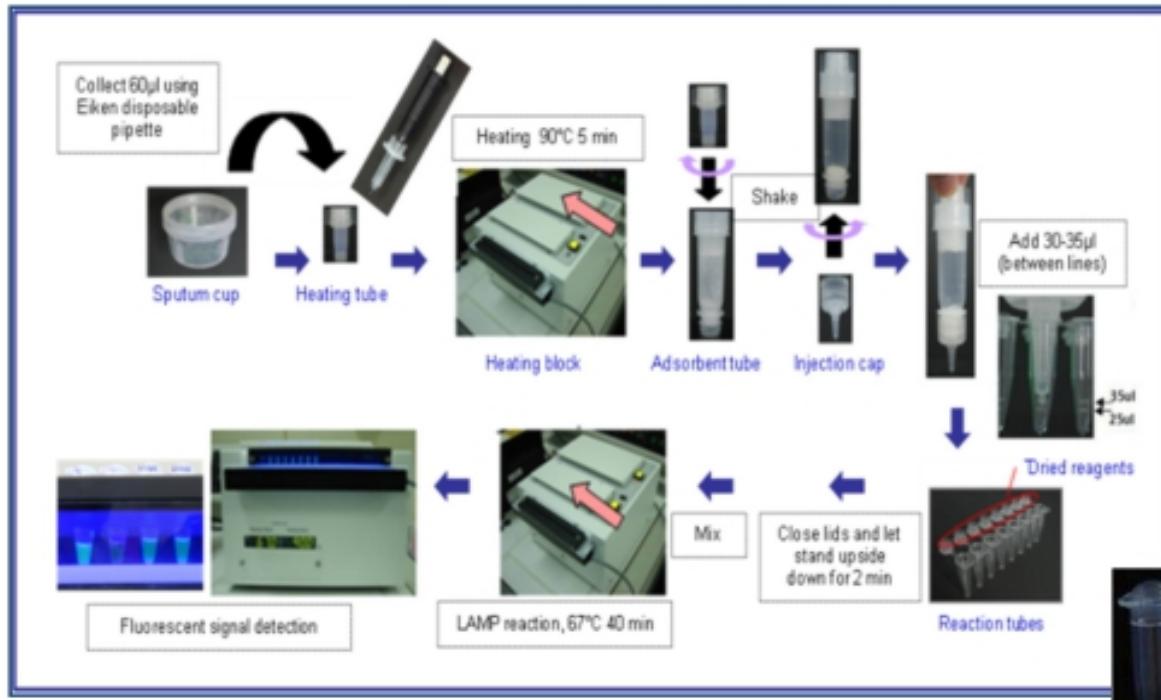
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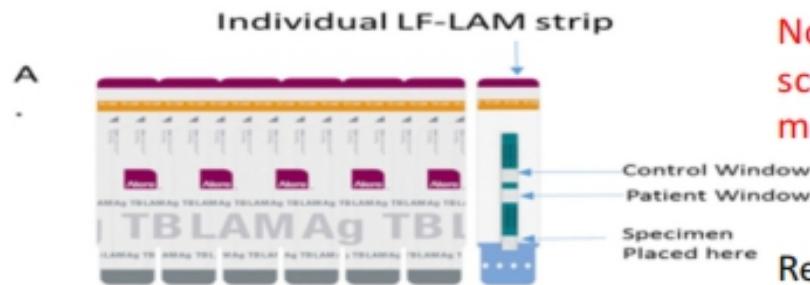


# Loop-mediated Isothermal Amplification Assay (TB-LAMP)

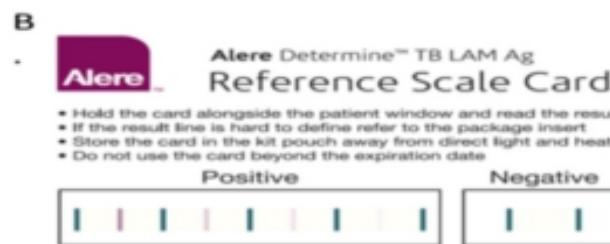


TB-LAMP may be used as a replacement test for sputum smear microscopy for the diagnosis of pulmonary TB in adults with signs and symptoms consistent with TB

# Lateral flow- Urine Lipoarabinomannin assay (LF-LAM)



Not recommended by WHO for TB screening or diagnosis of active TB disease in most population groups



Recommended to help with the diagnosis of TB in two specific population groups:

- People living with HIV who have signs or symptoms of TB and a CD4 cell count less than or equal to 100 cells/ $\mu\text{L}$
- People living with HIV who are “seriously ill” regardless of CD4 count or if the CD4 count is unknown.



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# Next Generation Sequencing(NGS)

A “high-throughput, massively parallel” sequencing method used to determine the nucleotide sequence of a whole genome. ( WGS or Targeted)



2018

Technical Guide:  
**The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in *Mycobacterium tuberculosis* complex**

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Because diagnosis matters  
WHO collaborating centre for the evaluation of new diagnostic technologies

# Whole genome sequencing (WGS) Vs Targeted

## sequencing **Whole genome sequencing (WGS)**

- Determines the complete genome sequence for a given organism at one time through NGS.
- The sequences obtained can be used to detect any variations relative to a reference genome using bioinformatics analyses.

## Targeted sequencing

- Focused on sequencing a select set of genes or gene regions that have known or suspected associations with a specific pathogen.



NGS

# Strengths

- Provides detailed sequence information for multiple gene regions or whole genomes of interest
- confirm the presence or absence of indels
- assess the occurrence of rare mutations
- heteroresistance, or a mix of multiple genetic populations can be determined



# Limitations

- cost
- specialized and well-trained staff
- data analysis and data storage
- Lack of regulatory standards and guidance

# Molecular methods for the diagnosis of DR-TB - limitations

There remains imperfect correlation between phenotypic and genotypic methods.

Molecular methods had high specificity but lower sensitivity which varies for different drugs

Rifampicin – *rpoB* 95% sensitivity, 99% specificity

Isoniazid – *inhA* and *katG* ~90% sensitivity, 99% specificity

Fluoroquinolones – *gyrA* and *gyrB* ~86% sensitivity, 99% specificity

Secondline injectable agents – *rrs* and *eis* ~86% sensitivity, 99% specificity

The predictive values of imperfect tests depend on the pre-test probability of resistance



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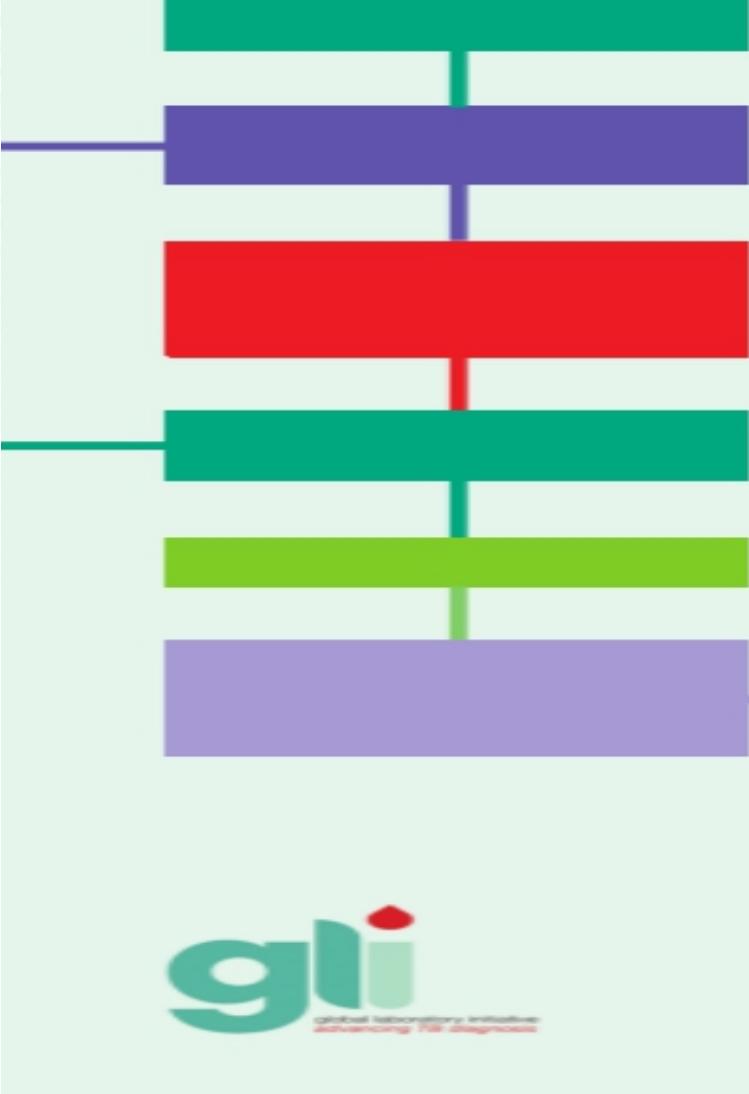
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# Chest radiography in tuberculosis detection

- Cxr is a sensitive tool for triaging and screening for active tb with higher sensitivity than screening for tb symptoms.
- useful to aid diagnosis when pulmonary TB cannot be confirmed bacteriologically.
- CXR can be used for selecting individuals for referral for bacteriological examination
- radiology remains important when bacteriological tests cannot provide a clear answer
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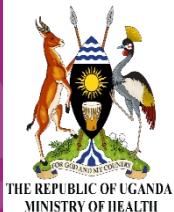
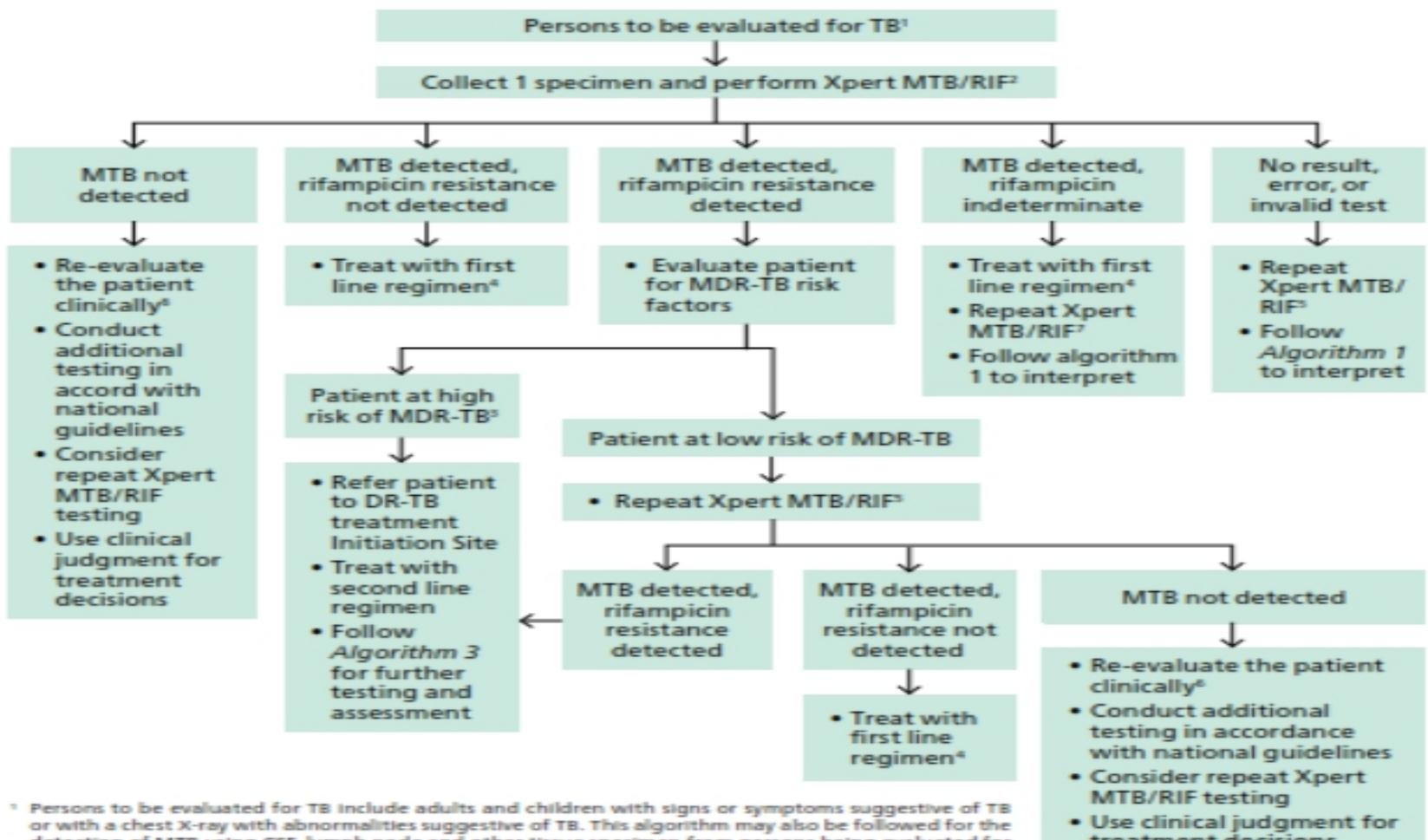




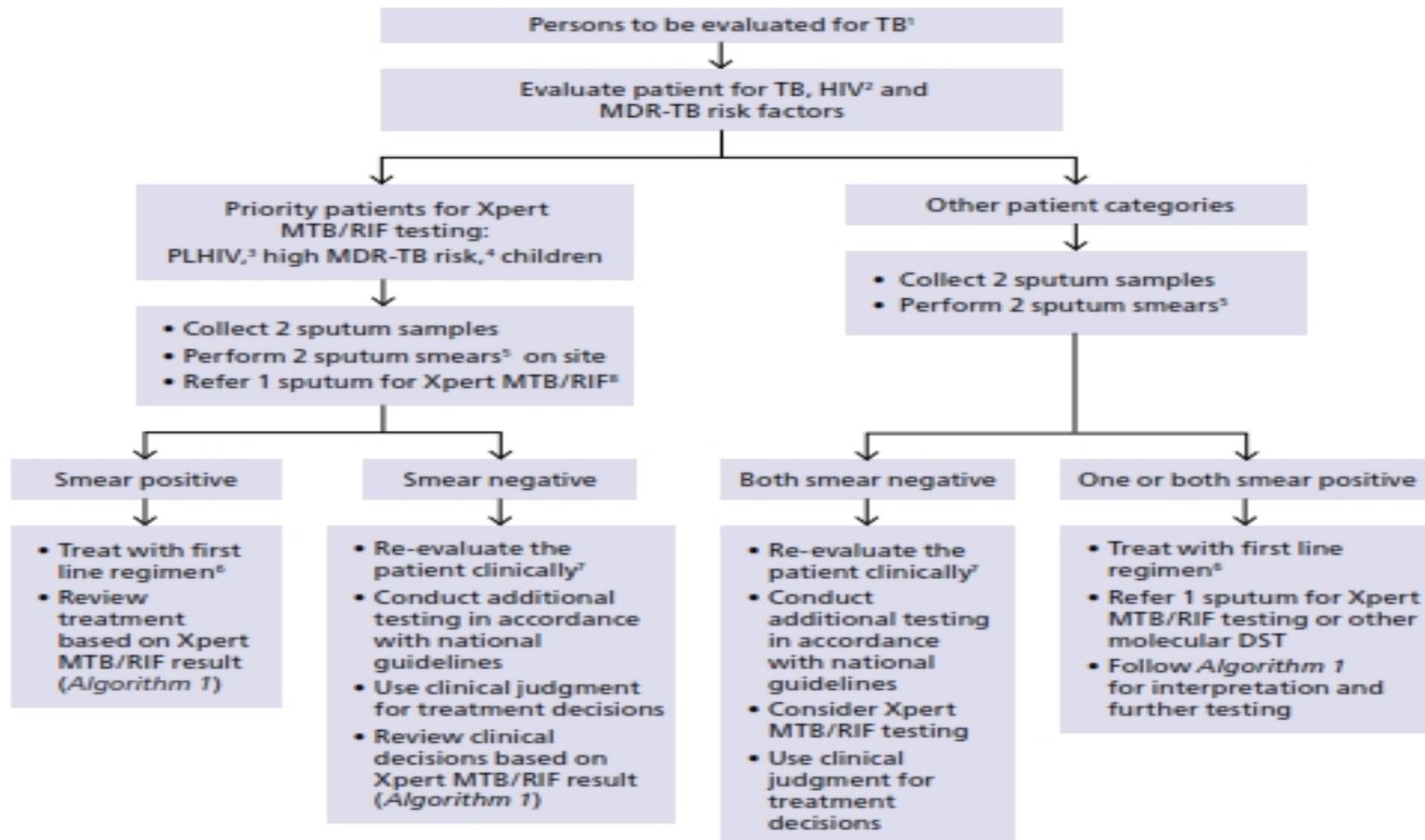
# **GLI model TB diagnostic algorithms**



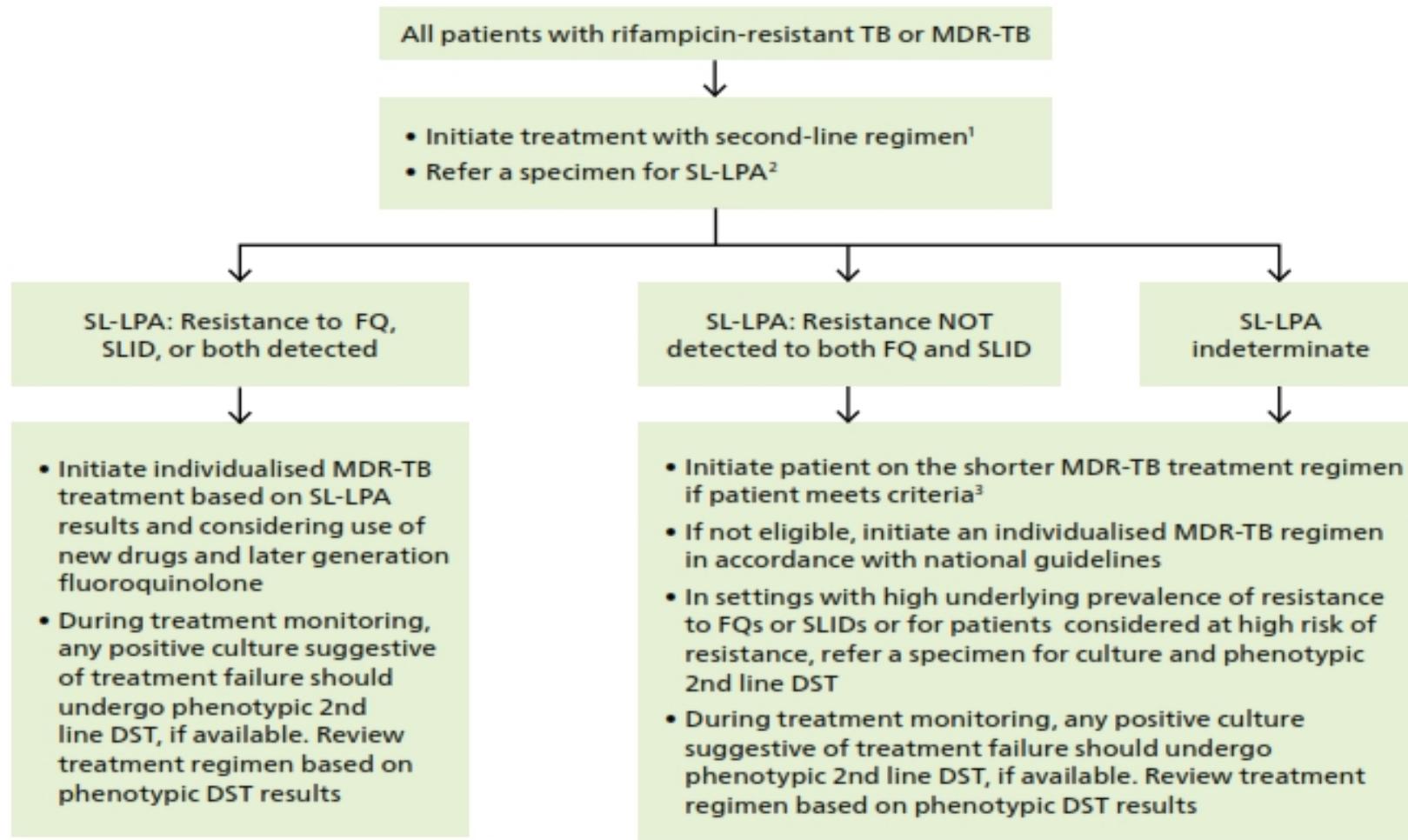
## Algorithm 1: Preferred algorithm for universal patient access to rapid testing to detect MTB and rifampicin resistance



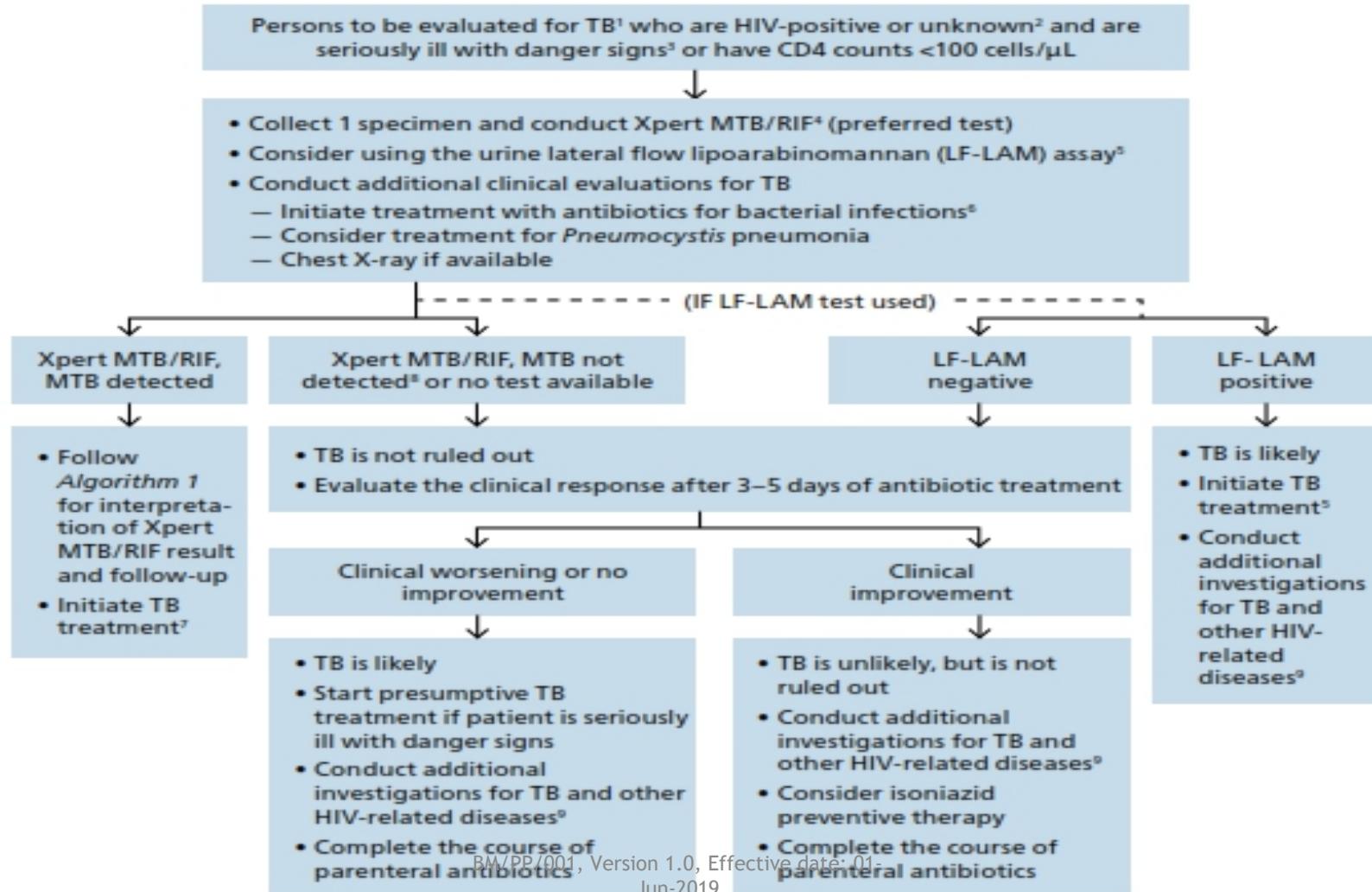
## Algorithm 2: Interim algorithm moving towards universal access, with rapid testing for priority populations



## Algorithm 3: Algorithm for testing for second-line drug resistance among rifampicin-resistant TB or MDR-TB patients



## Algorithm 4: Algorithm for evaluating persons for TB, among PLHIV who are seriously ill with danger signs or have CD4 counts ≤ 100 cells/µL



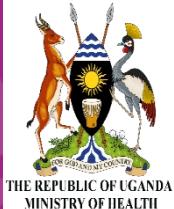
# Assessment

1. Outline the end tb strategy Objectives
2. List atleast five WHO recommended TB diagnostics



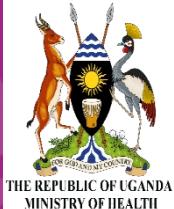
# Summary

1. Laboratories play a **significant role** under the End TB Strategy
2. Increasing access to **rapid detection** of TB and reaching **universal access to DST** will require major efforts
3. **Future diagnostics** will play a role in reaching targets of End TB Strategy, but we also need to make the **best use** of the currently available diagnostics
4. **Multi-disease testing platforms** will provide opportunities for laboratory integration
5. Adoption of **WHO policy guidance** on new TB diagnostics combined with use of **GLI implementation guidance** can help countries reach the targets of the End TB Strategy for laboratory strengthening



# References

- [WHO End tb strategy](#)
- [www.gliafricatb.org](#)
- [www.who.int/tb](#)
- [http://www.who.int/tb/publications/molecular-test-resistance/en](#)
- [http://www.who.int/tb/dots/laboratory/policy/en](#)



# Acknowledgments



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