



Training on Tuberculosis Drug and Susceptibility Testing (MGIT DST – Liquid Method)

Module 3: WHO recommended TB diagnostic assays & algorithms

Date:
Uganda Supranational Reference Laboratory

Content Outline

- WHO endorsed TB diagnostic techniques until 2017
- WHO processes for new technology evaluation
- Existing and recent WHO policies on TB diagnosis
- Next steps and knowledge gaps



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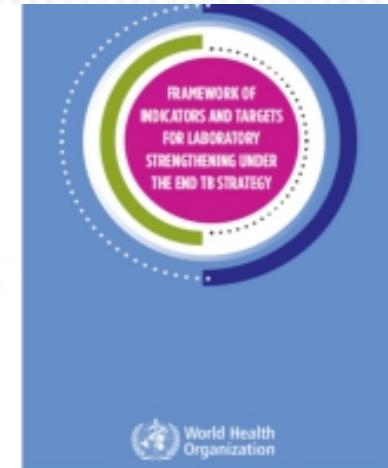
Group exercise-10 minutes

1. List all the WHO approved TB diagnostic tests that you know
2. Identify the role of each of those tests in the TB diagnostic algorithm.
3. At what level (peripheral/community, intermediate/district/sub district and central/reference) of diagnosis is each of the above assays recommended for deployment



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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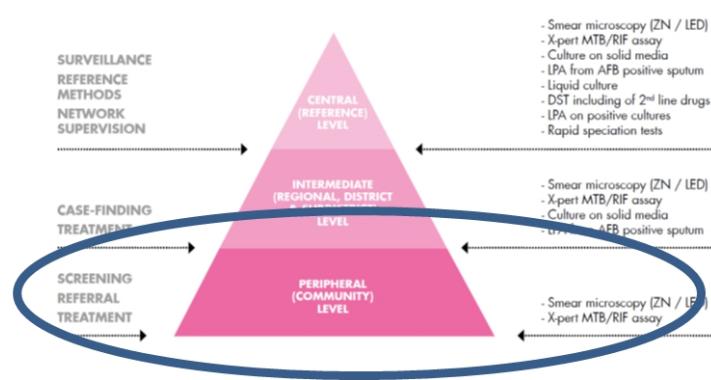
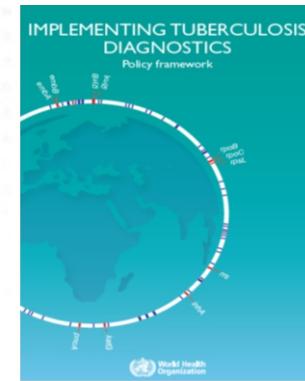
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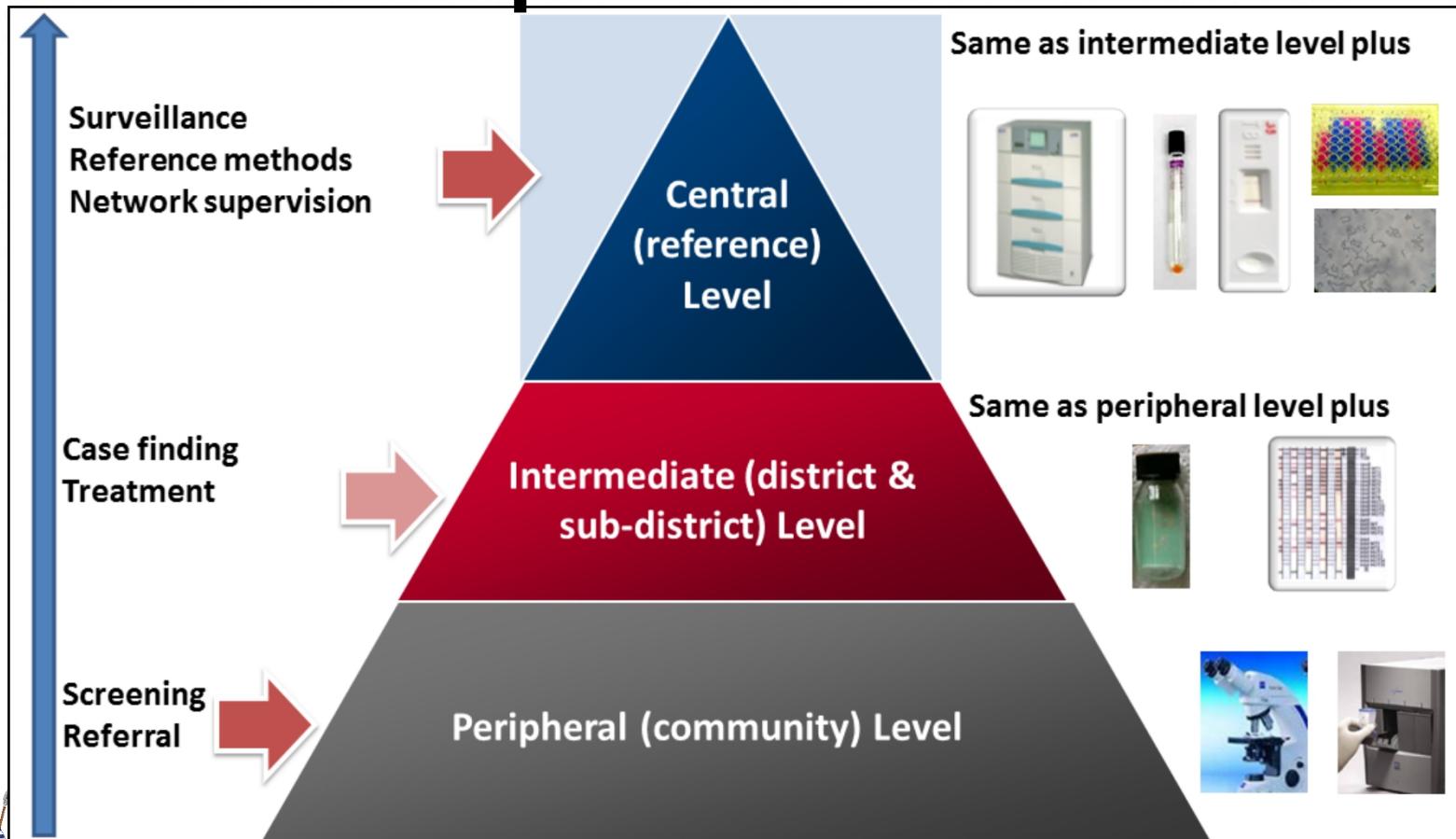
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WHO's recommended techniques for diagnosing TB: until 2017

- **Microscopy**
 - Conventional light microscopy
 - Light-emitting diode fluorescent microscopy
- **Culture**
 - Culture on solid media
 - Commercial liquid culture systems and rapid speciation
- **Drug-susceptibility testing**
 - DST first-line anti-TB agents
 - DST for second-line anti-TB agents
 - Non-commercial methods
- **Molecular testing**
 - LPA (**first and second-line**)
 - TB-LAMP
 - Xpert MTB/RIF assay (**Ultra+others**)
- **LF-LAMP Urine test for PLHIV**



Placement of different tests at the levels of laboratory sophistication



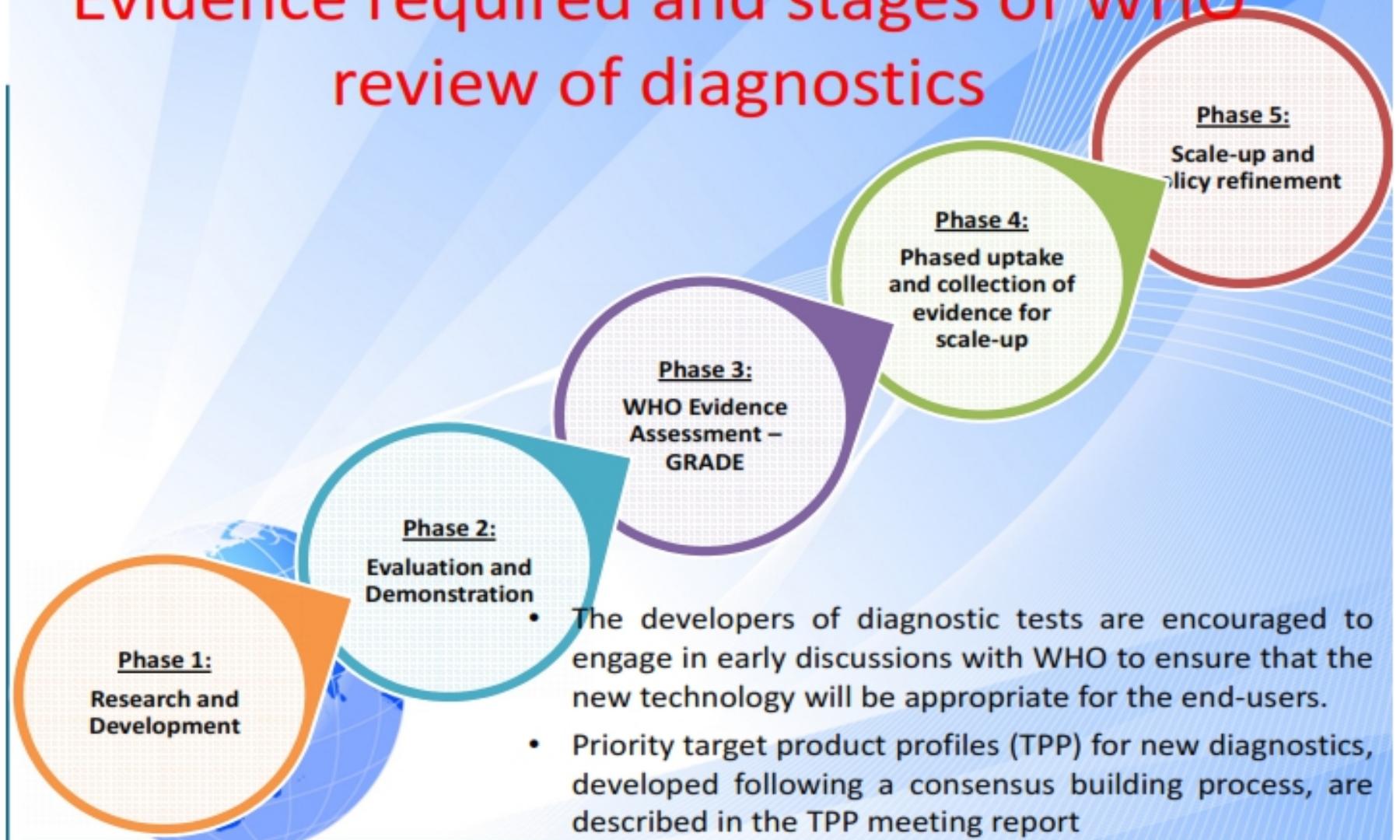
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Evidence required and stages of WHO review of diagnostics



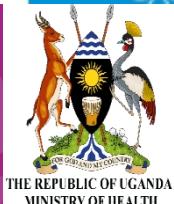
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Molecular methods for the diagnosis of DR-TB

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



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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**



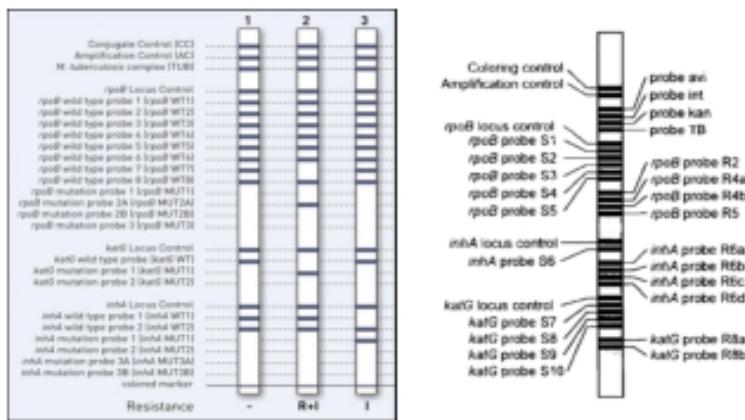
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First-line LPAs



Examples of different line probe assays strip readouts:

- Hain GenoType MTBDRplus V1 and V2 strip readout
- 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**



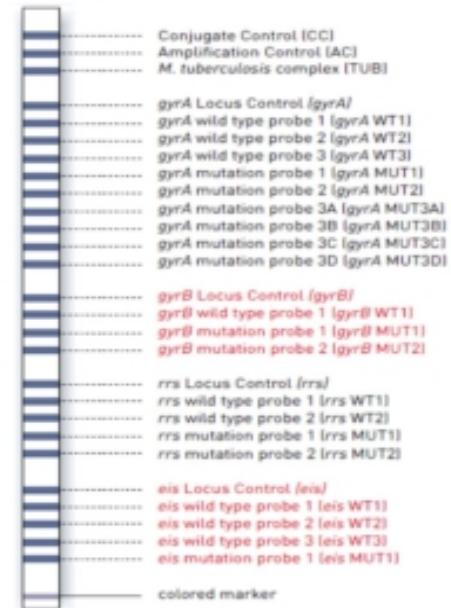
Second-line LPAs



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).



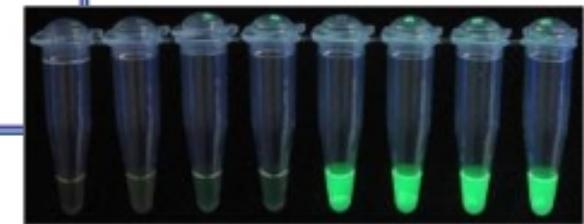
GenoType MTBDRsl VER 2.0



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



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



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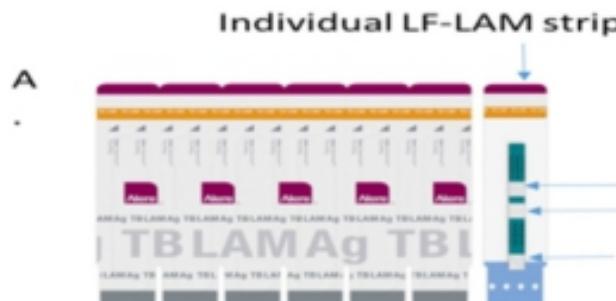
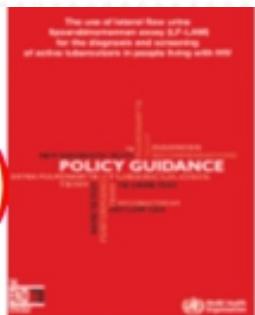


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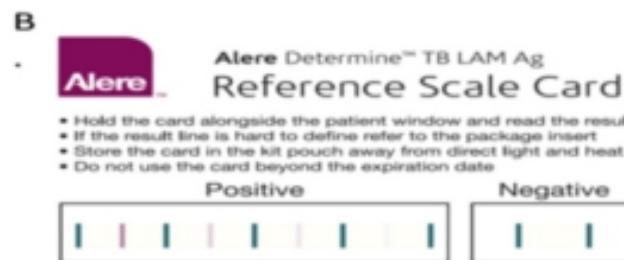
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Lateral flow- Urine Lipoarabinomannin assay (LF-LAM)



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

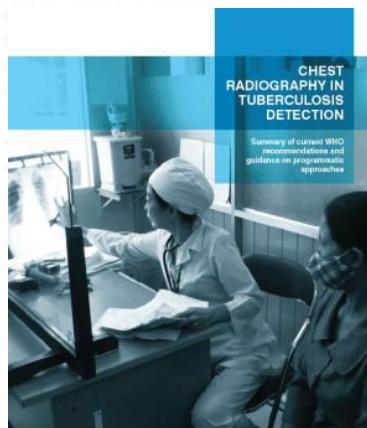


Recommended to help with the diagnosis of TB in two specific population groups:

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



Chest radiography in tuberculosis detection



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Chest radiography as a **triage tool**.
Chest radiography as a **diagnostic aid**.
Chest radiography as a **screening tool**.

- **CXR IS A SENSITIVE TOOL FOR SCREENING FOR ACTIVE TB** with higher sensitivity than screening for TB symptoms.
- **CXR CAN IMPROVE THE EFFICIENCY OF XPERT MTB/RIF USE**
- **CXR HELPS RULE OUT ACTIVE TB BEFORE TREATING LATENT TB INFECTION**
- **WHO has recommended that CAD (Computer aided detection)** should be used only as part of research designed to contribute to the required evidence base for future guideline development.



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Technical Expert Group on Xpert MTB/RIF Ultra Assay

WHO Meeting Report of a Technical Expert Committee, Reviewing the results of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF



- Ultra has a **higher sensitivity** than Xpert MTB/RIF particularly in **S-C+** specimens (**+17%**) and in specimens from HIV-infected patients with **at least as good accuracy** for rifampicin resistance detection;
- Greatest benefit was in the increased yield for the detection of MTB in **S-C+ specimens, paediatric specimens, extra-pulmonary specimens** (notably **cerebrospinal fluid**) and especially for **HIV + patients** whose specimens are frequently paucibacillary;
- The group recognized that the **impact of increased sensitivity** results in **decreased specificity** for TB detection (as with any other test) and becomes a **trade-off** between increased diagnosis and overtreatment.



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Interpretation of Ultra – *trace call* results

- Much of the **increase in sensitivity** for MTB detection with the Ultra assay was attributed to the “**trace calls**” but these represented less than 1% of all results in the study;
- A “**trace call**” positive result was sufficient to initiate therapy in those with known or suspected HIV infection, children and for extrapulmonary samples from persons;
- Performing a repeat Ultra test on a fresh sputum specimen from **adults with signs and symptoms of TB and not at risk for HIV infection** that initially tested as “**trace call**” positive would contribute to increasing the specificity of Ultra without losing much of the benefits of increased sensitivity.

WHO Meeting Report of a Technical Expert
Consultation: Non-inferiority analysis of Xpert MTB/RIF
Ultra compared to Xpert MTB/RIF



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2017



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Areas of diagnostic integrations

- **Additional Xpert assays**

- Xpert cartridges **for other diseases** and conditions can be used on the same GeneXpert instrument as for Xpert MTB/RIF
- WHO Prequalified: Xpert HIV-1 Qual assay (**EID**), Xpert Hepatitis C and **HIV Viral Load assays**
- Potential **areas for integration**: Testing site personnel, trainings, maintenance, supply systems, specimen referral, quality assurance, etc.

See *WHO Information Note on adoption and use of multi-disease testing devices* ([Download the document](http://www.who.int/tb) [on](http://www.who.int/tb) [Supranational Reference Laboratory](http://www.who.int/tb))
www.who.int/tb)



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 – *gyr A* 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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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**).

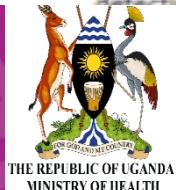
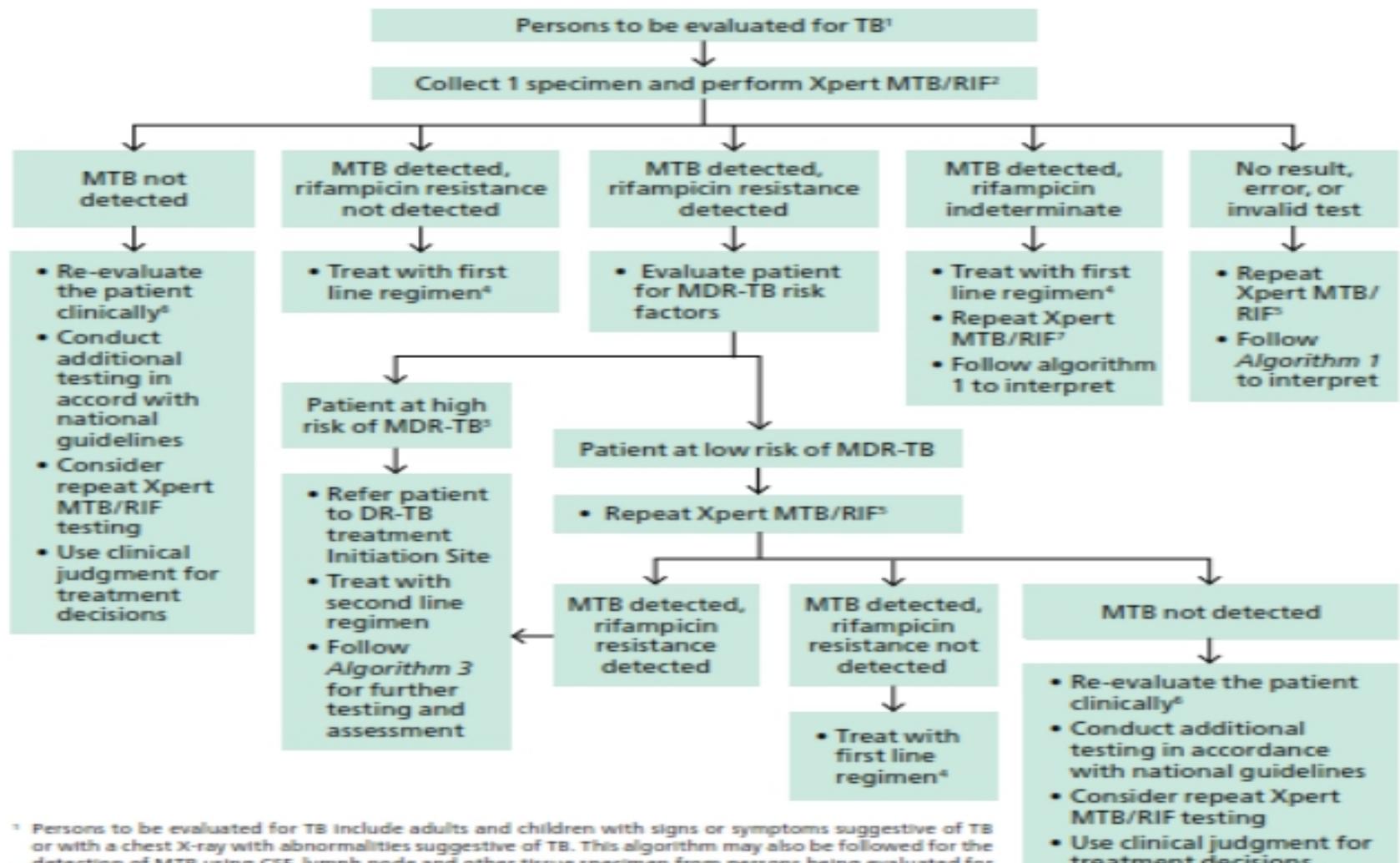
The indirect proportion method is the most common method

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

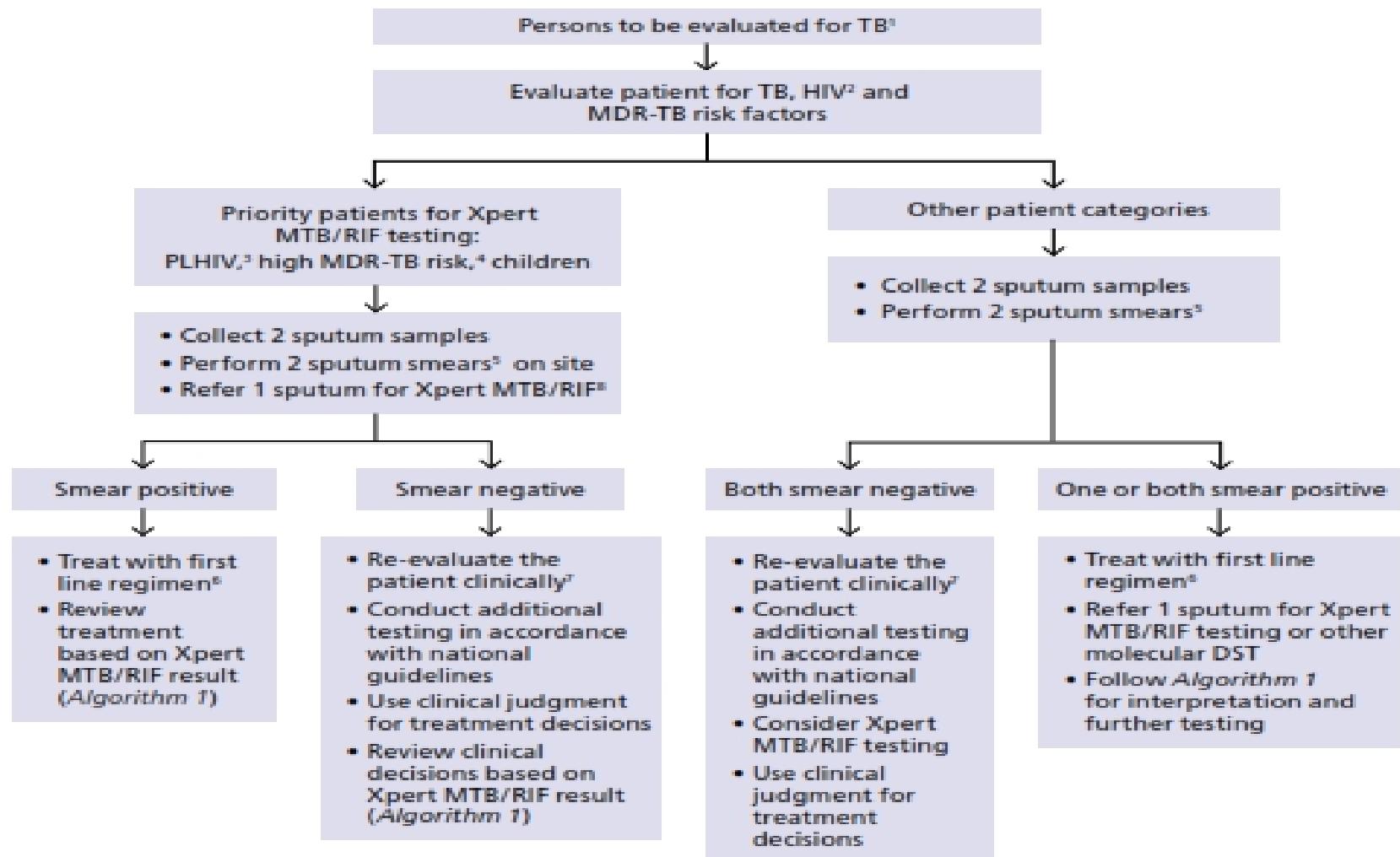
Commercial liquid culture systems for DST reduce the time to result to as little as 10 days, compared with the 28–42 days needed for DST using solid media



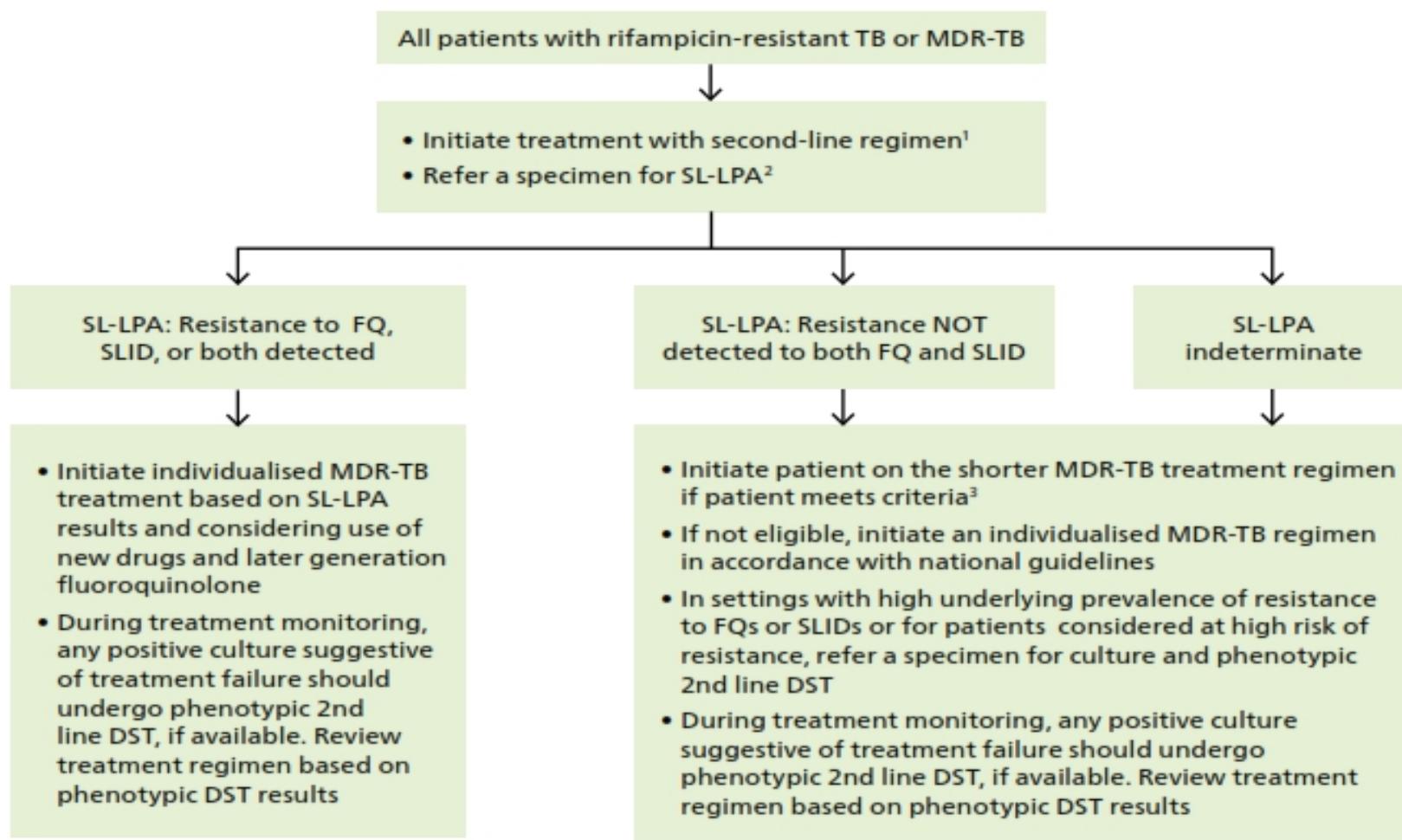
Algorithm 1: Preferred algorithm for universal patient access to rapid testing to detect MTB and rifampicin resistance



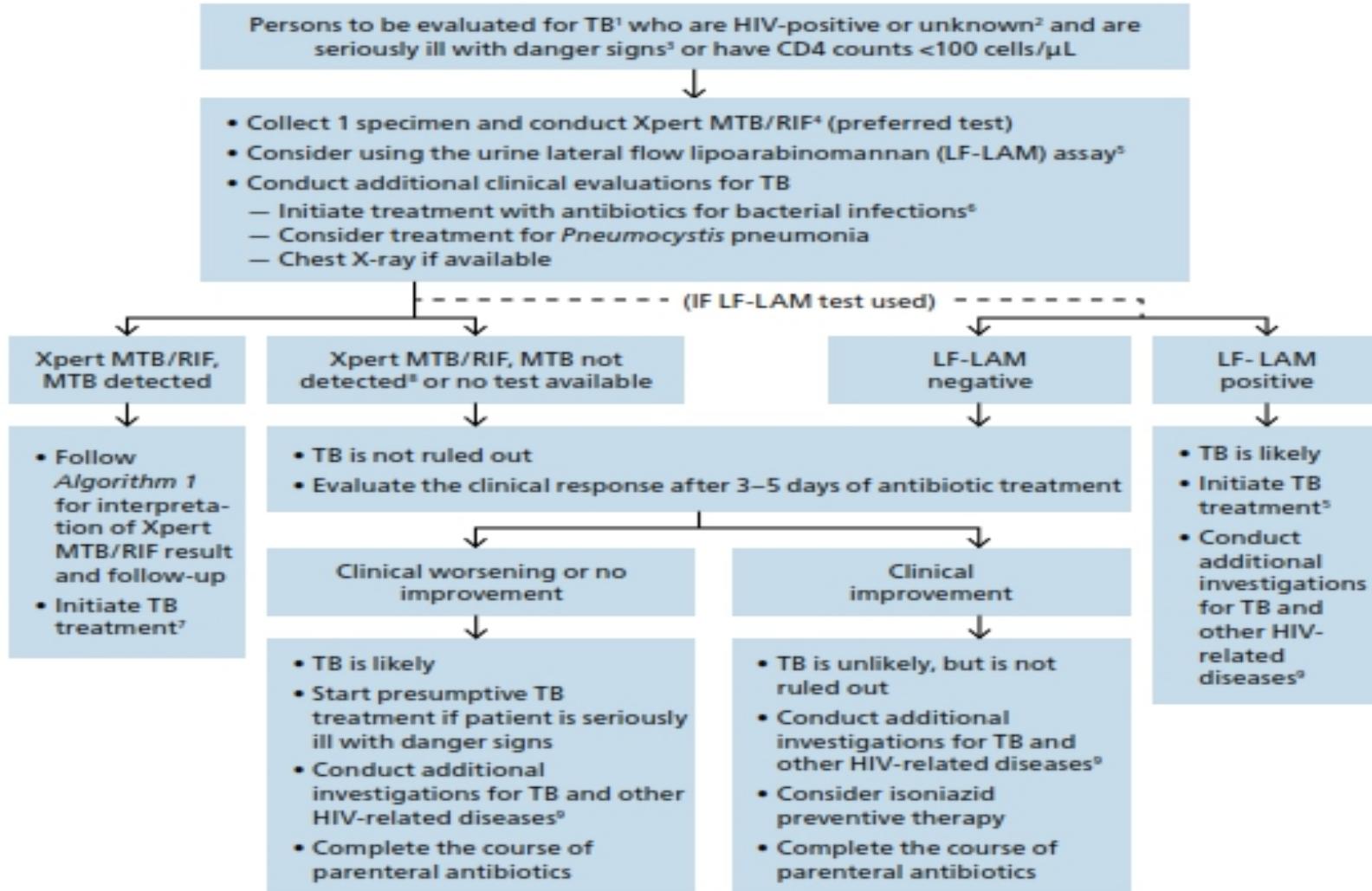
Algorithm 2: Interim algorithm moving towards universal access, with rapid testing for priority populations



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 \leq 100 cells/ μ L



GeneXpert Omni

- Small and Portable
- Durable
- Low Power Consumption
- Automatic Connectivity
- Solid State
- Integrated Battery



Evaluation the Omni instrument delayed until Quarter 2
2018



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Assessment

1. What is the role of microscopy in the diagnosis & management of TB with the advent of the rapid and more sensitive TB diagnostic assays?
2. What is the role of culture & phenotypic DST in the diagnosis & management of TB with the advent of the rapid and more sensitive TB diagnostic assays?
3. What is the WHO recommendation for the use of both the 1st & 2nd line LPA in the diagnosis of TB.
4. What are some of the limitations of the molecular TB diagnostic assays?



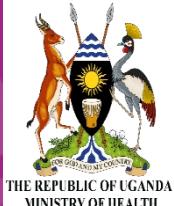
Summary

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. **Connectivity** provides opportunities for improved quality assurance and patient care
6. 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 through **laboratory strengthening**



References

- 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>



Acknowledgement



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