TAG

Treatment Action Group

AN ACTIVIST'S GUIDE TO DIAGNOSTIC TOOLS *FOR TUBERCULOSIS*

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INTRODUCTION

Tuberculosis (TB) has reclaimed its status as the world's leading cause of death from a single infectious agent, a position it held for years before being temporarily surpassed by COVID-19. In 2023, TB was responsible for an estimated 1.25 million deaths globally — a staggering toll for a disease that is both curable and preventable. That year, an estimated 10.84 million people developed TB, but only 8.16 million were diagnosed and reported, leaving a diagnostic gap of 2.7 million "missing cases."[1] These "missing cases" refer to individuals who remain undiagnosed, untreated, and/or unreported by health systems.

The TB diagnostic gap has narrowed over the past decade due to improved diagnostics and increased case detection, but it widened during the COVID-19 pandemic and remains significant, with millions still undiagnosed each year. Despite recent recovery, the persistent gap highlights ongoing challenges in TB detection and care. Notably, just ten countries accounted for over half of undiagnosed persons in 2023, with India (16%), Indonesia (11%), Pakistan (7.8%), China (6.5%), and Myanmar (6.5%) leading the list.[2]

Persistent structural, technological, and financial barriers continue to undermine global efforts in TB detection, particularly in resource-limited and high-burden settings, leaving significant gaps in timely diagnosis and care. Limited access to diagnostic services, especially in rural areas or at the **point of care**, remains a significant barrier. Many commonly used TB diagnostic tests rely on sputum samples that are difficult to obtain from certain population groups. Health systems in high-TB burden countries are often under-resourced and overwhelmed by large patient volumes and hampered by weak infrastructure, including fragile laboratory networks, frequent supply shortages, and a lack of trained personnel. Priority populations — such as children, pregnant women, and people living with HIV (PLWHIV) — are frequently overlooked in the research and development of new diagnostic tools and treatments, resulting in technologies that do not adequately address their needs. Moreover, accurate and efficient TB tests are often exorbitantly priced, making them unaffordable for national TB programs to implement at scale. The global funding crisis sparked by cuts to foreign assistance made by the United States and European governments have exacerbated this situation and threaten to further widen gaps in TB detection and care.[3]

POINT-OF-CARE: the location where a person presents to care for a disease or condition

BOX 1: ASSESSING THE ACCURACY OF A TEST

The accuracy of a test is evaluated by two key measurements: sensitivity and specificity.

- Sensitivity is how well a test correctly identifies people who have TB. It measures the proportion of true positives — meaning the test gives a positive result when someone actually has TB.
- Specificity is how well a test correctly identifies people who do not have TB. It measures the proportion of true negatives — meaning the test gives a negative result when someone actually does not have TB.

In simple terms:

- Sensitivity answers: "If someone has TB, how likely is the test to detect it?"
- Specificity answers: "If someone doesn't have TB, how likely is the test to say they don't have it?"

While sensitivity and specificity are important for understanding how accurate a test is, other factors also matter, such as: How many people can access the test? How quickly does the test provide results? Does the test use sample types that are easy for most people to provide? All these aspects together help determine how impactful a test will be in real-world settings.

The **TB cascade of care** is a complex, multistep process. Navigating this pathway is often challenging for people with TB, with substantial delays and **losses to follow-up** occurring at each stage — from the onset of symptoms and initial care-seeking, through testing and diagnosis, to treatment initiation and eventual treatment success. Notably, about half of people with TB drop out of the cascade before starting treatment, underscoring the need to improve diagnosis and linkage to treatment specifically.[4]

This Activist's Guide is designed to help readers navigate the TB diagnostic pathway and to provide TB advocates and community members with the information they need to advocate for access to the highest standard of TB diagnostic testing. Organized by steps along the diagnostic pathway (see figure 1), the Activist's Guide details the latest World Health Organization (WHO) recommendations for TB screening and diagnosis, the array of available diagnostic tools and how they should be optimally used in country programs, key access considerations, and actions activists can take to ensure that all people at risk of TB receive quality TB diagnostic testing. Table 2 provides an overview of the currently available tests for TB screening and diagnosis, along with their corresponding WHO recommendations.

TB CASCADE OF CARE: the entire pathway of TB care, starting with screening and diagnosis, then treatment, and ending with cure and post-TB treatment support.

LOSS-TO-FOLLOW-UP: when people with TB drop out of the cascade of care.

FIGURE 1: KEY ACTIONS, OUTCOMES, AND AVAILABLE TOOLS ALONG THE TB DIAGNOSTIC PATHWAY

	SCREENING	DIAGNOSIS	RESISTANCE TESTING	TREATMENT MONITORING
KEY ACTIONS	• Raise community awareness of TB • Screen all household contacts, PLWHIV, and high-risk groups for TB • Use chest X-ray for screening where possible	• Test everyone who screens positive for TB • Use mWRDs as the first test in all clinics • Ensure all clinics have have access to mWRDs	• Test all people diagnosed with TB for drug resistance • Use mWRDs to guide treatment	• Monitor everyone on TB treatment to ensure all people with TB are cured • Recognize treatment failure and appropriately changing regimens
OUTCOME	• High-risk individuals identified and referred for confirmatory testing	• Individuals diagnosed and linked to treatment + resistance testing	• Drug resistance detected + timely and optimal treatment initiated	• Relapse-free cure
AVAILABLE TOOLS	• Symptom Screening • Chest X-ray +/- CAD	• Rapid Molecular Tests (NAATs) • LF-LAM	• Molecular Tests (NAATs) • Line Probe Assays • tNGS • Culture	• TB Culture • Smear Microscopy

Adapted from WHO standard: universal access to rapid tuberculosis diagnostics[5]

PLWHIV: People living with HIV; CAD: Computer-Aided Detection; mWRDs: molecular WHO-recommended rapid diagnostic tests; NAATs: Nucleic acid amplification tests; LF-LAM: Lateral flow urine lipoarabinomannan assay; DST: Drug-susceptibility testing; tNGS: Targeted next-generation sequencing

This 2025 update to *An Activist's Guide to TB Diagnostic Tools* builds on the previous version released in 2020, adding classes of tests and specific technologies that have since been reviewed and endorsed by the WHO. Updates include the use of computer-aided detection software for digital chest X-rays used for TB screening, molecular diagnostic tests now categorized by level of complexity and automation, targeted next-generation sequencing for detection of drug resistance, and new concurrent testing of multiple sample types and tests to improve diagnosis of TB among children and PLWHIV of all ages.

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1. TB SCREENING

TB screening is designed to identify individuals who may have **active TB** disease, enabling timely diagnostic evaluation and treatment initiation. Screening methods include symptom assessment and chest X-ray. Screening may be integrated into routine care at health facilities or conducted in communities, similar to screening for noncommunicable diseases like diabetes or hypertension. The goal is to detect TB early, especially among populations at increased risk, to reduce transmission and improve outcomes.

ACTIVE TB: TB that actively reproduces in thebody, causes tissue damage that makes people sick, and is capable of being transmitted from one person to another.

1.1. Who to Screen

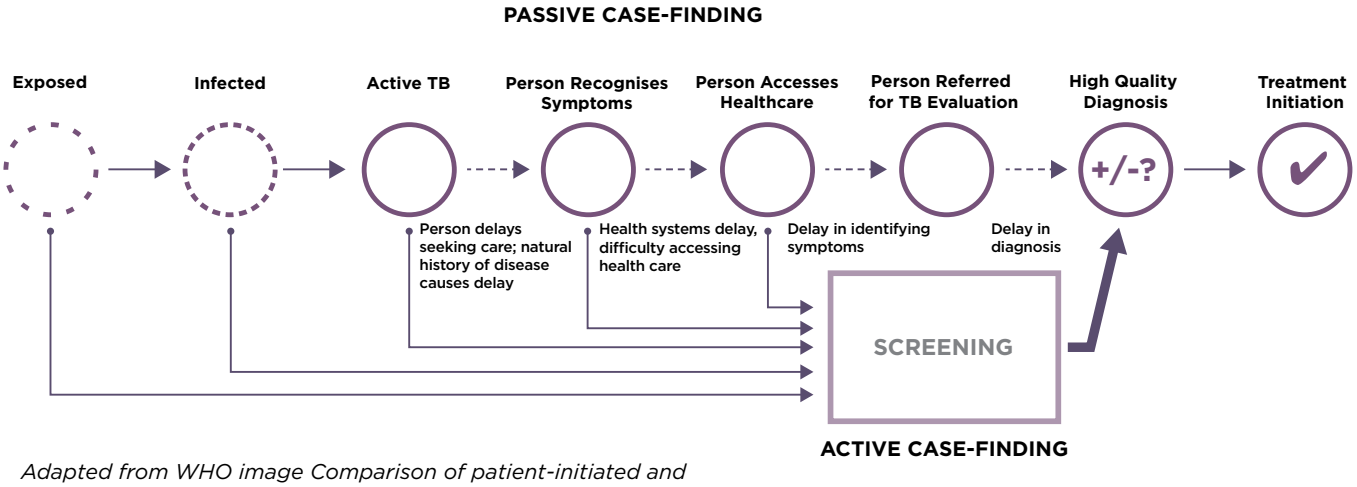
Screening should prioritize people at high risk for TB. A positive screening result is not a diagnosis and must be followed by confirmatory diagnostic testing and evaluation. High-risk groups include:

- Household and close contacts of people with active TB
- PLWHIV
- Individuals in prisons or detention centers
- Miners exposed to silica dust
- People with clinical risk factors such as diabetes, chronic lung disease, malnutrition, or a history of TB
- Populations with limited access to healthcare, such as those living in urban slums, refugees, migrants, and unhoused individuals

The WHO recommends systematic screening among high-risk groups and in the general population (in addition to high-risk groups) in settings where the prevalence of TB is 0.5% or higher.[6] Systematic screening is an organized, proactive approach targeting individuals who belong to high-risk groups or live in settings with high TB prevalence, regardless of symptoms. Another term for this is "active case finding," which includes **contact tracing** and outreach in communities.[7] In contrast, passive case finding depends on people seeking medical care typically after they develop symptoms, which usually appear when TB is already quite advanced. Figure 2 lays out the comparison between active case finding (initiated by providers) and passive case finding (initiated by patients).

CONTACT TRACING: The process of identifying and notifying people who have been in close contact with someone diagnosed with TB to provide testing and treatment or preventive therapy as needed.

FIGURE 2: ACTIVE VERSUS PASSIVE CASE FINDING



Adapted from WHO image Comparison of patient-initiated and provider-initiated screening pathways for the diagnosis and treatment of tuberculosis (TB)[8]

1.2. How to Screen

Tools and algorithms used for screening depend on the target population, setting, and available resources. The main WHO-recommended screening tools are:

- Symptom Screening: Involves evaluating for classic symptoms of pulmonary TB, such as cough (of any duration), night sweats (drenched), weight loss, fever (low grade), and hemoptysis (coughing up blood).[9] A positive symptom screen typically prompts further evaluation, such as chest X-ray or diagnostic testing.
- Chest X-Ray: Chest X-rays, or **radiographic imaging**, produce an image of the internal structures of the lungs to enable the identification of any lung abnormalities that may be suggestive of TB. On the chest X-ray images, air in the lungs appears as black space and lung abnormalities (such as lesions caused by TB) appear as gray or white shadows.

RADIOGRAPHIC IMAGING: an imaging technique that projects small amounts of radiation through the body to produce an image of its internal structures (e.g.,X-ray).

Symptom screening has low sensitivity and will miss people with asymptomatic TB and/or people with early TB disease who have yet to develop symptoms; symptoms could also be the result of another illness. Chest X-rays are highly sensitive for detecting lung abnormalities suggestive of TB but are not very specific, as other diseases can produce similar abnormalities. Therefore, positive symptom screens or abnormal X-ray findings both require confirmatory diagnostic testing.

Since 2021, WHO recommends the use of computer-aided detection (CAD) software for automated interpretation of digital chest X-rays among people aged 15 years and older.[10] CAD leverages artificial intelligence (AI) to help identify TB-related abnormalities and is particularly useful in places where there are few trained radiologists.

There are multiple types of proprietary CAD software available that can automatically read digital chest X-rays and determine the likelihood of TB disease. This information is then used by health workers to determine whether a patient should be referred for further TB diagnostic evaluation.

CAD performance is comparable to that of human readers for TB detection, so the WHO recommends CAD as an alternative to human interpretation of digital chest X-ray for screening for TB. However, specialist interpretation remains important for complex cases (refer to section 7.1 to learn more about what makes a case complex). Further research into developing CAD software that performs well in young children (<15 years) is a diagnostic research priority.

LEARN MORE ABOUT THE AVAILABLE CAD PRODUCTS ON STOP TB PARTNERSHIP'S RESOURCE CENTER AI4HLTH.ORG.

2. TB DIAGNOSIS

The WHO recommends that those who screen positive for TB — they have an abnormal chest X-ray and/or signs and symptoms suggestive of TB — should be further evaluated with a TB diagnostic test.[11] Diagnosis is the confirmation that a person has active TB and results in the clinical decision to initiate treatment. Diagnosis can be determined following a **bacteriological confirmation**, which detects the presence of TB bacteria in a sample using a WHO-recommended diagnostic test. Or, in some cases, diagnosis can be made and treatment can be initiated solely through clinical judgment based on signs and symptoms and other risk factors of TB — even in the absence of a positive TB diagnostic test

BACTERIOLOGICAL CONFIRMATION: detecting the physical presence of TB bacteria in a given sample.

result. This is especially important for children and PLWHIV, for whom current tests aren't as accurate. Following a positive TB diagnosis, further **drug-susceptibility testing** is required to identify the most appropriate drugs or regimens for treatment.

DRUG-SUSCEPTIBILITY TESTS (DST): tests used to determine resistance to TB drugs.

BOX 2: MOLECULAR TESTS — NUCLEIC ACID AMPLIFICATION TESTS — EXPLAINED

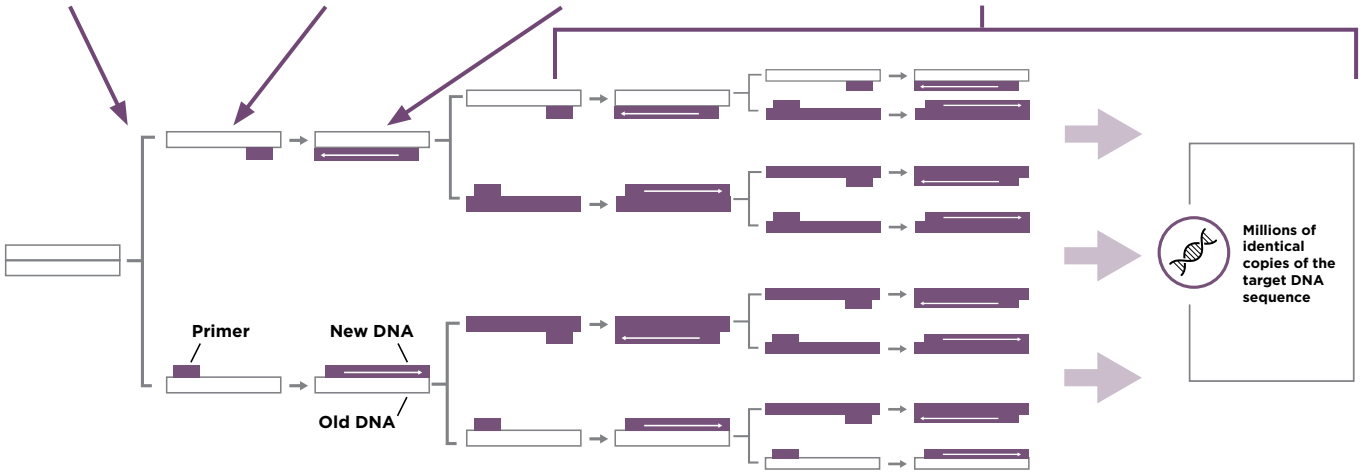
The most commonly used technologies for bacteriological confirmation of TB disease are molecular tests or nucleic acid amplification tests (NAATs). NAATs detect the presence of ***Mycobacterium tuberculosis (MTB)*** bacteria in a sample by extracting and amplifying the bacteria's genetic material (DNA), even if there's only a small amount present. The sample is first collected from the patient. Typically, this is a **sputum** sample, but other sample types can be collected, especially if the clinician wants to test for TB outside the lungs (referred to as extrapulmonary TB). The sample is then prepared and the DNA of the bacteria is extracted. The extracted DNA is then amplified, meaning many copies are made using techniques like polymerase chain reaction (PCR). Figure 3 visually demonstrates the PCR technique, which uses a series of controlled temperature changes to create copies of the DNA of the bacteria. The amplified DNA is then detected, indicating the presence of the TB bacteria. NAATs are highly sensitive and specific tests that can produce results within a few hours. Many NAATs may be used to simultaneously detect both TB and the presence or absence of mutations associated with resistance to certain drugs.[12]

MYCOBACTERIUM TUBERCULOSIS (MTB): The full name of the bacteria that causes TB.

SPUTUM: a mixture of saliva and mucus that is coughed up from the lungs.

FIGURE 3: POLYMERASE CHAIN REACTION (PCR) TECHNOLOGY

STEP 1	STEP 2	STEP 3	STEP 4
Double-stranded DNA separates into single strands when the temperature is raised to 95°C.	Fragments of DNA called primers attach to the target DNA sequence of the single-stranded DNA when the temperature is lowered to 60°C.	DNA polymerase enzymes complete the double strand of the target DNA sequence when the temperature is raised to 72°C, resulting in two identical copies.	This process of temperature change is repeated about 40 times, resulting in millions of identical copies of the target DNA sequence.



There are multiple tests that can be used to diagnose TB. These can be organized into three categories:

1. Initial tests for TB diagnosis with drug-resistance detection.
2. Initial tests for TB diagnosis without drug-resistance detection.
3. Follow-on tests for detection of TB drug resistance.

Each category contains multiple classes of TB diagnostic technologies, and each class contains one or more test(s). Tests in the same class share a purpose (e.g., detection of TB and rifampicin resistance), mechanism of action (e.g., NAAT), level of complexity, method of reporting a result, and setting of use (see figure 4). New tests are rapidly assessed by the WHO Global TB Program (WHO GTB) to determine whether they belong to an existing diagnostic class or if a new class is needed. If the new test belongs to an existing class, it can be referred for WHO Prequalification (WHO PQ). WHO PQ evaluates individual health products, including TB diagnostics, to ensure they meet international standards for quality, safety, and effectiveness. Only products that pass this rigorous assessment are prequalified and WHO-recommended for global procurement. If a prequalification assessment procedure is not yet available for an existing class of tests, the WHO GTB assesses new within-class products and releases updated policy guidance on their use. Alternatively, if a new test does not belong to an existing class, then WHO GTB conducts a first-in-class evidence assessment. If recommended by WHO GTB, the new class is established with the new testing product, which is then referred for WHO PQ.[13]

WHO GTB recommendations hold until the prequalification assessment procedures are available and successfully completed. This means that countries can procure and begin using new tests recommended by the WHO GTB even if they haven't yet been evaluated by WHO PQ. This approach allows new tests to be categorized, assessed, and introduced more rapidly.

FIGURE 4: COMPLEXITY OF THE CLASSES OF TB TECHNOLOGIES

COMPLEXITY	LC-ANAAT	LC-MNAAT	LF-LAM	MC-ANAAT	LINE PROBE ASSAYS	TNGS
Equipment & Infrastructure	Basic laboratory; Minimal equipment; basic power supply; no special infrastructure required	Basic laboratory; Manual assay; basic power supply; no special infrastructure required	No equipment or laboratory infrastructure; true point-of-care test	Specialized machines and infrastructure; stable power requirements; manual specimen preparation	Molecular laboratory; special infrastructure required;	Centralized, molecular laboratory; special infrastructure
HR Skill Level	Basic technical skills (e.g. basic pipetting, precision not critical)	Basic technical skills (e.g. basic pipetting, precision not critical)	Minimal training (health workers); potential use as a self-test	Moderate technical skills (i.e., multiple sample or reagent handling steps, precision pipetting and molecular workflows may be required)	Advanced technical skills (i.e., multiple sample or reagent handling steps, precision pipetting, molecular workflows may be required)	Highly skilled lab personnel with specialized skills
Results Reporting	Automated	Automated or manual	Manual; visual interpretation of results	Automated	Manual	Automated
WHO-recommended products	Xpert MTB/RIF Ultra, Xpert MTB/XDR (Cepheid), Truenat MTB, MTB Plus and MTB-RIF Dx (Molbio)	TB LAMP (Eiken Chemical)	Determine TB LAM Ag (Alere/Abbott)	RealTime (Abbott), BD MAX (Becton Dickinson), Cobas (Roche), FluoroType (Hain Lifesciences)	GenoType MTBDRplus, GenoType MTBDRsl (Hain Lifescience), Genoscholar NTM+MDRTB II, Genoscholar PZA TB II (Nipro)	Deeplex Myc-TB (GenoScreen), amPORE TB (Oxford Nanopore), TBseq (ShenTing)

Adapted from WHO Consolidated guidelines on tuberculosis: module 3: diagnosis: rapid diagnostics for tuberculosis detection, 3rd ed.[14]
LC-aNAAT: Low-complexity automated NAATs; LC-mNAAT: Low-complexity manual NAATs; LF-LAM: Lateral Flow urine lipoarabinomannan assay; MC-aNAAT: Moderate-complexity automated NAATs; tNGS: Targeted next-generation sequencing; HR: Human resource

2.1. Initial Tests for TB Diagnosis with Drug-Resistance Detection

2.1.1. Low-Complexity Automated NAATs

The WHO recommends using low-complexity automated NAATs — namely GeneXpert (Cepheid) and Truenat (Molbio) — as initial tests for TB and detecting rifampicin resistance. Low-complexity automated NAATs are usually automated tools that can be placed in decentralized laboratories, enabling testing at the peripheral levels of the healthcare system. The testing instruments use software and hardware (such as computers) to report results, and they require basic laboratory infrastructure with stable power supplies and temperature control but without other specialized equipment. They also require basic technical skills to operate. These tests are ideal for resource-limited settings with basic laboratory infrastructures in place.[15]

Xpert MTB/RIF Ultra (Cepheid, USA)

Xpert MTB/RIF Ultra is a rapid, automated tests that can diagnose TB and detect resistance to rifampicin, one of the most important TB drugs. The WHO recommends Xpert Ultra as the initial test for TB and rifampicin resistance for all people being evaluated for pulmonary TB. The test is also recommended for use in adults and children with signs and symptoms of extrapulmonary TB.

Xpert Ultra has high sensitivity, meaning it can find TB bacteria when it is present in a sample at very low quantities. It can also test for TB using many different sample types, making it useful for a wide range of patients. The most common sample is sputum, but the tests can also use samples like gastric aspirate (fluid taken from the stomach, often used for children who can't cough up sputum), stool, and nasopharyngeal aspirate (fluid from the back of the nose and throat). For people presumed to have extrapulmonary TB, Xpert can be used on fluids from around the brain and spinal cord (cerebrospinal fluid), lymph node samples, and other body fluids such as pleural (lung), peritoneal (abdomen), or pericardial (heart) fluid. This flexibility helps doctors diagnose TB in adults, adolescents, and children who may experience different forms of TB disease, including those who may not be able to provide sputum samples. However, one shortcoming is that the test has imperfect specificity, which means it can sometimes give a positive result when active or live TB isn't present, especially in people who have previously recovered from TB or are currently improving on treatment (which is why these tests are not recommended for treatment monitoring — more on this in section 4).

The Xpert Ultra test is a cartridge-based, fully automated test that is run on GeneXpert instruments that require continuous electricity, limiting its use to basic laboratories at the peripheral health center level. The tests cost US$7.97 per test and provide results for both TB and rifampicin resistance in under two hours.

Truenat MTB Plus and MTB-RIF Dx (Molbio, India)

Truenat MTB Plus and MTB-RIF Dx are rapid, portable molecular tests that can diagnose TB and detect resistance to rifampicin through **reflex testing**. In 2020, the WHO-recommended Truenat MTB Plus and MTB-RIF Dx as an initial test for TB and rifampicin resistance for all people being evaluated for pulmonary TB.[16] The WHO approval of Truenat tests introduced much-needed competition into the global market for rapid molecular tests, which was dominated by Cepheid between 2010 and 2020.

REFLEX TESTING: if an initial test finds TB bacteria, an additional test is done on the same sample to check if the TB is resistant to certain drugs.

The Truenat system includes a sample preparation device and a real-time PCR analyzer. Both instruments can be optionally battery-operated. Unlike Xpert tests, which simultaneously test for TB and rifampicin resistance, Truenat uses separate test chips for TB and rifampicin resistance, which are tested sequentially (referred to above as reflex testing). Truenat MTB Plus chips detects the presence of TB bacteria, while the MTB-RIF Dx chip detects rifampicin resistance. Upon positive results for TB, the same sample may be added to the MTB-RIF Dx chip

to test for rifampicin resistance. The entire testing process, including the reflex testing for resistance, takes two hours (similar to Xpert Ultra), and costs US$7.90.

Truenat tests are semi-automated and lightweight, can be battery-operated (for up to eight hours), and are designed for use in places with basic laboratories (at the peripheral level) and minimal infrastructure. These design features enable use at even lower levels of the healthcare system than is possible with Xpert Ultra, expanding access to rapid molecular tests to more remote and resource-limited settings.[17] However, Truenat does have some limitations. The test is currently validated only for sputum samples and is not recommended for extrapulmonary TB or for use with other specimen types, unlike the Xpert platform, which has broader validation for various sample types for children and extrapulmonary TB.

The Truenat MTB Plus/RIF tests by Molbio (India) and the Xpert Ultra tests by Cephied (USA) are comparable in price per test ($7.90 for Truenat and $7.97 for Xpert), time to result (~two hours), and placement and infrastructure requirements (basic laboratories). Both tests are recommended for use in the concurrent testing strategies for the initial detection of TB among PLWHIV and children (read more about concurrent testing strategies in section 7.2). However, while Truenat's accuracy for detecting TB is generally comparable to Xpert MTB/RIF, Xpert Ultra has demonstrated higher sensitivity, especially in challenging cases, for example where the amount of TB bacteria in the sample is too low to be detected by conventional tests (which is common for children and PLWHIV).[18]

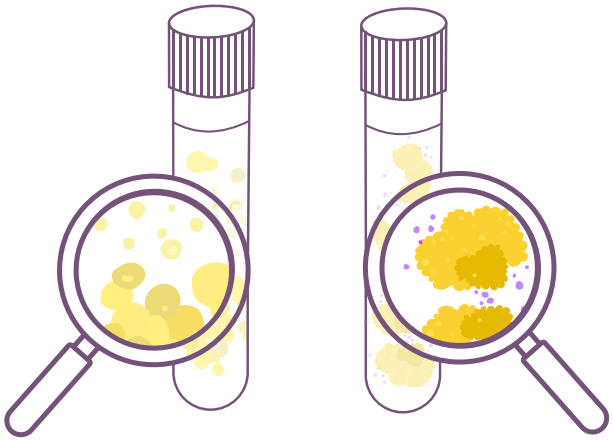
2.1.2. Moderate-Complexity Automated NAATs

Current moderate-complexity automated NAATs can also be used as an initial test for the detection of TB and resistance to rifampicin and isoniazid (simultaneously) and for follow-on testing for fluoroquinolones (FQs; specifically levofloxacin). These tools are categorized as moderate complexity because the sample preparation stage requires a higher level of technical skill than is required for Xpert or Truenat tests. The NAATs that fall under this category run on larger instruments that can test multiple samples simultaneously (sometimes referred to as high throughput instruments), offering the potential for rapid provision of accurate results. This makes these tests well suited for use in areas with high population density and/or rapid sample referral systems.[19] They require well-established laboratory infrastructure, which usually entails test-specific equipment and skilled personnel with training in laboratory procedures. Moderate-complexity NAATs recommended by the WHO are RealTime MTB and RealTimeMTB RIF/INH (Abbott), BD MAX MDR-TB (Becton Dickinson), cobas MTB and cobas MTB-RIF/INH (Roche), and FluoroType MTB and Fluorotype MTBDR (Hain Lifesciences/ Bruker).

2.1.3. TB Culture

In some cases, TB culture is also used for TB diagnosis. TB culture is a phenotypic test that physically grows TB from patient samples using a liquid or solid media. To conduct phenotypic testing, the sample (typically sputum) is first collected and put into a tube or onto a plate with a substance that helps the bacteria grow. This substance is called culture medium. The sample is then incubated under controlled conditions to promote bacterial growth, after which the culture is visually inspected to confirm the presence or growth of TB. Figure 5 depicts a culture test with TB bacteria visible in it.

FIGURE 5: A VISUAL REPRESENTATION OF TB GROWING IN CULTURE



Culture is widely regarded as the "gold standard" for TB diagnosis due to its high specificity (around 98–99%) and considerable sensitivity (typically 80–93%). Liquid culture is commonly used as the microbiological reference standard (MRS) for most test accuracy studies, making it the reference method against which the performance of other TB diagnostic tests are evaluated. Additionally, culture with drug compounds added is also used to test for drug resistance by determining whether TB grows in the presence of the drug (resistant) or not (not resistant). This makes culture a critical tool for effective TB management.

However, the major limitation of culture is the lengthy turnaround time — results for TB detection can take anywhere from two to six weeks because TB grows very slowly. Culture-based drug-resistance testing takes even longer because positive, pure TB samples need to be available to set up new culture tests that include TB drugs. This delay can be problematic, as patients may continue to transmit TB and experience disease progression while awaiting results, leading to delayed treatment initiation and potentially worse outcomes, including increased risk of **treatment failure** and drug resistance. For these reasons, while culture remains an essential tool, especially for drug-resistance testing, NAATs are the preferred initial tests for diagnosing TB.

TREATMENT FAILURE: the persistence of TB disease despite treatment.

2.2. Initial Tests for TB Diagnosis without Drug-Resistance Detection

2.2.1. Low-Complexity Manual NAATs

TB-LAMP

The WHO also recommends loop-mediated isothermal amplification (TB-LAMP) as an initial test for TB diagnosis. TB-LAMP is classified as a low-complexity manual NAAT (LC-mNAAT) and is a test-tube-based molecular test appropriate for use in basic laboratories at the peripheral level of the healthcare system. TB-LAMP targets bacterial DNA (as explained in highlight box 2) and employs a loop-based nucleic acid amplification technique to multiply DNA sequences to enable TB detection. The loop-based amplification technique is different from the PCR technique used by Xpert and Truenat because it is done at a single temperature. Another difference is that the detection of TB is done visually, using an ultraviolet (UV) lamp. The method requires only

basic equipment and can be implemented at the peripheral levels of the health system. However, the current technology cannot detect the genetic mutations that cause drug resistance, so the tool cannot be used for drug-susceptibility testing. TB-LAMP is less expensive (US$6) than other NAATs, and it takes just 40 minutes to produce a result, but it also has lower sensitivity for TB detection.[20] Given the lower sensitivity and the inability to test resistance to first-line drugs, the WHO only recommends the use of TB-LAMP as an initial test for the detection of TB in areas with low prevalence of drug-resistant TB.

2.2.2. Antigen-Based Lateral Flow Tests

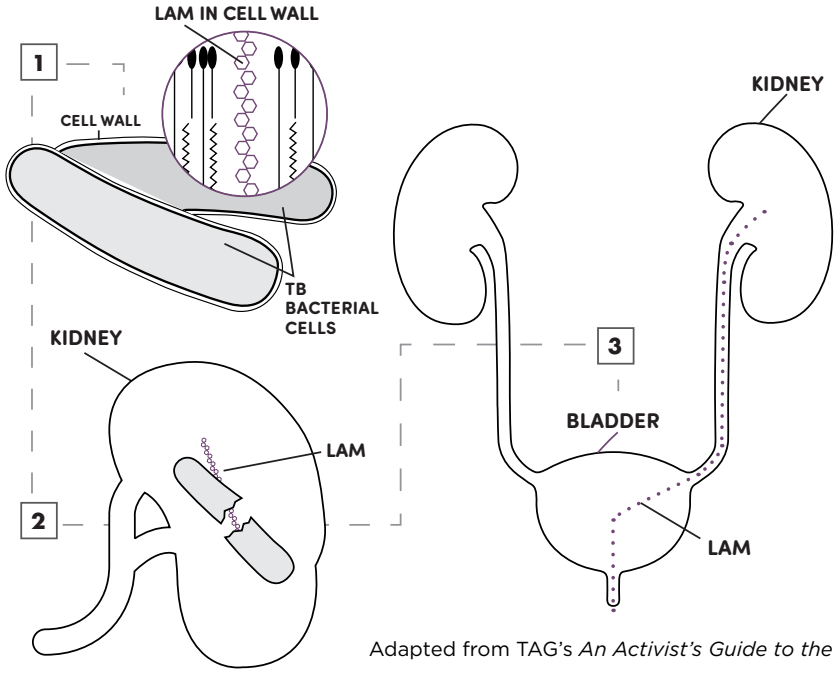
LF-LAM

The urine **lipoarabinomannan (LAM)** test is a rapid, instrument-free point-of-care TB test currently only recommended by the WHO for use in PLWHIV. It is unique in that it uses urine — a sample that is easy to collect, especially from people with advanced HIV or AIDS who may have trouble producing sputum. The urine LAM test is the only TB diagnostic shown in clinical trials to reduce mortality by enabling faster diagnosis and earlier initiation of treatment among PLWHIV, especially those with advanced disease.[21] The **antigen**-based test detects the presence of LAM, a component of the TB bacteria's cell wall, which is released into the urine when TB spreads throughout the body, particularly in people with advanced immunosuppression. Figure 6 depicts how LAM is detected in urine.

LIPOARABINOMANNAN (LAM): a component of the outer cell wall of TB bacteria. Its presence shows that TB bacteria are in the body, so detecting LAM can help identify people who have TB.

ANTIGENS: molecules or components of a pathogen such as bacteria that induce an immune response.

FIGURE 6: DETECTING LAM IN URINE



Adapted from TAG's *An Activist's Guide to the LAM Test*[22]

The WHO recommends using the Abbott Determine TB LAM Ag test — the only available LAM test — for diagnosing TB in PLWHIV. This rapid urine-based test helps quickly identify TB in this high-risk group when other tests may be less effective. The WHO first recommended this test in 2015 for PLWHIV who have advanced disease but expanded its guidance in 2019 to include all PLWHIV with TB symptoms, serious illness, or advanced immunosuppression (CD4 count below 200 cells/mm^3 for inpatients and below 100 cells/mm^3 for outpatients), covering adults, adolescents, and children.[23] The test does not have any laboratory or equipment requirements. It is also simple to use, similar to a pregnancy test: a small amount of urine is applied to a paper strip, and results are available in about 25 minutes. The darkness of the line on the strip indicates a positive result, with darker lines linked to higher risk of death. The Abbott LAM test is affordable, costing about US$3.70 per test, and is cost-effective for health systems.[24] Its sensitivity is highest in people with advanced HIV, increasing as CD4 counts decrease. The currently available LAM test is only recommended for PLWHIV and as a component of a concurrent testing approach (learn more about concurrent testing in section 7.2).

3. FOLLOW-ON TESTS FOR DETECTION OF TB DRUG RESISTANCE

Drug-resistant TB (DR-TB) is a form of TB disease that is caused by strains of TB that have mutated or changed in a way that helps them escape or resist being killed by a specific drug or multiple drugs. Drug resistance can be transmitted and/or develop over time as a result of inadequate or irregular TB drug exposures, caused by factors such as incorrect prescription by healthcare provider, poor quality drugs resulting in inadequate drug levels or exposures, drug shortages resulting in treatment interruption or discontinuation, and/or lack of adherence to the treatment (which is common due to lack of counseling and the toxicity and side effects of some medications). Transmitted resistance is the primary form of resistance and is more common than secondary resistance, which is developed.

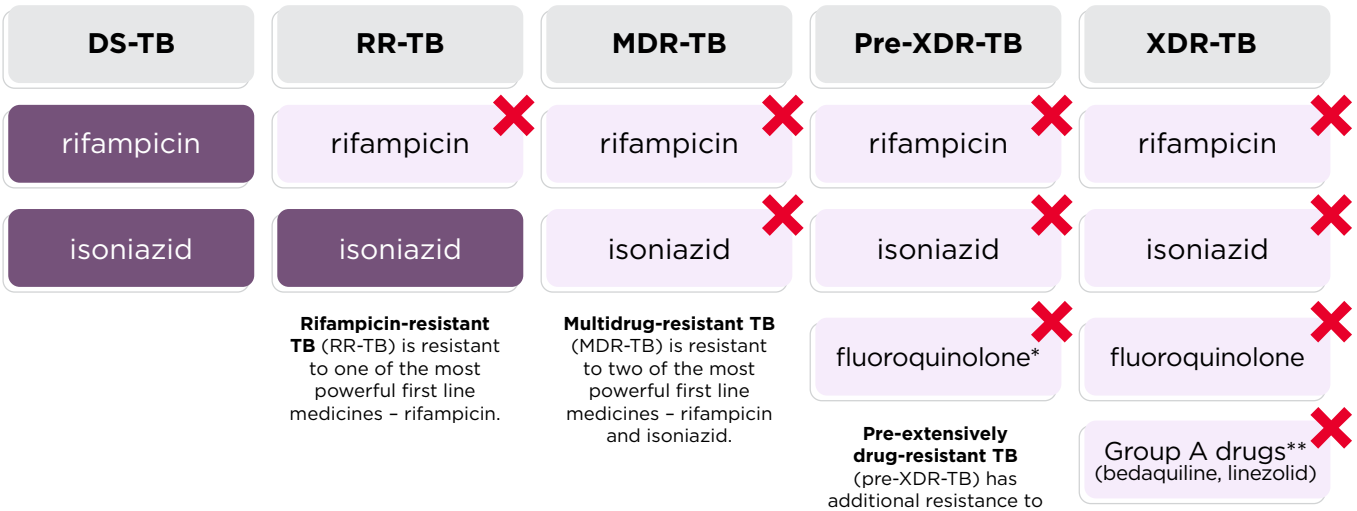
DR-TB is generally defined in the context of treatment regimens and separated into four groups defined by the medicine(s) to which TB bacteria are resistant. Figure 7 demonstrates the resistant drugs under each group.

- Drug susceptible-TB (DS-TB) is when the TB bacteria is sensitive to the two most powerful first-line medicines — rifampicin (RIF) and isoniazid (INH).
- Rifampicin-resistant TB (RR-TB) is when the TB bacteria is resistant to RIF.
- Multidrug-resistant TB (MDR-TB) is when the TB bacteria is resistant to both RIF and INH.
- Pre-extensively drug-resistant TB (Pre-XDR-TB) is when the TB bacteria is resistant to RIF, INH, and at least one FQ (moxifloxacin, levofloxacin).
- Extensively drug-resistant TB (XDR-TB) is when the TB bacteria is resistant to RIF, INH, FQs, and bedaquiline (BDQ) and/or linezolid (LZD).

Drug-susceptibility testing (DST) can be performed **genotypically** using molecular tests or phenotypically using culture. Genotypic DST methods include NAATs, line probe assays, and targeted next-generation sequencing. Accurate and timely DST is essential to ensure that a person diagnosed with TB receives an appropriate treatment regimen with drugs that will work against their strain of TB. For additional information about DR-TB and how it is treated, see ***An Activist's Guide to Shorter Treatment for Drug-Resistant Tuberculosis***.[26] The WHO calls for universal DST, which means that all individuals with bacteriologically confirmed TB should receive DST for at least RIF, with additional follow-up DST as needed. In practice, this means that all individuals are tested at least for rifampicin resistance alongside their initial test to confirm their TB diagnosis.

GENOTYPIC TESTING: detects TB bacteria by analyzing their genetic material (DNA) in the lab and can also identify genetic changes linked to drug resistance.

FIGURE 7: DRUG-RESISTANT TB IN THE CONTEXT OF TREATMENT REGIMENS



Rifampicin-resistant TB (RR-TB) is resistant to one of the most powerful first line medicines – rifampicin.

Multidrug-resistant TB (MDR-TB) is resistant to two of the most powerful first line medicines – rifampicin and isoniazid.

Pre-extensively drug-resistant TB (pre-XDR-TB) has additional resistance to the fluoroquinolones (i.e., moxifloxacin, levofloxacin).

Extensively drug-resistant TB (XDR-TB) has additional resistance to the fluoroquinolones AND at least one other group A drug (i.e., bedaquiline, linezolid).

Adapted from TAG's Activist's guide to shorter treatment for drug-resistant tuberculosis[25]

**Fluoroquinolone drugs refer to moxifloxacin, levofloxacin*

***Group-A drugs refer to bedaquiline and/or linezolid*

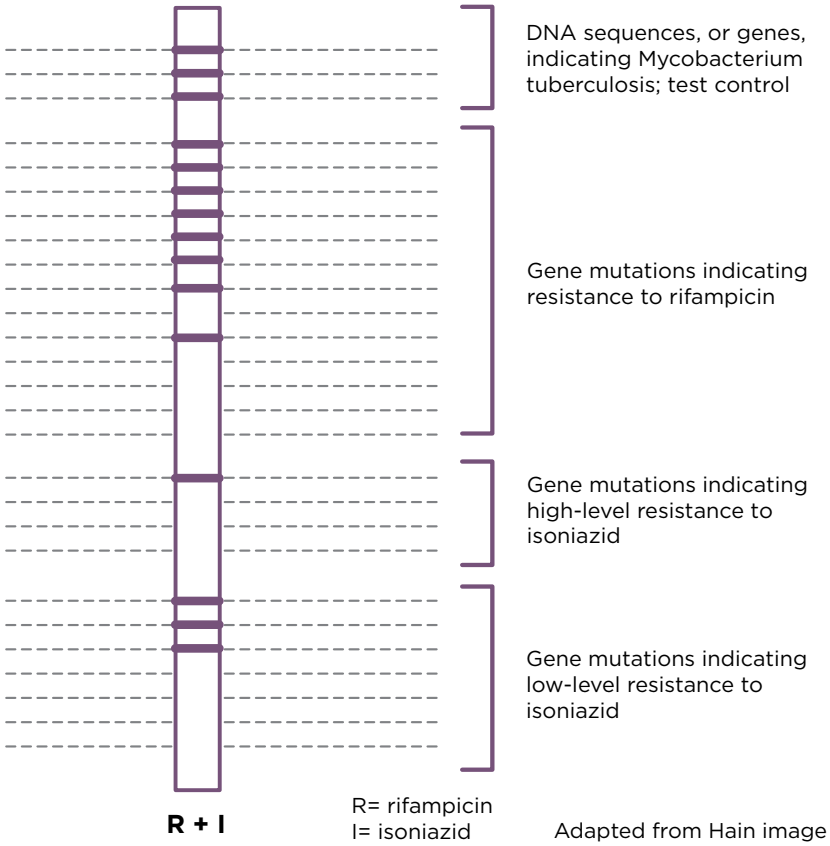
3.1. Low-Complexity Automated NAATs

There is also a low-complexity automated NAAT option to detect resistance to INH and FQs in people with bacteriologically confirmed pulmonary TB, which is Xpert MTB/XDR (Cepheid). This test was WHO-recommended in 2021 as a follow-on test to detect resistance to INH and FQs in people with TB, as well as ethambutol (ETH) and amikacin (AMK) in people with rifampicin-resistant TB. Xpert MTB/XDR uses a single cartridge to analyze sputum samples that have already tested positive for TB and RR-TB, delivering results in under 90 minutes. These tests can be performed at peripheral healthcare centers (at the same level as Xpert Ultra and Truenat tests). The assay can differentiate between low- and high-level resistance to INH and FQs. Low-level resistance means the TB bacteria can survive lower doses of a drug, while high-level resistance means they can survive even the highest safe doses — knowing the level of resistance helps healthcare providers choose the most effective treatment and decide if higher drug doses might still work or if a different drug is needed. However, the high cost of the test — US$14.90, nearly double the price of the Xpert MTB/RIF Ultra — limits its widespread scale-up and adoption in countries. The test also requires a newer model GeneXpert instrument or an expensive upgrade to older model GeneXpert instruments that countries currently have in place. The Xpert MTB/XDR assay has demonstrated high sensitivity and specificity for detecting resistance to these drugs, and its rapid provision of results can significantly improve clinical decision-making and reduce delays in starting appropriate treatment for drug-resistant TB.[27]

3.2. Line Probe Assays

Line probe assays (LPAs) were recommended by the WHO in 2016 as the initial DST for first- and second-line TB drugs. These molecular tests use PCR to amplify TB bacterial DNA and then use a strip-based technology with binding patterns that indicate the presence of certain genetic changes (mutations) linked to drug resistance. This makes it easy to see if the TB bacteria are likely to be resistant to specific drugs. Figure 8 demonstrates the detection of drug resistance on an LPA. LPAs use PCR technology to multiply the target DNA sequences in test tubes and to amplify them so they can be detectable. Once the DNA has gone through enough amplification cycles, an LPA strip is placed in the solution filled with amplified DNA. The LPA strip has several probes for specific target DNA sequences at different locations along the strip. If these target DNA sequences are present in the solution, they bind to the probes and form colored bands on the strip, indicating a positive result for resistance to specific TB drugs. Figure 8 demonstrates the detection of drug resistance on an LPA.

FIGURE 8: LINE PROBE ASSAYS



An example of Hain's GenoType MTBDRplus Version 2.0 LPA strip indicating a positive result for resistance to rifampicin and isoniazid. Adapted from Hain/Bruker image[28]

LPA sensitivity is limited to a set of prominent target DNA sequences associated with resistance to certain TB drugs. This means that LPAs do not detect all of the DNA mutations that may contribute to resistance. These tests require well-equipped labs with multiple dedicated instruments and trained technicians to perform a number of manual steps. They can detect resistance to first-line drugs (RIF and INH) and second-line drugs (FQs and AMK).[29] The turnaround time can be as short as five hours, but it can take one to two days for results to reach the patient since the samples need to be transported to the central laboratories, tested over the five hours using the multistep procedure, and manually interpreted before results are returned.

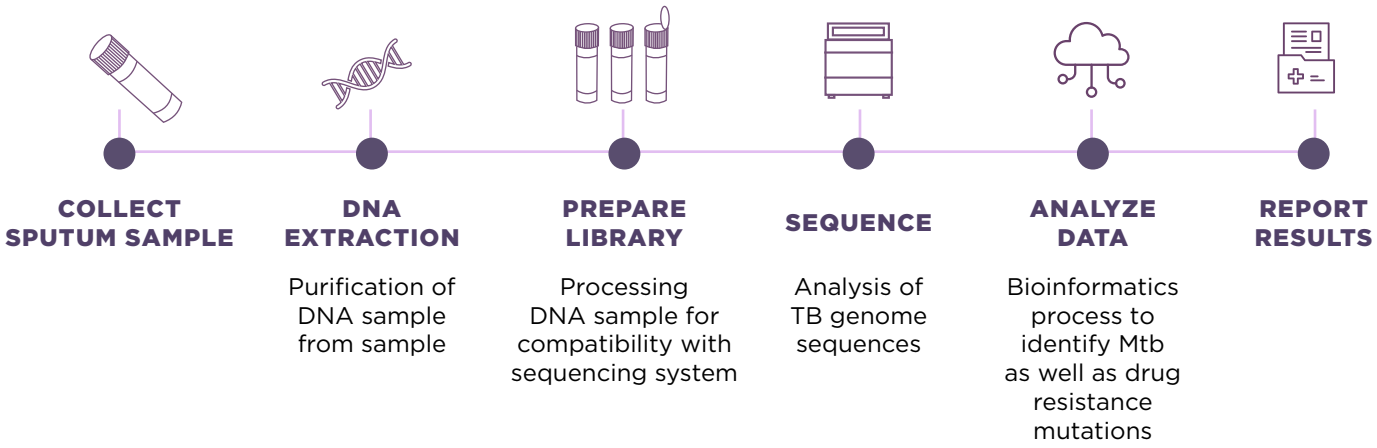
LPAs recommended by the WHO are GenoType MTBDRplus and GenoType MTBDRsl (Hain Lifescience), Genoscholar NTM+MDRTB II and Genoscholar PZA TB II (Nipro). Genoscholar PZA TB II (Nipro) is the only LPA that can detect resistance to more drugs than the Xpert MTB/XDR test, namely pyrazinamide (PZA).

3.3. Targeted Next-Generation Sequencing

Targeted next-generation sequencing (tNGS) solutions were recommended by the WHO in 2024 for follow-on detection of resistance to a broad range of TB drugs after the initial detection of TB or of rifampicin resistance. Figure 9 describes the process of conducting a tNGS test. New tests based on tNGS can detect resistance to up to 16 drugs from a single sample, including second-line drugs not currently included in any of the aforementioned NAATs or LPAs (such as BDQ, LZD, and clofazimine [CFZ]).[30] This comprehensive resistance detection is done by coupling the nucleic amplification technique with next-generation sequencing to scan the TB bacteria's genetic code for mutations known to cause drug resistance. Once a sputum sample is collected from the patient,

DNA from the TB bacteria is isolated from the sample and targeted amplification is performed, where specific gene regions linked to drug resistance are copied millions of times using the PCR technique. The DNA copies are then treated so that their sequences can be read. Sequencing then takes place, where amplified and treated DNA is read using high-tech machines. The sequences are then read by software that compares them to the sequence of a TB bacteria without drug resistance to identify changes or mutations. If mutations are identified, they are compared to a standardized list of those known to be associated with TB drug resistance. If resistance-associated mutations are present, the software automatically flags resistance to specific drugs and reports all results in user-friendly testing reports.

FIGURE 9: THE TNGS PROCESS EXPLAINED



Adapted from Illumina's World TB Day Infographic[31]

These tests are placed in central laboratories and require complex lab infrastructure along with highly skilled lab technicians. They provide results in one to two days and have a high accuracy for commonly used TB drugs (such as INH and RIF) but a lower sensitivity for second-line drugs (such as BDQ, delamanid [DLM], pretomanid [Pa], cycloserine [CS], and LZD).[32] tNGS platforms that are recommended by the WHO are Deeplex Myc-TB (GenoScreen), AmPORE-TB (Oxford Nanopore), and TBseq (ShenTing).

It is important to note that switching to molecular tests does not mean we can stop using traditional culture-based tests (phenotypic DST), which are still needed to check for resistance to certain TB medicines — especially newer or less common ones — where molecular tests may not work well or don't exist yet. In particular, culture-based testing is still important to rule out resistance to drugs like BDQ, DLM, Pa, CS, and LZD.

4. TREATMENT MONITORING

Healthcare workers use treatment monitoring to determine how an individual is responding to treatment and to make adjustments as needed. To ensure a successful outcome, it is important to regularly monitor how the individual is responding to their TB medication. Detecting signs of failure early allows healthcare providers to adjust the regimen promptly and improve the chances of cure. Currently available genotypic diagnostic tests (such as NAATs, LPAs, and tNGS) cannot be used for treatment monitoring because they cannot differentiate between live and dead TB bacteria and thus may remain positive even when treatment is working. There are two phenotypic tools that are currently recommended by the WHO for treatment monitoring: mycobacterial culture and smear microscopy.

Culture, as explained in section 2.1.3, is a highly accurate but slow test, taking two to six weeks to produce results. It is the gold standard for confirming that TB bacteria are no longer present in a patient's sputum, which shows that treatment is working. The conversion of samples from culture-positive to culture-negative (called "culture conversion") is a key sign that TB treatment is effective and helps guide decisions about the length and success of therapy. However, because results take

time, this information is usually available only after two to three months of treatment. Despite the wait, a negative culture at the end of treatment is the most reliable proof that a person is cured of TB.

Smear microscopy, on the other hand, provides rapid results but has very limited accuracy. It has been the most prominent method of diagnosing and microbiologically confirming TB throughout history and is done by looking directly for TB bacteria in samples using a microscope. This method, however, is insufficiently sensitive, detecting TB in only 50 percent of sputum samples with TB bacteria present. Smear microscopy is especially unreliable when there are only small amounts of TB bacteria in the sample, such as in children, PLWHIV, or those with early or less severe disease.

Because of this low sensitivity, the WHO does not recommend smear microscopy as an initial TB diagnostic test; smear microscopy is only recommended for treatment monitoring.

5. TB INFECTION TESTING

Most people with TB infection do not have symptoms and are not contagious, but they carry the bacteria in their bodies and could develop active TB disease later, especially if their immune system becomes weak. About one-quarter of the world's population (one in four people) is estimated to have been infected with TB, but only a small percentage (about 5%) will ever develop active TB disease in their lifetime. The risk is much higher for PLWHIV or people who have recent close contact with someone with active TB.[33]

Testing for TB infection helps identify people who might benefit from **TB preventive treatment (TPT)**, which can stop the infection from turning into active disease (for more information on TPT, see ***An Activist's Guide to Rifapentine – TB Preventive Treatment: 3HP and 1HP***.[34]) However, it is important to know that a person does not always need a TB infection test to start preventive treatment. People at high risk for TB, like children living with someone who has TB or PLWHIV, can start TPT as long as active TB disease has been ruled out.

TB PREVENTIVE TREATMENT (TPT): treatment taken by people with TB infection to prevent progression from infection to active TB disease; TPT is also sometimes given to protect people who are uninfected but at risk of TB exposure and infection.

There are two main types of tests for TB infection: skin tests and blood tests. Both tests work by checking how the immune system reacts to proteins from the TB bacteria, which indicate if someone has been exposed to TB in the past.

- Tuberculin skin test (TST): For this test, a small amount of testing fluid is injected just under the skin of the forearm. After two or three days, a healthcare worker checks the spot for swelling. If there is swelling, it may mean a TB infection. However, people who have had the TB vaccine (Bacille Calmette-Guérin or BCG) can sometimes have a positive result even if they do not have TB infection — due to these "false positive" results, the test is not very specific. TST is simple and inexpensive, making it common in many countries, but it requires a return visit to read the results. The WHO recommends Tuberculin purified protein derivative (PPD) products for TSTs.

- TB antigen-based skin tests (TBSTs): These are newer tests that were recommended by the WHO in 2022. These tests use special proteins found only in TB bacteria, so they do not react with the BCG vaccine and are more specific than traditional TSTs. TBSTs are given in the same way as the regular skin test and also require a return visit, but they help reduce false positives in people who have received the BCG vaccine. TBSTs that have WHO recommendations are Diaskintest (Generium), Siiltibcy (Serum Institute of India), and C-TST (Anhui Zhifei Longcom).

- Interferon-gamma release assays (IGRAs): These are blood tests that measure the immune system's response to TB proteins in the lab. A small sample of blood is mixed in the lab with proteins found only in TB bacteria, and if the immune system recognizes these proteins, the blood cells release a substance called interferon-gamma, which the test measures. IGRAs are not affected by the BCG vaccine, so they are more specific, but they are more expensive and need special lab equipment. Results are usually available within a day. IGRA tests that are recommended by the WHO are T-Spot (Revvity), TB-IGRA (Wantai BioPharm), QuantiFERON-TB Gold Plus (QIAGEN), STANDARD E TB-Feron ELISA (SD BIOSENSOR), and LIAISON QFT-Plus CLIA (Diasorin).

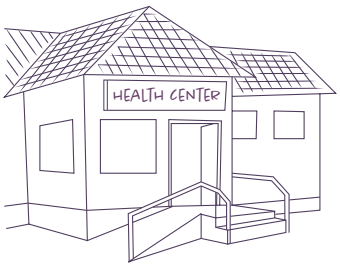
All above listed tests can tell if someone has been previously exposed to and/or infected with TB bacteria, but they cannot predict if the infection will become active TB disease, and they cannot be used to diagnose active TB. Also, because these tests are looking for indicators that the immune system has seen TB before, these tests can be less accurate in children and people with weakened immune systems, such as PLWHIV. TB infection testing is a useful tool for finding people who have been exposed to TB bacteria and could benefit from preventive treatment. However, these tests have limitations, and in many high-risk situations, preventive treatment can be started without waiting for a test result, as long as active TB disease has been ruled out.

6. PLACEMENT OF SCREENING AND DIAGNOSTIC TESTS

The placement of TB diagnostic tools is closely tied to the infrastructure available at different levels of the health system. While most individuals first present to care at primary healthcare centers at the peripheral level (subdistrict and community levels) of the healthcare system, oftentimes more complex tests capable of providing comprehensive diagnostic results are only available at district or central level hospital laboratories. This requires transport of samples to centralized laboratories, which delays the time to result and treatment initiation, resulting in loss-to-follow up from the healthcare system, disease progression, poor outcomes, and continued transmission. Figure 10 explores where the currently available and recommended screening and diagnostic tools can be placed in the healthcare system.

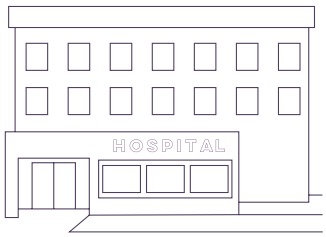
FIGURE 10: PLACEMENTS OF TB SCREENING AND DIAGNOSTIC TOOLS AT DIFFERENT LEVELS OF HEALTH SYSTEMS

PERIPHERAL HEALTH CENTER



- Symptom screening
- Chest X-ray +/- CAD
- Rapid molecular tests (LC-aNAATs and LC-mNAATs)
- LF-LAM tests for PLWHIV
- C-reactive protein for PLWHIV
- Sample referral for complex DST
- Smear Microscopy

DISTRICT HOSPITAL



- MC-aNAATs
- Line probe assays for DST
- Mycobacterial culture for DST

CENTRAL HOSPITAL

- tNGS for DST

Adapted from TAG's Community-led Monitoring for Access to Tuberculosis Screening and Diagnostic Testing.[35]
Note: This figure indicates the lowest level of the health system where a tool can be implemented.

TABLE 1: AN ALGORITHM, DETAILING THE DIFFERENT TESTS THAT CAN BE USED FOR DIFFERENT SCENARIOS OF DIAGNOSTIC AND DRUG-SUSCEPTIBILITY TESTING

Scenario	Purpose	Recommended Test
Initial Screening	Identify high-risk individuals and refer for further confirmatory testing	• Symptom Screening • Chest X-ray +/- CAD
Initial Diagnosis	Detect TB + RR	• Low-complexity automated NAATs (Xpert MTB/RIF Ultra, Truenat MTB Plus, and Truenat MTB-RIF Dx) • Moderate-complexity automated NAATs
Confirmed RR-TB	Detect resistance to INH, FQs	• Low-complexity automated NAATs (Xpert MTB/XDR) • Moderate-complexity automated NAATs • LPAs
Presumptive XDR-TB (Confirmed FQ-R TB)	Detect resistance to other group-A drugs	• tNGS • Culture
Treatment monitoring	Evaluate treatment success	• Smear Microscopy • Culture

Note: Diagnostic tools without DST ability are not included in this algorithm because they are recommended to be used in very specific situations or populations. Low-complexity manual NAATs (TB-LAMP) can be used as a replacement for smear microscopy in low-burden settings. In PLWHIV, C-reactive protein, a blood-based test, can be used for screening and LF-LAM can be used for initial diagnosis.

7. ENSURING ACCESS TO TB TESTS

Achieving the highest standard of care in TB diagnosis requires national TB programs to adapt and implement WHO-recommended diagnostic tools and strategies. However, several major barriers continue to impede universal access to rapid and accurate TB testing. These include the high cost of many diagnostic tools, slow adoption of new WHO recommendations at the country level, insufficient donor and domestic funding to scale up and sustain the use of these tools, inefficiencies within health systems, and the catastrophic out-of-pocket costs faced by people with TB. Without addressing these obstacles, the global TB diagnostic gap will persist, undermining efforts to detect, treat, and ultimately end unnecessary suffering and death from TB.

7.1. Special Populations

While no TB diagnostic test is perfect, ongoing investments in TB research and development (R&D) continue to improve the technologies and tools used to diagnose TB. However, this R&D process still falls short in serving some key vulnerable populations who could benefit the most from innovation.

People Living with HIV

One such vulnerable group is PLWHIV, who are at especially high risk of developing active TB disease. PLWHIV are much more likely than HIV-negative individuals to progress from TB infection to active disease, with TB being the leading cause of death among PLWHIV worldwide, causing approximately 161,000 deaths in 2023.[36] Additionally, PLWHIV often have disseminated TB, where the infection spreads throughout the body, or paucibacillary TB, characterized by low and variable concentrations of TB bacteria in samples. This leads to lower sensitivity of TB diagnostic tests in this group. Moreover, PLWHIV, especially those with advanced HIV or AIDS, frequently have difficulty producing sputum, making sputum-based tests less accessible. To address this, the WHO recommends the use of the urine-based LF-LAM test for PLWHIV, which, despite not being the most accurate, has proven mortality benefits by enabling faster diagnosis and treatment initiation.

The WHO also recommends C-reactive protein (CRP) testing for TB screening in PLWHIV. CRP is a non-specific marker of inflammation that can be measured in the blood and is used as a screening tool for TB in PLWHIV. Elevated CRP levels are associated with active TB, and using CRP testing alongside symptom screening can improve the accuracy of TB detection in this population. CRP testing is quick, inexpensive, and can be done at the point of care, making it particularly useful in settings where other diagnostic tools like chest X-ray are less available. The WHO recommends CRP as part of the TB screening algorithm for PLWHIV because it has higher specificity than symptom screening alone and helps identify individuals who are more likely to have active TB, reducing unnecessary confirmatory testing.

Children

Children represent another high-risk population underserved by current TB diagnostics. They are more likely than adults to progress to active TB disease and to develop extrapulmonary TB. Due to the generally low bacterial load in children (paucibacillary TB), the sensitivity of TB diagnostic tests is lower compared to adults. Consequently, even highly sensitive rapid molecular tests may fail to detect TB in many children.

To address these challenges, the WHO recommends use of a treatment decision algorithm for diagnosing pulmonary TB in children under 10 years. Treatment decision algorithms combine clinical assessment, history of contact with a person with TB, chest X-ray findings, and results from available laboratory tests. Bacteriological confirmation should be sought whenever possible, but if not available or feasible, a diagnosis can be made based on symptoms, exposure history, and chest X-ray abnormalities.[37] This approach ensures that children at high risk — such as those under five years old, living with HIV, or severely malnourished — can start treatment promptly, even if laboratory confirmation is not possible or lab tests produce negative results but there are clinical or other indicators of TB present.

Extrapulmonary TB

Extrapulmonary TB occurs when TB infection is present outside the lungs, affecting areas such as lymph nodes, the pleural cavity, the brain and spinal cord (TB meningitis), bones or joints, and the abdominal cavity. Since TB bacteria are located outside the lungs in these cases, sputum samples are not as useful, and alternative samples can be collected from the affected body sites for testing. According to WHO's recommended treatment decision algorithms, diagnosis of extrapulmonary TB should use a combination of clinical evaluation, imaging (such as ultrasound or X-ray), and laboratory testing of samples from the affected area, with rapid molecular tests (like Xpert Ultra) prioritized, when possible, to support timely and accurate diagnosis.

To better serve these vulnerable groups, there is a pressing need for diagnostic tools that use nonsputum sample types that are easier to collect. It is also essential to actively involve these underserved populations in the development and evaluation of new diagnostics to ensure new tools are accurate and effective for all.

7.2. Concurrent Testing

In April 2025, the WHO updated its guidelines to improve the detection of TB and drug resistance, especially for people who are at higher risk, such as those PLWHIV and children. One of the key updates is the recommendation for concurrent testing, which means using more than one type of test or sample at the same time to increase the chances of diagnosing TB accurately and quickly.

The new WHO guidelines now recommend that, for adults and adolescents living with HIV, both a molecular test on a respiratory sample (like sputum) and a urine-based LAM test should be done at the same time. This combination has been shown to find more individuals with TB than using just one test alone, and it is still affordable for many health systems.[38] Similarly, for children, the guidelines recommend using a molecular test on a respiratory sample together with a stool test. For children living with HIV, the recommendation is to use all three — a molecular test on a respiratory sample, a stool test, and a urine LAM test — at the same time. These combined approaches help find TB in

people who might otherwise be missed, especially when it is hard to get a good sputum sample, as is often the case with young children or people who are very sick.

WHO's concurrent testing recommendations mean that, for people at higher risk of TB, health workers should use several tests and sample types at the same time. This approach helps maximize the value of combining sample types (e.g., sputum with stool or urine) using WHO-recommended rapid diagnostics for higher **diagnostic yield** among high-risk groups. Such refined testing algorithms help diagnose TB more accurately and quickly, ensuring that people get the right treatment as soon as possible.

DIAGNOSTIC YIELD: the proportion of people who are identified with a disease using a diagnostic test (or a combination of tests) out of the total number of people who were eligible to be tested.

7.3. Fair and Affordable Pricing

Advocating for fair and affordable pricing of TB diagnostics is critical to improving access, especially in resource-limited settings. While increased competition has brought new and improved tests to the market, prices remain high due to factors beyond manufacturing costs, such as distributor markups, sales and marketing expenses, and supply chain inefficiencies. To address this, the global health community is calling for a shift toward a cost-of-goods-sold (COGS)-plus pricing model, where manufacturers set prices based on transparent production costs plus a reasonable margin for profit and reinvestment rather than undisclosed or arbitrary markups. Campaigns like **Time for $5** have highlighted the gap between production costs and sale prices, successfully pressuring companies like Cepheid to reduce the price of the Xpert MTB/RIF test from nearly US$10 to US$7.97 per test in September 2023.[39] This 20 percent price reduction translates to US$32 million in savings, enough to purchase 3.6 million more tests, demonstrating the power of collective advocacy in securing more affordable prices and equitable access to diagnostics.[40]

7.4. Adequate and Sustainable Funding

Despite these gains, TB programs remain chronically underfunded, and the current political and global health funding landscapes have put additional strain on already limited resources. There is an urgent need for alternative and sustainable financing solutions to ensure countries can strengthen their health systems, expand laboratory capacity, and procure essential TB diagnostic tools at scale. Increased investment from domestic budgets and from global donors, innovative financing mechanisms, and more efficient use of available funds are all necessary to close the gap and maximize the impact of TB program efforts.

8. CALL TO ACTION: DEMANDING ACCOUNTABILITY

This Activist's Guide offers practical information on how to use TB diagnostic tools most effectively and how to integrate them into health systems to achieve the highest standard of TB care. It also identifies critical gaps in the adoption and implementation of these diagnostics and highlights barriers that prevent many people from accessing quality TB testing. Overcoming these obstacles and closing the TB diagnostic gap requires coordinated action and accountability from country governments, global donors, and diagnostics companies.

1

Country governments must increase domestic funding for national TB programs, ensuring strong investment in health system infrastructure, particularly laboratories, and in the full scale-up of quality TB diagnostic tools. Updating national TB and HIV program policies in line with the latest WHO recommendations is essential, as is integrating TB screening and diagnostic testing at all levels of care. Governments should also prioritize comprehensive training for health workers on new diagnostic tools and algorithms to ensure effective implementation.

2 **Global donors** need to boost financial support to help countries strengthen health systems, expand laboratory capacity, and procure TB diagnostic tools at scale. Donors should also invest in R&D for new diagnostics, making funding conditional on commitments to cost transparency and fair pricing. Leveraging collective procurement power can help drive down prices and improve access. Investment in local manufacturing and technology transfer is also important to build sustainable diagnostic capacity by ensuring reliable supply chains, reducing dependence on imports, and building local expertise. This approach enhances health security, fosters innovation, and improves equitable access to quality TB diagnostics in low- and middle-income countries.

3 **Diagnostics companies** should invest in the development of new TB diagnostic tools that meet WHO target product profiles (TPPs) and involve affected communities and vulnerable populations early in the R&D process. The TPPs emphasize the need for new diagnostics that are affordable, easy to use at the point of care, accurate in all populations, including children and PLWHIV, and work with nonsputum samples.[41] Companies must commit to transparent, fair pricing that reflects actual production costs and public investments and ensure that new diagnostics are accessible in all high-burden countries. This includes providing affordable service and maintenance plans and scaling up manufacturing to meet global needs.

By pushing for these changes and demanding accountability, activists can help remove barriers to TB diagnosis and ensure that everyone, everywhere, has access to timely, accurate, and affordable TB testing.

TABLE 2: CURRENTLY AVAILABLE TESTS FOR TB SCREENING AND DIAGNOSIS WITH WHO RECOMMENDATIONS

Category	WHO Classification	Test	Manufacturer	Accuracy (SE = sensitivity; SP = specificity)	Price (USD)	WHO Recommendation
TB Screening	NA	Symptom Screening	NA	SE: 71% (any TB symptom), SP: 64% (any TB symptom)[42]	NA	People who screen positive for TB symptoms should be screened for HIV and receive chest X-ray (CXR) as a second screening test
		Chest X-ray	Multiple	SE: 82–93%, SP: 14–63% (with human reader)[43]	Multiple	People with an abnormal CXR suggestive of TB should be given a TB diagnostic test
		Computer-Aided Detection		Pooled Accuracy: SE: 90–92%, SP: 90–91%[44]		CAD is recommended as an alternative to human interpretation of digital CXR for screening and triage for TB
TB Diagnosis	LC-aNAAT	Xpert MTB/RIF Ultra	Cepheid, USA	**MTB**: SE: 90%, SP: 96%; **RIF**: SE: 94%, SP: 99%	$7.97[45]	Initial tests for TB diagnosis with rifampicin-resistance detection (RIF)
		Truenat MTB Plus	Molbio, India	SE: 80%, SP: 96%	$7.90[a,46]	
		Truenat MTB-RIF Dx		**RIF**: SE: 84%, SP: 97%		

a. *Price for MTB Plus and MTB-RIF Dx combined*

Category	WHO Classification	Test	Manufacturer	Accuracy (SE = sensitivity; SP = specificity)	Price (USD)	WHO Recommendation
TB Diagnosis	LC-mNAAT	TB-LAMP	Eiken Chemical	SE: 78%, SP: 98%[47]	≥$6[48]	Initial tests for TB diagnosis without drug-resistance detection
	LF-LAM	Determine TB LAM Ag	Abbott	**0–100 CD4 cells/m**m: SE: 56%, SP: 93.6%; **101–200 CD4 cells/mm**: SE: 25.3%, SP: 96.7%	$3.70[49]	
Drug-Susceptibility Tests	MC-aNAAT	RealTime MTB	Abbott	Overall pooled accuracy – **TB Detection**: SE: 93.0%, SP: 97.7%; **RIF**: SE: 96.7%, SP: 98.9%; **INH**: SE: 86.4%, SP: 99.2%[50]	NA	Initial tests for TB diagnosis with drug-resistance detection (RIF and INH)
		RealTime MTB-RIF/INH				
		BD MAX™ MDR-TB	Becton Dickinson		$8.59[51]	
		cobas MTB	Roche		~$8.90[b,52]	
		cobas MTB-RIF/INH	Roche			
		FluoroType MTB	Hain Lifescience / Bruker		NA	
		FluoroType MTBDR			$9.71–$14.09[c,53]	
	LC-aNAAT	Xpert MTB/XDR	Cepheid	Overall pooled accuracy: **INH**: SE: 94%, SP: 98%; **FQ**: SE: 93%, SP: 98%[54]	$14.90[55]	Follow-on tests for detection of TB drug resistance (INH, FQ, EMB, AMK)
	LPAs	GenoType MTBDRplus v1 and v2	Hain Lifescience / Bruker	Overall pooled accuracy: **INH**: SE: 89%, SP: 98%; **FQ**: SE: 86%, SP: 99%, (Genoscholar PZA-TBII) **PZA**: SE: 81.2%, SP: 97.8%[56]	$8.44[d,57]	Follow-on tests for detection of TB drug resistance (INH, FQ, PZA)
		GenoType MTBDRsl				
		Genoscholar NTM+M-DRTB II	Nipro		NA	
		Genoscholar PZA TB II				
	tNGS	Deeplex Myc-TB	GenoScreen / Illumina	For people with bacteriologically confirmed pulmonary TB, pooled SE: **INH, MFX, EMB**: ≤95%, **RIF, LFX**: >93%, PZA: 88%; pooled SP: ≤96% for all drugs For people with bacteriologically confirmed RIF-resistant pulmonary TB, pooled SE: **INH, LFX, MFX, STR, EMB**: ≥95%, **BDQ**: 68%, **LZD**: 69%, **CFZ**: 70%, **AMK**: 87%, PZA: 90%; pooled specificity for all drugs: 95%, **STR**: 75%[58]	$100 - $110[e,59]	Follow-on tests for detection of TB drug resistance (INH, FQ, EMB, AMK, PZA, BDQ, LZD, CFZ)
		AmPORE-TB	Oxford Nanopore Technologies		NA	
		TBseq	Shengting Medical Technology Company			

b. Roche's global access pricing is ~US$8.90 for MTB combined with MTB RIF/INH. The price of MTB is US$7.90 and if one in ten are positive and require a reflex test for RIF/INH, the price per person tested comes up to US$8.90.; ***c.*** 8.99–13.05 euros (conversion rate: 1 Euro = 1.08 USD). Price depends on DNA extraction workflow selected (manual vs. automated); ***d.*** 7.81 euros (conversion rate: 1 Euro = 1.08 USD); ***e.*** Global access pricing provided by Illumina/Genoscreen. The price depends on the instrument selected and there are other reagents required. This price is just for the reagents provided by Genoscreen/Illumina

Category	WHO Classification	Test	Manufacturer	Accuracy (SE = sensitivity; SP = specificity)	Price (USD)	WHO Recommendation
Treatment Monitoring	TB Culture	BACTEC MGIT liquid culture	BD Max	SE: 100%, SP: 100%	NA	A combination of culture and smear microscopy is recommended to monitor patient response to MDR/RR-TB treatment[60]
	Smear Microscopy	NA	Multiple	SE: 50% (sputum), SP: 98% (sputum)	NA	

Abbreviations: RIF: rifampicin; INH: isoniazid; FQs: fluoroquinolones; PZA: pyrazinamide; MFX: moxifloxacin; EMB: ethambutol; LFX: levofloxacin; STR: streptomycin; BDQ: bedaquiline; LZD: linezolid; CFZ: clofazimine; AMK: amikacin; DLM: delamanid; Pa: pretomanid

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