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Diagnosing Tuberculosis: Traditional and New Methods Against an Old Enemy

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ABSTRACT

Tuberculosis (TB) poses significant health challenges globally, contributing significantly to illness and mortality. Approximately one-fourth of the world's population is believed to carry the tuberculosis bacterium, with a 5–10% lifetime risk of developing active TB disease. Timely identification of TB and rapid detection of drug resistance are crucial in mitigating its global impact. Clinical, radiological, bacteriological, and molecular methods are used to screen and diagnose TB. Microscopy, culture, and immunological methods are commonly employed. Although microscopy has been used for approximately a century, it has limitations, necessitating its use and evaluation alongside other diagnostic techniques for a complete and accurate diagnosis. Bacterial culture in solid and liquid media is still the gold standard recommended by the World Health Organization (WHO) for the diagnosis of TB, as it enables the isolation of *Mycobacterium tuberculosis* (MTB) as well as the detection of drug resistance. In recent years, promising modern solutions have emerged that aim to overcome the limitations of culture and microscopic examination methods in molecular diagnostic testing. In routine laboratories in resource-wealthy countries, methods such as *Mycobacterium Tuberculosis Drug Resistance Plus* (MTBDRplus), loop-mediated isothermal amplification (LAMP), line probe assay (LPA), GeneXpert, and whole genome sequencing are increasingly being used to diagnose and characterize TB and determine anti-TB drug susceptibilities. Our goal is to explore and assess the evolution, advancements, and future prospects in TB diagnosis, focusing particularly on the current laboratory methods used for diagnosing the disease.

Keywords: Diagnosis, laboratory, *Mycobacterium tuberculosis*, traditional and new methods, tuberculosis.

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INTRODUCTION

Tuberculosis (TB), believed to have emerged more than 150 million years ago, was referred to as "phthisis" by the ancient Greeks, "tabes" by the ancient Romans, and "schachepheth" by the ancient Hebrews. The TB bacillus was first discovered by the German bacteriologist Robert Koch on March 24, 1882.[1–4] *Mycobacterium tuberculosis* (MTB) belongs to the family *Mycobacteriaceae* and is a part of the *Mycobacterium tuberculosis* complex (MTBC), which includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canettii*, *M. caprae*, *M. microti*, *M. pinnipedii*, *M. mungi*, and *M. orygis*. Approximately 98% of TB cases are caused by MTB. Its cell wall is unique, with a thick peptidoglycan layer and an outer membrane composed of various lipopolysaccharides and fatty acids. This forms a low-permeability

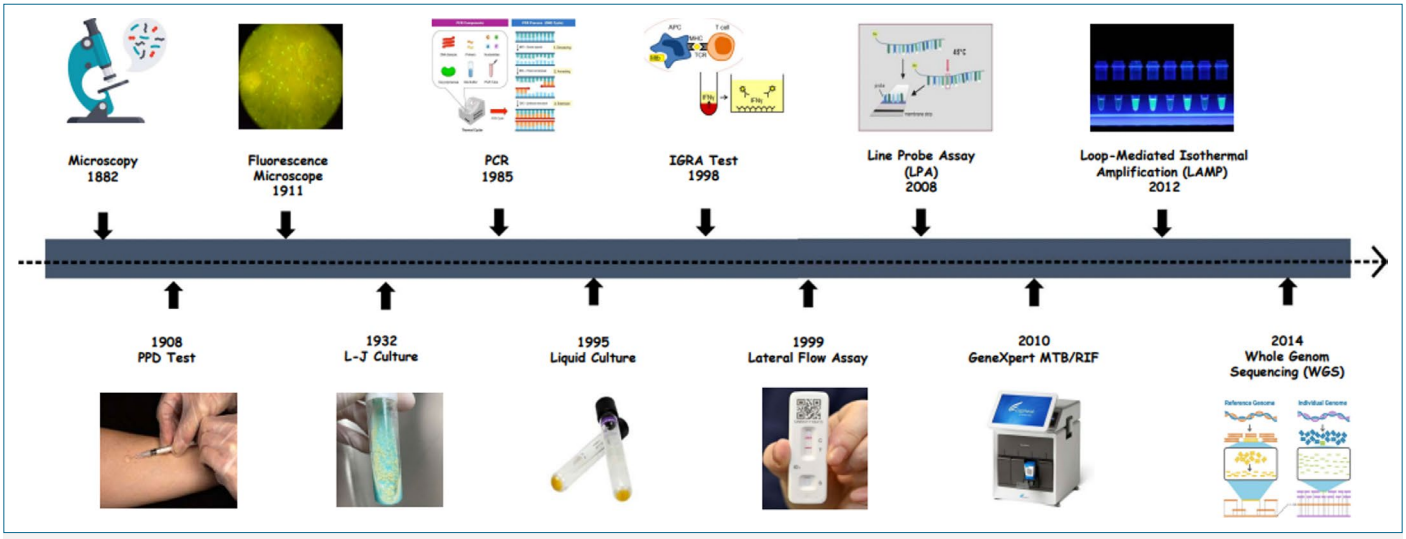

Figure 1. Chronological diagram of methods used in tuberculosis (TB) diagnosis.

barrier that protects MTB from most antibiotics. The cell wall of MTB is highly complex and distinctive, characterized by a thick peptidoglycan layer and an outer membrane primarily composed of diverse lipopolysaccharides, fatty acids, glycolipids, and wax esters. This lipid-rich structure creates a formidable barrier with low permeability, offering significant protection to MTB against a wide range of antibiotics.[5,6]

Despite advancements in diagnostics and care, TB remains a significant global burden, with 10.6 million new cases and 1.3 million deaths reported in 2022. An estimated 410,000 cases are currently multidrug-resistant TB (MDR-TB).[7]

Tuberculosis continues to be a leading cause of mortality attributable to a single infectious agent and has evolved into a serious global public health challenge, further complicated by the rise in drug-resistant cases. Early and effective treatment is pivotal in preventing the emergence of drug-resistant strains. This necessitates accessible and reliable diagnostic methods that are both rapid and exhibit high diagnostic accuracy for effective case management. Currently, the most effective strategies for combating MTB infection are to control the source of infection, interrupt transmission pathways, and protect susceptible populations. However, it is also essential to develop preventive, diagnostic, and therapeutic interventions to reduce the disease burden.[8]

Regarding the prevention of MTB infection, the Bacillus Calmette-Guérin (BCG) vaccine, which is the only vaccine with partial protective effects, has limited efficacy and duration of protection in adults. Consequently, developing multiple vaccines with enhanced preventive efficacy could provide new strategies for safeguarding vulnerable populations. In terms of diagnosing MTB infection, existing diagnostic technologies lack the specificity and sensitivity required to distinguish between latent tuberculosis infection (LTBI) and active tuberculosis (ATB). Therefore, identifying biomarkers linked to immune mechanisms could be a crucial step in improving the accuracy and efficiency of TB diagnosis. Conventional chemotherapy agents are ineffective against multidrug-resistant tuberculosis. Combining immunomodulatory cytokines, immune checkpoint inhibitors, and vaccines with anti-tuberculosis chemotherapy may offer a more effective treatment.[4,5,7]

The chronological diagram of the methods used in TB diagnosis is shown in Figure 1.

The acid-fast staining (AFS) method, which has been employed in the laboratory diagnosis of TB for over 100 years, provides the first evidence for the presence of mycobacteria in smears of clinical specimens. This method provides valuable information for confirming the clinical diagnosis and assessing the patient's infectiousness.[9,10] Acid-fast staining, also known as Ziehl-Neelsen (ZN) stain microscopic detection, developed over a century ago, is a basic, fast, and inexpensive diagnostic method and is still the most widely used method for TB diagnosis. However, it has very low and variable sensitivity that depends on the bacterial load, which can be a significant issue, particularly among children and immunocompromised individuals.[11,12]

The sensitivity and specificity of AFS depend on several factors, including the quality of the reagents, the experience of the technical staff, and the duration of the primary and counterstaining steps. The sensitivity of the staining process

ranges from 20% to 60%. Ziehl-Neelsen staining sensitivity depends on bacillary counts in the sputum. A positive acid-fast bacillus (AFB) diagnosis from a TB patient's sputum requires 5,000 to 10,000 colony-forming units (CFU) per milliliter of bacilli.[8,11,12]

The gold standard for diagnosing TB is culture, which is a more sensitive method than bacilloscopy, allowing for the detection of 10–100 colony-forming units of bacilli from a concentrated clinical sample. Culture sensitivity is 80–85%, with a specificity of 99%. However, the slow growth rate of MTB causes isolation, identification, and drug susceptibility testing to take several weeks. Despite this delay, culture remains crucial for drug sensitivity testing, research, and strain preservation.[8–10,13]

Molecular tests diagnose TB rapidly and accurately and determine drug sensitivity. Recent developments in childhood TB diagnosis using non-invasive samples have shown promise. The World Health Organization (WHO) has approved the Xpert® MTB/RIF Ultra test for diagnosing TB in children using samples from the nose and other body sites. However, it is not yet a replacement for traditional techniques such as microscopy and culture. Combining traditional and molecular methods for diagnosing TB or testing drug sensitivity has demonstrated improved diagnostic outcomes.[9]

According to the 2022 Global Tuberculosis Report, extrapulmonary TB (EPTB) accounts for 16% of the 7.5 million reported TB cases worldwide.[14] In 2020, it was reported that 12% of the global TB burden and 16% of all TB-related deaths were child cases, with most deaths occurring in children without access to TB treatment.[15] Diagnosing EPTB and pediatric TB presents challenges due to non-specific clinical findings, difficulty in obtaining clinical samples, paucibacillary counts in clinical samples, and variable sensitivity and specificity of diagnostic tests. Delayed diagnosis of EPTB and pediatric TB may lead to untimely treatment, more severe outcomes, and increased mortality.[16,17]

To prevent transmission in pulmonary TB (PTB) and reduce mortality in EPTB and childhood TB, rapid and high-performance diagnostic methods must be employed.

Our goal is to explore and assess the evolution, advancements, and future prospects in TB diagnosis, focusing particularly on the current laboratory methods used for diagnosing the disease.

Clinical Diagnosis

Only 5-10% of infected individuals exhibit symptoms. Tuberculosis primarily affects the lungs. The primary symptoms of PTB include cough, fever, night sweats, and weight loss. Tuberculosis can also affect other organs.[8,18]

Radiography

Chest X-rays (CXR) are used to diagnose and screen for TB. Primary tuberculosis typically presents as a lesion in the middle or lower right lobe, with enlarged lymph nodes. Reactivation TB generally shows a single lesion in the apical region, with normal lymph nodes and multiple secondary tubercles. Miliary TB is characterized by small granulomas scattered throughout the lung fields. CXR can identify PTB but not latent infections.[8]

Microbiological Diagnosis

The WHO recognizes culture-based methods as the gold standard for TB diagnosis due to their high sensitivity and ability to confirm active TB. Culture tests can detect even low levels of MTB in various types of specimens, making them extremely reliable. These tests also allow for drug susceptibility testing (DST) to determine the most effective treatment regimens, which is crucial for managing drug-resistant TB.[19]

Despite being the gold standard, culture tests have limitations, including longer turnaround times—typically several weeks—as MTB is a slow-growing bacterium. To address these limitations and provide faster results, the WHO recommends using rapid molecular tests like the Xpert *Mycobacterium tuberculosis*/rifampicin (MTB/RIF) assay and line probe assays (LPAs) alongside culture methods. These molecular tests offer faster detection of TB and drug resistance, supporting early diagnosis and treatment initiation. The Xpert MTB/RIF assay is a widely endorsed molecular test that simultaneously detects MTB and rifampicin resistance, providing results in less than two hours. Additionally, the WHO advocates for the use of LPAs for the rapid identification of drug-resistant TB, particularly for first-line drugs such as isoniazid (INH) and RIF. Since 2016, the tuberculosis loop-mediated isothermal amplification (TB-LAMP) test has been recommended as a potential replacement for smear microscopy due to its superior diagnostic performance and suitability for use in peripheral healthcare settings. Collectively, these tests enhance TB diagnosis, enabling timely and accurate detection, which is crucial for effective disease management and control.[19,20]

In summary, while culture-based methods remain the gold standard due to their high sensitivity and comprehensive diagnostic capabilities, the WHO also endorses the use of rapid molecular tests to improve the speed and efficiency of TB diagnosis and treatment.

Microscopic Diagnosis

The laboratory diagnosis of PTB plays a crucial role in disease management, particularly in managing patients and isolating contagious individuals. In many developing countries with high

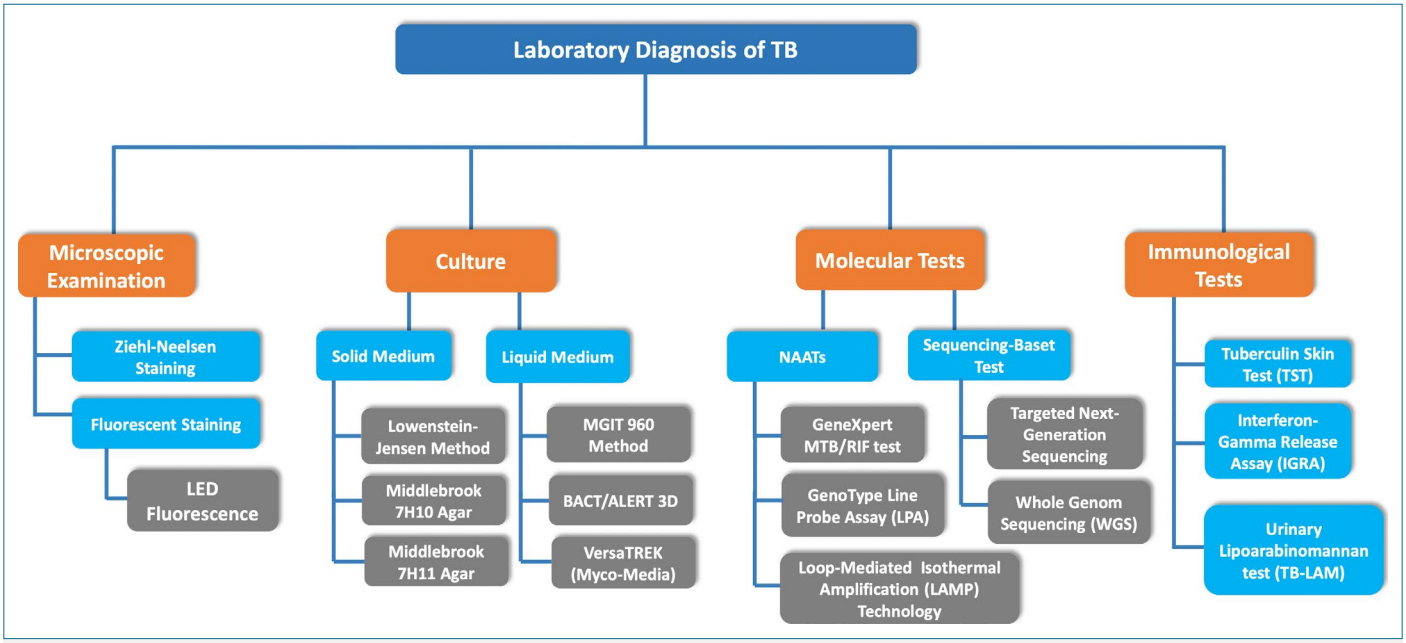

Figure 2. Diagram of laboratory diagnosis methods for tuberculosis (TB), including microscopic examination, culture, molecular tests, and immunological tests.

TB incidence rates, advanced diagnostic techniques and DST are often unavailable. As a result, these countries still heavily rely on traditional methods, such as microscopic examination and culture, despite challenges in management and resource limitations that hinder progress in TB control (Fig. 2).

Microscopic examination of sputum smears, a technique in use for over a century, remains a standard method in laboratory diagnosis. This method provides valuable diagnostic confirmation and helps assess the contagiousness of patients. Ziehl-Neelsen staining microscopy is a critical component of TB diagnosis, especially where facilities and equipment for bacterial culture are limited. This method is rapid and effective in detecting cases with a high bacterial load and significant transmission risk.[9] However, its sensitivity is limited, typically ranging from 50% to 60% in confirmed PTB cases. In cases of EPTB co-infected with Human Immunodeficiency Virus (HIV) or in children with low bacterial counts, ZN staining sensitivity can drop to approximately 30%. Despite these limitations, ZN staining is cost-effective, straightforward to perform and interpret, has a quick turnaround time (1 day), and correlates well with the infectiousness of the case.[9,10,21]

Traditional AFB staining methods, including ZN (hot staining) and Kinyoun (cold staining) techniques, rely on the resistance of the bacteria to acid discoloration after staining. This resistance results from the high lipid content, which makes up approximately 60% of the cell wall's dry weight. This lipid-rich structure also contributes to the bacteria's slow growth and acid resistance. The ZN staining technique involves staining the bacilli with Carbol Fuchsin, which they retain even after de-staining with dilute mineral acid.[8,22]

Fluorescence microscopy (FM) provides an alternative method for detecting MTB in samples. This technique utilizes fluorochromes such as Auramine O and Rhodamine, with Auramine-O, introduced in the 1940s, being particularly effective as a fluorescent marker. The sensitivity of direct microscopy can be enhanced by concentrating sputum sediment and applying Auramine-O fluorescence staining.[10] This method detected AFB in 358 out of 3,000 sputum samples, compared to only 274 detected by the ZN method.[8,22] However, this method alone may not distinguish MTB from other mycobacteria. Additionally, the equipment needed for FM is expensive, limiting its use to regions with sufficient financial resources. Moreover, fluorescence fades over time, necessitating that slides be read within 24 hours of preparation.[10]

Light-emitting diode fluorescence microscopy (LED-FM) is a diagnostic method for TB that utilizes LED light to enhance the visualization of MTB in fluorescently stained sputum samples. This method offers higher sensitivity and faster processing times compared to ZN smear microscopy, making it beneficial for high-throughput settings. However, LED-FM has lower specificity, potentially leading to more false positives. Effective

implementation requires proper training and ongoing quality management, and initial setup costs are higher than those for conventional microscopy.[23]

Overall, microscopy with ZN staining is a rapid, inexpensive, and straightforward method capable of detecting between 5,000 and 10,000 bacteria per milliliter, with a sensitivity ranging from 46% to 78%. Its specificity is nearly 100%, though this varies with the sample source and type of mycobacteria involved. However, smear-negative but culture-positive results are not uncommon.[24]

Cultural Media: Solid and Liquid Forms

Sputum culture is a highly sensitive diagnostic method employed to isolate mycobacteria, capable of detecting as few as 10 to 100 viable bacilli per cultured volume (typically a few tenths of a milliliter). This provides a stark contrast to smear microscopy, which requires at least 5,000 AFB per milliliter to achieve a 50% probability of observing one or more bacilli per 100 fields for a positive result. The specificity of sputum culture for diagnosing TB exceeds 99%.[8,22]

Mycobacterium tuberculosis, an obligate aerobe, thrives optimally at 37°C (with a range of 25–40°C) and a pH of 6.4–7.0. It exhibits a slow growth rate, with a generation time of approximately 14–15 hours, and colonies typically become visible within about two weeks, although delays of 6-8 weeks can occur. On solid media, MTB forms dry, rough, raised, irregular colonies with a wrinkled surface, initially creamy white and later developing a yellowish or buff color, characterized by their tough and tenacious nature.[8]

Solid Medium

Lowenstein-Jensen (LJ) Medium

Lowenstein-Jensen medium is widely used for cultivating and isolating MTB. Lowenstein-Jensen medium incorporates malachite green to inhibit contaminating bacteria and provides nutrients that support the slow growth of mycobacteria. Traditionally egg-based, LJ medium offers a rich environment conducive to MTB growth.

One of its key advantages is selectivity, achieved through malachite green, which inhibits most other bacteria. Lowenstein-Jensen medium is also well-established and standardized, ensuring reliability in clinical settings. Furthermore, it is cost-effective and straightforward to prepare, making it suitable for resource-limited settings.

However, LJ medium has drawbacks. Mycobacterium tuberculosis grows slowly on this medium, taking 4-8 weeks for colonies to appear, which delays diagnosis and treatment. It may be less sensitive than liquid cultures or molecular methods, potentially leading to false negatives, especially with paucibacillary specimens. Lowenstein-Jensen medium also requires manual inspection, which is labor-intensive and prone to human error. Despite the inclusion of selective agents, contamination remains a risk, complicating result interpretation.[25]

Middlebrook (MB) 7H10 and 7H11

Middlebrook 7H10 and 7H11 solid media are specialized agar formulations used for isolating and cultivating *Mycobacterium* species, particularly MTB. These media are essential tools in clinical microbiology for diagnosing and studying TB and other mycobacterial infections.[22,26]

Middlebrook 7H10 agar contains a combination of oleic acid, albumin, dextrose, catalase (OADC), and glycerol, providing a nutrient-rich environment for mycobacterial growth. The oleic acid and albumin enhance the growth of tubercle bacilli by supplying essential fatty acids and proteins. Dextrose serves as a primary energy source, while catalase helps detoxify peroxides that may inhibit bacterial growth. This medium is particularly useful for primary isolation from clinical specimens, allowing for robust and rapid mycobacterial growth compared to other media.

A modification of 7H10, MB 7H11 agar includes casein hydrolysate, which stimulates the growth of fastidious mycobacterial strains, including drug-resistant strains. MTB is highly selective and effective for isolating mycobacteria from contaminated specimens. The inclusion of casein hydrolysate provides additional nutrients, enhancing the recovery and growth of mycobacteria, particularly in clinical settings where contamination is a concern.

Both media types are incubated in a CO_2-enriched atmosphere and are monitored over several weeks due to the slow-growing nature of mycobacteria. They play a critical role in the laboratory diagnosis of TB, aiding in the identification, cultivation, and susceptibility testing of mycobacterial pathogens.[22,26]

Liquid Automatic Continuous Monitoring Systems

Mycobacteria Growth Indicator Tube (MGIT 960)

MGIT 960 system includes a modified MB 7H9 liquid medium with a fluorescence quenching-based oxygen sensor (silicone rubber impregnated with pentahydrate ruthenium) to detect mycobacterial growth. The presence of oxygen in the environment extinguishes the sensor's fluorescence. As mycobacteria or other organisms grow in the medium,

oxygen levels decrease, causing the indicator to fluoresce brightly when illuminated with 365 nm UV light. The medium is enriched with 0.5 mL of oleic acid, albumin, dextrose, and catalase (OADC) and 0.1 mL of PANTA antibiotic mixture (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin). In the BACTEC MGIT 960 system, tubes are continuously monitored by the instrument.[22,27]

BacT/Alert MP System

The BacT/Alert MP system by bioMérieux, France, uses bottles containing modified MB 7H9 medium and growth factors for effective mycobacterial recovery, achieving high recovery rates for MTB (91.3% to 98.7%). The MB/BacT ALERT 3D system monitors microbial growth through pressure changes in closed bottle environments, where CO_2 production from microbial metabolism alters the sensor's pH, causing a color shift from dark green to light green or yellow. This process, monitored by a light-emitting diode system and photodetector, records increasing reflection units to determine growth positivity or negativity.[28] The primary disadvantage of this system is that it cannot be used to study anti-TB drug resistance.

VersaTREK Myco

The VersaTREK Myco system consists of a bottle containing modified MB 7H9 broth, oleic acid-albumin-dextrose-catalase enrichment, and automated instrumentation for mycobacterial recovery and susceptibility testing. Each bottle includes a cellulose sponge to enhance the surface area for growth, simulating the structure of human lung alveoli. Additionally, the medium is supplemented with antibiotics to prevent bacterial contamination. The system automates detection by measuring oxygen consumption changes during growth.[28]

Molecular Tests

Tuberculosis diagnostics have advanced over the past decade with the development of new molecular tests. These tests, known as nucleic acid amplification tests (NAATs), amplify specific MTBC regions through polymerase chain reaction (PCR). NAATs detect TB and conduct DST for critical drugs like rifampicin and isoniazid faster than traditional mycobacterial cultures. Molecular methods are especially useful in cases of smear-negative tuberculosis and drug resistance, facilitating effective case management. Molecular diagnostic tests provide timely results, allowing clinicians to promptly initiate case management and prescribe suitable treatment.[8,22,29]

In 2021, the WHO published updated guidelines on using NAATs for the detection of TB and drug-resistant TB. This update, part of the WHO's consolidated guidelines on TB, highlights several WHO-endorsed molecular tests, including LPAs, Xpert Ultra, LAMP, and Truelab.[29] These tests are crucial for the rapid and accurate diagnosis of TB and the identification of drug resistance.

The WHO specifically recommends using Xpert Ultra as the initial diagnostic test for TB in children presenting with symptoms suggestive of PTB. This recommendation includes the use of various sample types, such as sputum, gastric aspirate, nasopharyngeal aspirate, or stool, to rapidly detect MTB and assess RIF resistance. This approach is preferred over traditional methods like smear microscopy, culture, and phenotypic DST due to its speed and accuracy.[30]

The rapid and accurate detection of MTBC and its drug susceptibility patterns is of great importance for timely treatment initiation and disease control. Numerous molecular methods are currently used for these purposes. There are two main methods for molecular analyses: probe-based tests and sequencing analyses.[8] The main difference between the two is that probe-based tests can detect target gene mutations but cannot provide sequence information for other mutations. This difference is significant for detecting drug resistance and conducting molecular epidemiological studies.

Probe-Based Tests

Nucleic Acid Amplification Test (NAAT): Due to challenges in the conventional bacteriological diagnosis of TB, NAATs have emerged as a potential alternative. In NAATs, a specific nucleic acid probe amplifies the target DNA region. Some NAATs can also detect genes encoding drug resistance. These tests, with rapid turnaround times, can provide results at the time of patient presentation and facilitate the initiation of treatment, thereby preventing losses during patient follow-up.[31] Most probe-based NAATs detect the mycobacterial insertion element IS6110 for the identification of MTBC.[32]

NAATs can directly detect MTBC DNA from patient samples, whether smear-positive or smear-negative. Studies indicate that these tests demonstrate very high sensitivity (98–100%) in smear-positive patients, though sensitivity in smear-negative patients ranges from approximately 51% to 65%.[33] The NAATs recommended by the WHO for TB and drug resistance detection include Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA), Truenat MTB-RIF Dx (Molbio Diagnostic, Goa, India), BD MAX MDR-TB (Becton Dickinson, Sparks, MD, USA), Real Time MTB RIF/INH (Abbott, Molecular, DesPlaines, IL, USA), FluoroType MTB (Bruker, Hain Life Science, Germany), and Cobas MTB RIF/INH (Roche Molecular Diagnostics, Pleasanton, CA, USA) (Table 1).[30]

Table 1. World Health Organization-recommended molecular rapid diagnostic tests for tuberculosis detection[30]

Test	Manufacturer	Description	Type of approval	Resistance approval
Initial diagnostic tests for TB diagnosis without detection of drug resistance				
Loopamp™ MTBC detection kit	Eiken Chemical, Tokyo, Japan	Manual or automated NAAT	Individual	None
FluoroType® MTB	Bruker/Hain Lifescience, Nehren, Germany	Automated NAAT	MC-NAAT	None
Initial diagnostic tests for TB diagnosis with detection of drug resistance				
Xpert® MTB/RIF	Cepheid, Sunnyvale, CA, USA	Automated NAAT	Individual	RIF
Xpert MTB/RIF Ultra	Cepheid, Sunnyvale, CA, USA	Automated NAAT	Individual	RIF
Truenat MTB-RIF-Dx	Molbio Diagnostics, Goa, India	Automated NAAT	Individual	RIF
RealTime MTB RIF/INH	Abbott Molecular, Des Plaines, IL, USA	Automated NAAT	MC-NAAT	RIF, INH
BD MAX™ MDR-TB	Becton Dickinson, Sparks, MD, USA	Automated NAAT	MC-NAAT	RIF, INH
FluoroType MTBDR	Bruker/Hain Lifescience, Nehren, Germany	Automated NAAT	MC-NAAT	RIF, INH
Cobas® MTB-RIF/INH	Roche Molecular Diagnostics, Pleasanton, CA, USA	Automated NAAT	MC-aNAAT	RIF, INH

AMK: Amikacin; DR-TB: Drug-resistant tuberculosis; ETO: Ethionamide; FL-LPA: First-line line-probe assay; TB: Tuberculosis; INH: Isoniazid; RIF: Rifampicin; MC-aNAAT: Moderate Complexity Automated Nucleic Acid Amplification Test; USA: United States of America.

NAATs are generally more sensitive than smears but less sensitive than cultures. A sample with as few as 1 to 10 organisms per milliliter can yield a positive NAAT result. A positive NAAT result supports the diagnosis of TB under appropriate clinical and epidemiological conditions, and positive NAAT results combined with smear positivity are considered sufficient for a TB diagnosis. However, a negative NAAT result is not sufficient to rule out active TB or drug resistance. NAATs do not replace the roles of smear and culture in the TB diagnostic algorithm; culture is essential for confirmation of identification and drug susceptibility testing.[34]

Xpert® MTB/RIF Assay

The Xpert® MTB/RIF assay uses real-time multiplex PCR to amplify an 81-base pair core region of the rpoB gene, which is specific to MTB and associated with RIF resistance mutations. These mutations are detected using molecular beacon technology. This "laboratory in a cartridge" is straightforward to perform, poses a lower biohazard risk, is robust, and provides rapid results within approximately 90 minutes. The assay demonstrates a high sensitivity of 98% for detecting RIF resistance and the presence of tubercle bacilli in smear-positive cases, and a sensitivity of 70% for smear-negative samples.[8]

In 2010, the WHO endorsed the use of Xpert MTB/RIF on the GeneXpert platform developed by Cepheid, based in Sunnyvale, United States. This endorsement was updated in 2013, and by 2017, the WHO recommended an advanced version, Xpert Ultra (also by Cepheid), as the initial diagnostic test for TB in both adults and children, regardless of HIV status. This recommendation superseded smear microscopy and culture-based methods. Similar to its predecessor, Xpert Ultra detects RIF resistance by targeting the rpoB gene with four specific probes and uses melting temperature analysis as part of its diagnostic process. This technology represents a significant advancement in TB diagnostics, offering improved sensitivity and specificity compared to traditional methods, thereby enhancing early detection and management of TB cases worldwide.[22]

The Ultra test cartridges for Xpert MTB/RIF feature a larger chamber for DNA amplification and incorporate two multi-copy amplification targets specific to TB: IS6110 and IS1081. These enhancements allow Ultra to detect TB at a lower limit of 16 CFU/mL.

These improvements have increased Ultra's overall sensitivity from 85% (with a 95% confidence interval [CI] ranging from 82% to 88%) to 88% (95% CI, 85% to 91%). However, the specificity of Xpert Ultra is slightly lower at 96% (95% CI, 90% to 98%) compared to the previous generation, which had a specificity of 98% (95% CI, 97% to 98%). This decrease in specificity is primarily due to Ultra's ability to detect nonviable bacteria, which is particularly notable in individuals with recent

TB. Despite this slight decrease in specificity, the enhanced sensitivity of Xpert Ultra represents a significant improvement in detecting TB cases, especially those with low bacterial loads. This makes Xpert Ultra a highly valuable tool in the early and accurate diagnosis of TB, contributing to better management and control of the disease globally.[29]

The WHO recommends using Xpert Ultra as the initial diagnostic test for TB in children presenting with symptoms suggestive of PTB. This recommendation prioritizes the Xpert Ultra test over traditional methods such as smear microscopy, culture, and phenotypic DST. Xpert Ultra is particularly valuable as it can rapidly and accurately detect MTB and assess RIF resistance using a variety of samples, including sputum, gastric aspirate, nasopharyngeal aspirate, or stool.

By adopting Xpert Ultra, healthcare providers can ensure more accurate and faster diagnosis, which is essential for reducing TB transmission and improving patient outcomes. The recommendation also emphasizes the importance of skilled and trained technicians to handle the test and interpret results accurately. The operational handbook serves as a comprehensive guide for implementing these recommendations in various healthcare settings.[9]

Truenat MTB Test

The Truenat MTB-RIF Dx system, developed by Bigtec Laboratories in Bangalore, India, and endorsed by the WHO, represents a significant advancement in TB diagnostics. This portable, battery-operated nucleic acid test swiftly identifies MTB and assesses RIF resistance. The test involves sample preparation, DNA extraction, and amplification using real-time PCR with a portable analyzer. Results from the Truenat MTB test can be printed or transmitted electronically via SIM card, Bluetooth, or WiFi. Studies show comparable accuracy to the Xpert MTB/RIF assay for MTB detection and RIF resistance and similar performance to TB-LAMP as a replacement for AFB smear microscopy. The system achieves a sensitivity of 91.1% and specificity of 100% for detecting MTB, making it a valuable tool for TB diagnosis in diverse settings.[35]

Line Probe Assay (LPA)

Line probe assays detect MTB and drug-resistant mutations through reverse hybridization on a strip containing specific oligonucleotide probes after PCR. A colored band indicates MTBC and drug resistance. The WHO recommends several LPA-based commercial tests, including the GenoType *Mycobacterium tuberculosis* drug resistance (MTBDR) plus 1.0–2.0 test and the Genoscholar™ nontuberculous mycobacteria + multidrug-resistant tuberculosis (NTM + MDRTB) Detection Kit. The latest LPAs target common mutations associated with resistance to first- and second-line anti-TB agents, as well as specific wild-type MTBC sequences.[36]

Mutations are detected by the binding of amplicons to oligonucleotides containing mutations or by the absence of amplicon binding to oligonucleotides corresponding to wild-type (WT) sequences.

However, LPAs have some limitations. While they detect the most common mutations in resistant strains, some resistance-causing mutations may lie outside the regions covered by the test, so resistance cannot be entirely ruled out if all WT probes are present. Additionally, in samples containing both drug-sensitive and resistant bacteria, LPAs are less effective than traditional culture-based DST in detecting resistance. If resistant bacteria constitute at least 5% of the total population, LPAs can be used to identify resistant bacteria with mutations. For first-line drugs, LPAs have demonstrated 95.8% sensitivity and 98.4% specificity for detecting RIF resistance, and 94.5% sensitivity and 99.3% specificity for detecting INH resistance. For second-line drugs, combined sensitivity and specificity are 87.0% and 99.5%, respectively.[8]

LPAs are recommended by the WHO as initial tests for detecting INH and RIF resistance in sputum-positive and/or culture-positive PTB and EPTB. They are endorsed for detecting MDR-TB, including resistance to first-line drugs like INH and RIF. Advancements in LPAs, such as the GenoTypeMTBDRsl version 2.0, enhance sensitivity to detect mutations associated with resistance to fluoroquinolones and second-line injectable drugs (kanamycin, amikacin, capreomycin). These improvements play a crucial role in guiding the initiation of treatment for MDR-TB.[8,29]

Loop-Mediated Isothermal Amplification (LAMP)

LAMP is a straightforward, rapid, precise, and economical nucleic acid amplification (NAA) method developed by Eiken Chemical, Japan. This method employs at least four distinct sets of primers, each designed to recognize a specific region of the target gene. Amplification is conducted in a single step via a strand displacement reaction at a constant temperature of 63–67°C for 15–60 minutes. The amplification efficiency is exceptionally high, and results are evaluated visually by inspecting fluorescence within the contents. No sophisticated equipment is required for results interpretation; evaluation under ultraviolet light is an option. This methodology has been effectively implemented for tuberculosis diagnosis in resource-limited, high-endemic settings. The sensitivity and specificity of the LAMP test have been observed to range between 76–80% and 97–98%, respectively. Additionally, the WHO has recommended this test for diagnosing active TB in peripheral laboratory settings.[37–39]

Sequencing-Based Tests

Targeted Next-Generation Sequencing (NGS): Targeted next-generation sequencing (NGS) can predict resistance to multiple anti-TB drugs simultaneously by amplifying various regions of the MTBC genome. This method addresses the critical need for rapid prediction of resistance to both first- and second-line TB drugs. In March 2024, the WHO approved targeted NGS for genotypic DST in drug-resistant TB cases, emphasizing its importance as a research and development priority for TB diagnostic tools.[40]

NAATs target a small number of known resistance-conferring mutations and can analyze resistance to a limited number of drugs. In contrast, targeted NGS examines multiple genomic regions associated with primary and secondary drugs simultaneously, providing a comprehensive approach to drug resistance surveillance. Studies have found the combined sensitivity and specificity of targeted NGS for all anti-TB drugs to be 94.1% (90.9–96.3) and 98.1% (97.0–98.9), respectively.[41] Targeted NGS, which can be performed directly on clinical samples, is an important diagnostic tool that identifies MTBC, provides genotypic resistance profiles to drugs in a short time, and supports clinical management.

Whole Genome Sequencing (WGS): Whole genome sequencing is the process of reading all nucleotide base pairs in an organism's genome. Since the first complete genome sequence of the H37Rv strain was published in 1998, WGS applications for MTBC have been significantly developed. WGS can guide all components of TB control, including diagnosis, resistance analysis, treatment, surveillance, and source investigation.[42] It can identify genotypes associated with drug resistance throughout the microbial genome and has the potential to aid clinical decision-making. Combining WGS data with epidemiological analyses enables the identification of disease spread patterns and risk factors, facilitating the development of TB control strategies.[43] Standard steps for WGS analysis include culturing samples, DNA extraction, library preparation, and sequencing using short-read (e.g., Illumina platforms) or long-read (e.g., Oxford Nanopore platforms) technologies.[42,44]

However, there are some limitations to the NGS technologies in use. The repeat-rich genome regions of MTBC complicate bioinformatic processes and can lead to erroneous sequencing.[45] Additionally, the need for culture, high costs, data-based inefficiencies, and the requirement for experienced personnel present further challenges.

The WHO is expected to release guidelines on DNA sequencing technology in TB diagnostics, which could potentially enhance WGS adoption in clinical settings, pending validation of high-confidence drug resistance mutations.

Immunological Methods

The Tuberculin Skin Test (TST)

The tuberculin skin test has been used for approximately a century to diagnose TB and detect exposure to *Mycobacterium tuberculosis*. The primary limitation of purified protein derivative (PPD) tuberculin is that its protein components are shared among mycobacterial and unrelated bacterial species. The PPD tuberculin test gauges the immune response by measuring the size of the resulting induration (≥5 mm is considered positive) at the injection site after 48 to 72 hours. Skilled personnel are needed for both the injection and interpretation of results.[8,46]

In regions where MTB is less common, the TST is effective at detecting exposure. The greatest drawback of PPD lies in yielding false positives in individuals from high-prevalence TB countries, those vaccinated with BCG, and those infected with NTM. Additionally, its sensitivity is notably low in immune-compromised individuals.[8,46]

Recent global guidelines recommend MTB antigen-based skin tests, such as the Early Secretory Antigenic Target 6-Culture Filtrate Protein 10 (ESAT6-CFP10 (EC)) skin test, as acceptable alternatives to the TST and interferon-gamma release assays (IGRA). The EC is a diagnostic test used for TB infection diagnosis, employing the antigens ESAT-6 and CFP-10 from MTB. These antigens are specific markers absent in BCG vaccine strains and most nontuberculous mycobacteria, making the EC particularly useful for distinguishing TB infection from other mycobacterial infections. The test is typically administered as a skin test, introducing the ESAT-6 and CFP-10 antigens into the skin to elicit an immune response. It serves as an alternative to traditional tests like the TST and QuantiFERON-TB Gold In-Tube test (QFT), offering high specificity and moderate to high sensitivity. The clinical utility of the EC is emphasized in populations where BCG vaccination is common or among immunocompromised individuals, such as those living with HIV/AIDS, where cross-reactivity or a reduced immune response may affect the accuracy of traditional TB testing. Globally, EC-based tests are recommended as viable options for TB diagnosis, particularly in regions with a high prevalence of HIV and BCG vaccination.[47]

Lu et al.[47] conducted a study to evaluate the diagnostic accuracy of the EC skin test among persons living with HIV (PLHIV) in Jiangsu Province, China. Recent global guidelines suggest EC as a viable alternative to the TST and QFT, but its performance in PLHIV remains uncertain. The study enrolled 350 PLHIV and compared EC, QFT, and TST results, stratifying by age, BCG vaccination status, and CD4 count. EC positivity rates were 16.6%, compared to 25.4% for QFT and 16.9% for TST. Sensitivity of the EC test at a 5-mm QFT cutoff was moderate (81.4%), with

high specificity (99.6%). Stratified by BCG vaccination status, sensitivity was 86.4% among vaccinated PLHIV and 76.2% among unvaccinated PLHIV, with consistently high specificity (99.1% to 100.0%). Overall, the EC skin test showed comparable performance to TST, suggesting its validity as an alternative diagnostic tool in high HIV prevalence and BCG vaccinated populations. This study represents the first evaluation of EC among PLHIV, highlighting its robust diagnostic potential across varied demographic and health status factors.

Interferon-Gamma Release Assays (IGRA)

New in vitro assays have been developed thanks to advances in molecular biology and genomics. These assays measure interferon-gamma (IFN-γ) released by T cells when stimulated with MTB antigens. Early versions used PPD as the stimulating antigen, while newer versions use antigens more specific to MTB. Newer antigens such as ESAT-6, CFP-10, and TB7.7 are encoded by genes in the MTB genome's region of difference 1 (RD1) segment. They are more specific than PPD because they are not shared with BCG strains or certain nontuberculous mycobacteria. Using these specific antigens in an ex vivo assay format distinguishes modern IGRAs.[48]

IGRAs are particularly useful in populations unlikely to return for a TST reading, or in individuals with known MTB exposure, HIV infection, or other immunocompromising conditions.[8]

Unlike TST, which requires skilled personnel for injection and interpretation, IGRAs involve collecting blood samples into tubes coated with MTB antigens. The IFN-γ release is then measured using an enzyme immunoassay technique. This method is widely available in countries like the United States and across Europe but is less accessible in many developing countries, limiting its global applicability.[8]

Although discordant results have been reported, most studies show a concordance of 60–90%. TST positives but IGRA negatives are likely due to previous BCG vaccination.[48]

Urinary Antigen Detection

A promising immune-based strategy involves detecting an MTB antigen, lipoarabinomannan (LAM), in urine. LAM is a glycolipid specific to mycobacteria and is released by metabolically active bacteria. It passes through the renal filtration system and can be detected in the urine of patients with active TB. Initially, LAM was detected in serum, but this method had limitations.[49]

LAM serves as a noninvasive biomarker for MTB detection in various body fluids. Urine is easier to obtain, more consistent, and safer to handle than sputum. Studies have assessed the accuracy of tests using enzyme-linked immunosorbent assay (ELISA) for detecting LAM in urine to diagnose TB. New LAM tests for urine include Fuji LAM and Alere LAM. The WHO recommends the urine LAM test for diagnosing PTB or EPTB in HIV-positive patients in critical condition or with low CD4+ cell counts.[19]

LAM ELISA tests have shown sensitivity ranging from 38% to 50.7% and specificity from 87.8% to 89% for TB detection. These findings were corroborated using smear microscopy, solid culture, and/or liquid culture as reference standards. Sensitivity was higher for patients with HIV-related TB and for females; however, it dropped to 28% for smear-negative patients.

Commercial LAM ELISA tests are often specific but lack sensitivity, making them unreliable for diagnosing or ruling out PTB in HIV-infected or non-infected patients. These assays can enhance case detection when combined with smear microscopy and/or culture, especially in regions with a high prevalence of HIV. Such tests may aid in diagnosing TB in HIV-coinfected patients with low CD4 cell counts. The urine LAM assay has a high negative predictive value, so a negative result excludes active TB. Urine-based tuberculosis diagnosis is a promising possibility, with ongoing research on improving the LAM assay. New LAM ELISAs are being developed, including in-tube and dipstick methods. Further refinement may produce a lateral flow test. Lateral flow tests can improve case detection by making testing more accessible. Techniques such as using larger urine samples, optimized extraction methods, and stabilizing agents at the time of collection could enhance the sensitivity of these tests. Additionally, exploring more urinary antigens may improve TB diagnostics. These efforts aim to make urine-based TB diagnostics more effective and accessible.[50,51]

Gold Nanoparticles (AuNPs)

AuNPs are increasingly used in TB diagnostic tests due to their unique properties. These nanostructures offer a high surface area-to-volume ratio, excellent biocompatibility, and remarkable optical properties, making them suitable for enhancing sensitivity and specificity in biosensing applications. In TB diagnostics, AuNPs can be functionalized with antibodies or antigens specific to MTB, enabling the detection of TB biomarkers with high accuracy. They enhance the performance of biosensors by improving signal transduction mechanisms, such as optical signal amplification or electrochemical detection, thereby facilitating rapid and sensitive detection of MTB in clinical samples. AuNPs continue to be explored and developed as integral components of next-generation TB diagnostic technologies aimed at improving early detection and management of the disease.[52]

CONCLUSION

In conclusion, pediatric TB presents a decreased microbiological diagnostic yield due to the difficulty of collecting respiratory samples and the paucibacillary nature of clinical samples. The GeneXpert Ultra assay has launched a new era of diagnostics for childhood TB. GeneXpert Ultra is a rapid test used for the detection of MTBC and identification of RIF resistance and is a cartridge-based NAAT. The WHO recommends using Xpert MTB/RIFTB in children with signs and symptoms of PTB in sputum, gastric aspirate, nasopharyngeal aspirate, and stool as the initial diagnostic test before smear microscopy, culture, and phenotypic DST for TB diagnosis and detection of resistance. Despite these encouraging developments in molecular tests for TB diagnosis, the application of conventional diagnostic methods, such as culture and microscopy, is essential for increasing diagnostic sensitivity. The LAM test, an immunological test based on detecting lipoarabinomannan, the MTB antigen, in urine as a non-invasive sample, is also considered promising.

At present, no single diagnostic test can accurately and universally detect tuberculosis in all patients. A combination of different tests must be employed and incorporated into a diagnostic algorithm to ensure the appropriate use of all available diagnostic modalities. This approach must consider factors such as the capacity of different healthcare levels and the requirements for infrastructure and manpower.

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REFERENCES

1. Daniel TM. The history of tuberculosis. Respir Med 2006; 100(11): 1862–70. [CrossRef]
2. Aslan G, Yapici G. Tuberculosis Immunology; the Ambivalence of the Ancient Disease Continues to Amaze. Turk J Immunol 2022; 10(3): 121–33. [CrossRef]
3. Barberis I, Bragazzi NL, Galluzzo L, Martini M. The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. J Prev Med Hyg 2017; 58(1): E9–E12.
4. U.S. Centers for Disease Control and Prevention (CDC). History of World TB Day. 2023 October 18. Available from: URL: http:/www.cdc.gov/tb/worldtbday/history.htm. Accessed Apr 14, 2024.
5. Zhang H, Liu M, Fan W, Sun S, Fan X. The impact of Mycobacterium tuberculosis complex in the environment on one health approach. Front Public Health 2022; 10: 994745. [CrossRef]
6. Vilchèze C. Mycobacterial cell wall: a source of successful targets for old and new drugs. Appl Sci 2020; 10(7): 2278.
7. World Health Organizations. Global Tuberculosis Report. 2023. Available from: URL: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023/tb-disease-burden. Accessed Apr 14, 2024.
8. Acharya B, Acharya A, Gautam S, Ghimire SP, Mishra G, Parajuli N, et al. Advances in diagnosis of Tuberculosis: an update into molecular diagnosis of Mycobacterium tuberculosis. Mol Biol Rep 2020; 47(5): 4065–75. [CrossRef]
9. Zaporojan N, Negrean RA, Hodișan R, Zaporojan C, Csep A, Zaha DC. Evolution of laboratory diagnosis of tuberculosis. Clin Pract 2024; 14(2): 388–416. [CrossRef]
10. Campelo TA, Cardoso de Sousa PR, Nogueira LL, Frota CC, Zuquim Antas PR. Revisiting the methods for detecting Mycobacterium tuberculosis: what has the new millennium brought thus far? Access Microbiol 2021; 3(8): 000245. [CrossRef]
11. Vilchèze C, Kremer L. Acid-fast positive and acid-fast negative mycobacterium tuberculosis: the koch paradox. Microbiol Spectr 2017;5(2): TBTB2-0003-2015. [CrossRef]
12. Gurusamy VR, Sundararajan K, Prakasam G, Vincy TM. Extrapulmonary tuberculosis-A cross-sectional study comparing the utility of Ziehl-Neelsen stain and immunohistochemistry. Asian J Med Sci 2024; 15(1): 142–9.
13. Leylabadlo HE, Kafil HS, Yousefi M, Aghazadeh M, Asgharzadeh M. Pulmonary tuberculosis diagnosis: where we are?. Tuberc Respir Dis (Seoul) 2016; 79(3): 134–42.
14. World Health Organizations. Global Tuberculosis Report 2022. Available from: URL: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022. Accessed Jul 4, 2024.
15. World Health Organizations. Global Tuberculosis Report 2021. Available from: URL: https://www.who.int/publications/i/item/9789240037021. Accessed Apr 14, 2024.
16. Wu X, Tan G, Gao R, Yao L, Bi D, Guo Y, et al. Assessment of the Xpert MTB/RIF Ultra assay on rapid diagnosis of extrapulmonary tuberculosis. Int J Infect Dis 2019; 81:91–6.
17. Gopalaswamy R, Dusthackeer VA, Kannayan S, Subbian S. Extrapulmonary tuberculosis—an update on the diagnosis, treatment and drug resistance. J Respir Med 2021; 1(2): 141–64. [CrossRef]

18. Golden MP, Vikram HR. Extrapulmonary tuberculosis: an overview. Am Fam Physician 2005; 72(9): 1761–8.
19. World Health Organizations. WHO consolidated guidelines on tuberculosis: module 4: treatment: drug-resistant tuberculosis treatment. Available from: URL: https://www.who.int/publications/i/item/9789240007048. Accessed Jul 1, 2024.
20. World Health Organizations. WHO treatment guidelines for drug-resistant tuberculosis, 2016 update. Available from: URL: https://www.who.int/publications/i/item/9789241549639. Accessed Apr 14, 2024.
21. Kapil A. Ananthanarayan & Paniker's Textbook of Microbiology. 9th edition. Orient Blackswan; 2013.
22. Natarajan A, Beena PM, Devnikar AV, Mali S. A systemic review on tuberculosis. Indian J Tuberc 2020; 67(3): 295–311. [CrossRef]
23. Cuevas LE, Al-Sonboli N, Lawson L, Yassin MA, Arbide I, Al-Aghbari N, et al. LED fluorescence microscopy for the diagnosis of pulmonary tuberculosis: a multi-country cross-sectional evaluation. PLoS Med 2011; 8(7): e1001057.
24. Schwarze K, Buchanan J, Taylor JC, Wordsworth S. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genet Med 2018;20:1122–30. [CrossRef]
25. Nambiar R, Chatellier S, Bereksi N, van Belkum A, Singh N, Barua B, et al. Evaluation of Mycotube, a modified version of Lowenstein-Jensen (LJ) medium, for efficient recovery of Mycobacterium tuberculosis (MTB). Eur J Clin Microbiol Infect Dis 2017; 36(10): 1981–8. [CrossRef]
26. Joloba ML, Johnson JL, Feng PJ, Bozeman L, Goldberg SV, Morgan K, et al. What is the most reliable solid culture medium for tuberculosis treatment trials? Tuberculosis (Edinb) 2014; 94(3): 311–6. [CrossRef]
27. Samant H, Desai D, Abraham P, Joshi A, Gupta T, Rodrigues C, et al. Acid-fast bacilli culture positivity and drug resistance in abdominal tuberculosis in Mumbai, India. Indian J Gastroenterol 2014; 33(5): 414–9. [CrossRef]
28. Procop GW. Laboratory diagnosis and susceptibility testing for Mycobacterium tuberculosis. Tuberculosis and Nontuberculous Mycobacterial Infections 2017. pp. 45–58.
29. MacLean E, Kohli M, Weber SF, Suresh A, Schumacher SG, Denkinger CM, et al. Advances in Molecular Diagnosis of Tuberculosis. J Clin Microbiol 2020; 58(10): e01582–19.
30. World Health Organizations. Manual for selection of molecular WHO-recommended rapid diagnostic tests for detection of tuberculosis and drug-resistant tuberculosis. Available from: URL: https://www.who.int/publications/i/item/9789240042575. Accessed Mar 10, 2024.
31. Nurwidya F, Handayani D, Burhan E, Yunus F. Molecular diagnosis of tuberculosis. Chonnam Med J 2018; 54(1): 1–9. [CrossRef]
32. Tiwari S, Nataraj G, Kanade S, Mehta P. Diagnosis of pediatric pulmonary tuberculosis with special reference to polymerase chain reaction based nucleic acid amplification test. Int J Mycobacteriol 2015; 4(1): 48–53.
33. Boldi MO, Denis-Lessard J, Neziri R, Brouillet R, von-Garnier C, Chavez V, et al. Performance of microbiological tests for tuberculosis diagnostic according to the type of respiratory specimen: A 10-year retrospective study. Front Cell Infect Microbiol 2023; 13: 1131241. [CrossRef]
34. UpToDate. Diagnosis of pulmonary tuberculosis in adults. Available from: URL: https://www.uptodate.com/contents/diagnosis-of-pulmonary-tuberculosis-in-adults. Accessed Oct 17, 2024.
35. Yadav R, Jyoti A, Manpreet B. Laboratory diagnosis of tuberculosis: conventional and newer methods. Int J Sci Res 2020; 9(10): 319–24.
36. World Health Organizations. Line probe assays for detection of drug-resistant tuberculosis Interpretation and reporting manual for laboratory staff and clinicians. 2022. Available from: URL: https://iris.who.int/bitstream/handle/10665/354240/9789240046665-eng.pdf?sequence=1. Accessed Apr 14, 2024.
37. Pandey BD, Poudel A, Yoda T, Tamaru A, Oda N, Fukushima Y, et al. Development of an in-house LAMP assay for detection of Mycobacterium tuberculosis and evaluation in sputum samples of Nepalese patients. J Med Microbiol 2008; 57(Pt 4): 439–43. [CrossRef]
38. World Health Organizations. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance. 2016. 2016 Available from: URL: https://iris.who.int/bitstream/handle/10665/249154/9789241511186-eng.pdf. Accessed Apr 14, 2024.
39. Boehme CC, Nabeta P, Henostroza G, Raqib R, Rahim Z, Gerhardt M, et al. Operational feasibility of using LAMPfor diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. J Clin Microbiol 2007; 45(6): 1936–40. [CrossRef]
40. World Health Organizations. WHO launches new guidance on the use of targeted next-generation sequencing tests for the diagnosis of drug-resistant TB and a new sequencing portal. Available from: URL: https://www.who.int/news/item/20-03-2024-who-launches-new-guidance-on-the-use-of-targeted-next-generation-sequencing-tests-for-the-diagnosis-of-drug-resistant-tb-and-a-new-sequencing-portal. Accessed Jul 2, 2024.

41. Schwab TC, Perrig L, Göller PC, Guebely De la Hoz FF, Lahousse AP, Minder B, et al. Targeted next-generation sequencing to diagnose drug-resistant tuberculosis: a systematic review and meta-analysis. Lancet Infect Dis 2024; S1473-3099(24); 00263–9. [CrossRef]
42. Meehan CJ, Goig GA, Kohl TA, Verboven L, Dippenaar A, Ezewudo M, et al. Whole genome sequencing of Mycobacterium tuberculosis: current standards and open issues. Nat Rev Microbiol 2019; 17(9): 533–45. [CrossRef]
43. Kizny Gordon A, Marais B, Walker TM, Sintchenko V. Clinical and public health utility of Mycobacterium tuberculosis whole genome sequencing. Int J Infect Dis 2021; 113 Suppl 1: S40–2. [CrossRef]
44. Dippenaar A, Goossens SN, Grobbelaar M, Oostvogels S, Cuypers B, Laukens K, et al. Nanopore sequencing for mycobacterium tuberculosis: a critical review of the literature, new developments, and future opportunities. J Clin Microbiol 2022; 60(1): e0064621. [CrossRef]
45. Thorpe J, Sawaengdee W, Ward D, Campos M, Wichukchinda N, Chaiyasirinroje B, et al. Multi-platform whole genome sequencing for tuberculosis clinical and surveillance applications. Sci Rep 2024; 14(1): 5201. [CrossRef]
46. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet 2000; 356(9235): 1099–104. [CrossRef]
47. Lu P, Wu K, Zhou H, Yu H, Yuan J, Dong L, et al. Evaluation of ESAT6-CFP10 skin test for Mycobacterium tuberculosis Infection among persons living with HIV in China J Clin Microbiol 2023; 61(4): e0181622. [CrossRef]
48. Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: part I. Latent tuberculosis. Expert Rev Mol Diagn 2006; 6(3): 413–22.
49. Parsons LM, Somoskövi A, Gutierrez C, Lee E, Paramasivan CN, Abimiku A, Spector S, et al. Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. Clin Microbiol Rev 2011; 24(2): 314–50.
50. Seid G, Alemu A, Tsedalu T, Dagne B. Value of urine-based lipoarabinomannan (LAM) antigen tests for diagnosing tuberculosis in children: systematic review and meta-analysis. IJID Reg 2022; 4: 97–104. [CrossRef]
51. Dhana A, Hamada Y, Kengne AP, Kerkhoff AD, Broger T, Denkinger CM, et al. Diagnostic accuracy of WHO screening criteria to guide lateral-flow lipoarabinomannan testing among HIV-positive inpatients: A systematic review and individual participant data meta-analysis. J Infect 2022; 85(1): 40–8. [CrossRef]
52. Eivazzadeh-Keihan R, Saadatidizaji Z, Mahdavi M, Maleki A, Irani M, Zare I. Recent advances in gold nanoparticles-based biosensors for tuberculosis determination. Talanta 2024; 275: 126099. [CrossRef]