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Evolution of Mycobacterium Tuberculosis Diagnostics: From Classic Methods to High-Resolution Molecular Platforms: A Literature Review

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Abstract

Despite the availability of effective therapies, tuberculosis (TB) remains one of the most challenging infectious diseases around the world. Accurate and early laboratory analysis was fundamental for sickness, treatment initiation and prevention of transmission to other peoples. The evaluation highlights detect of diagnostic strategies for Mycobacterium Tuberculosis (MTB) that integrate traditional, molecular, immunologic and superior techniques. Conventional methods, including acid-fast bacilli (AFB) by microscope and growth on Lowenstein–Jensen or Middle brook media, remain the cornerstone for confirmation and drug susceptibility testing, even though they may be time-consuming. The advanced and automated liquid culture systems like BACTEC MGIT 960 have markedly decreased detection time while keeping excessive sensitivity and specificity. By enabling rapid detection of MTB and rifampicin or multidrug resistance within hours, the molecular tests such as Expert MTB/RIF, Expert Ultra, True Nat MTB, line probe assays and polymerase chain reaction - based technologies offer more accurate methods and have transformed TB diagnosis. Histopathology and cytology remain important for extra pulmonary TB cases, although immunological diagnostics such as interferon-gamma release assays (IGRA) and T-spot methods that associated with the identification of latent TB infection. Although their routine use was still hindered by infrastructure and expense, emerging technologies such as whole genome sequencing (WGS), loop-mediated isothermal amplification (LAMP) and nano pore sequencing provide high-resolution data for strain typing of MTB and drug resistance profiling.

Keywords TB, Mycobacterium Tuberculosis, PCR, AFB, IGRAs, DST

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List of abbreviations: DST = Drug susceptibility testing, IGRAs = Interferon-gamma release assays, LPAs = Line probe assays, MTB = Mycobacterium Tuberculosis, MTBC = Mycobacterium Tuberculosis complex, NAATs = Nucleic acid amplification test, PCR = Polymerase chain reaction, TB = Tuberculosis, WHO = World Health Organization

Introduction

Tuberculosis (TB) is one of the oldest and most persistent infectious diseases affecting humanity worldwide [(1)]. It is ordinarily resulting from Mycobacterium Tuberculosis (MTB), gradually-growing, acid-fast bacilli (AFB) belonging to the Mycobacterium tuberculosis complex (MTBC) [(2)]. Despite being curable and preventable, TB remains a significant worldwide. According to the World Health Organization (WHO) Global TB Report 2024, an estimated 10.7 million people fell ill with TB worldwide, while about 1.3 million people will die– making it the second main infectious purpose of loss of life

after COVID-19 [3]. The laboratory diagnosis of TB plays a valuable position within the international approach to combat the disease [4,5].

Early and correct detection is crucial not only for initiating appropriate therapy response additionally for interrupting transmission, tracking remedy reaction and detecting of drug resistance, mainly in the generation of multidrug-resistant TB (MDR-TB) and extensive drug-resistant TB (XDR-TB). In the past, radiologic evidence and bacteriological confirmation using AFB microscopy and classical culture were used to diagnose TB primarily based on scientific suspicion [6,7]. Even though these conventional strategies are still critical, they have a few considerable drawbacks. For example, smear microscopy (AFB) is not always touchy enough to come across bacillary ailment of MTB and way of life may be very touchy and particular, but it takes several weeks for the organism to develop because MTB replicates slowly [8].

Diagnostic strategies have changed dramatically for the reason that improvement of cutting-edge laboratory medicine. Nucleic acid amplification test (NAATs) such as the Xpert MTB/RIF and Xpert Ultra, have completely modified the analysis of TB by using quickly detecting both MTB and rifampicin resistance within hours [9]. Precise the drug susceptibility testing (DST) was made possible by means of automatic liquid lifestyle structures (like MGIT 960) and line probe assays (LPAs), which similarly improve sensitivity. While histological and cytological investigations continue to be essential within the prognosis of extra pulmonary TB (EPTB), wherein microbiological yield was regularly inadequate, immunological procedures together with interferon-gamma release assays (IGRAs) have stepped forward the detection of latent TB infection (LTBI) [10,11].

The modern-day preferred for TB diagnosis was the aggregate of molecular and immunologic improvements with conventional microbiological techniques [12]. However, the problems still exist, especially in resource-poor environments where access to modern molecular systems is limited and the burden of disease was highest. Therefore, it is important for physicians, microbiologists and public health personnel involved in TB control to understand the concepts, techniques and limitations of various diagnostic tools [13].

Samples collection and processing of MTB

1. Pulmonary TB

Since in a single day accumulation of secretions from the bronchial tree incorporates the highest concentration of bacilli, early morning sputum amassed over to a few days was the suitable specimen [14]. Early morning sputum collected over three consecutive days, not saliva, expectorated right into a sterile, wide-mouthed, leak-proof container after the patient has rinsed their mouth with easy water to reduce oral contamination [15]. Gastric aspirates may be utilized in youngsters or unconscious sufferers who swallow sputum whilst they sleep, however, sputum induction the usage of hypertonic saline nebulization or bronchoscopic sampling; including Broncho alveolar lavage (BAL) or bronchial washings was usually recommended in sufferers who are not able to supply sputum on their very own [16]. To avoid the overgrowth of contaminants, the specimens have to be introduced to the laboratory within 1 to 2 hours of collection and stored in (2 to 8°C) if processing is postponed for greater than 24 hours [17].

2. Extra pulmonary TB

The type of specimen used in the laboratory diagnosis of EPTB depends on the affected site; examples include cerebrospinal fluid (CSF) for TB meningitis, pleural or pericardial fluid for serial involvement, fine needle aspiration cytology (FNAC) or lymph node biopsy, synovial fluid or tissue for musculoskeletal TB, urine for genitourinary TB, and tissue samples from liver and bone marrow was also tested [18]. Each sample should be collected aseptically, placed in an appropriate sterile cup, transported

rapidly to the laboratory (within 1-2 hours, or cooled at 2-8°C), and if solid tissue was recovered, it should be homogenized under sterile conditions or cut before processing [19].

3. Microscopic examination (AFB)

a. Ziehl-Nielsen (ZN) Stain

AFB test is a cornerstone in the laboratory diagnosis of TB, exploiting the unique mycolic acid-rich cell wall of MTB, which resists acid-alcohol discoloration and allows visualization in clinical specimens such as sputum, bronchoalveolar, lymphatic fluids, spinal fluid, urine and tissue biopsy [20]. Specimens were first treated with chemical digestion and decolonization using the N-Acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method to thin the mucus and suppress contaminating another's flora, then concentrated by centrifugation to increase bacillary yield before preparing smears for staining. ZN method stains when bacilli bright red on a blue background and was widely used because of its simplicity, rapid and low cost. In general, the sensitivity of this test was moderate with about 50-60% of pulmonary TB in detection of overall TB cases, while increase sensitivity of these laboratory methods in typically smear-positive pulmonary TB [21].

b. Auramine-Rhodamine fluorescent stain

This test that using of fluorescent stain and was an exceedingly touchy laboratory approach for detecting MTB and different AFB, exploiting the organism's mycolic acid-wealthy cell wall to bind the fluorochrome dyes, which emit brilliant yellow-orange fluorescence underneath ultraviolet or light-emitting diode (LED) mild, permitting speedy visualization of bacilli in clinical specimens which include sputum, bronchoalveolar lavage, CSF, pleural fluid, lymph node aspirates, synovial fluid, and tissue homogenates [22]. specimens are first concentrated after NALC–NaOH decontamination, implemented as thin smears, stained with Aura mine O (frequently mixed with Rhoda mine B), decolorized with acid-alcohol, optionally counterstained to lessen history, and examined at low magnification beneath a fluorescence microscope, supplying approximately 10-15% higher sensitivity than AFB , especially in smear-bad instances, at the same time as keeping high specificity (>95%) but unable to distinguish MTB from non-TB mycobacteria, making it a great speedy screening tool in high-quantity TB laboratories, regularly followed through tradition or molecular assays for definitive prognosis and drug susceptibility testing [23].

4. Culture techniques

The culture remains the gold standard because it not only confirms the presence of viable organisms but also provides isolates for comprehensive DST, which is crucial for guiding effective treatment regimens, preventing the spread of drug-resistant strains, and informing public health surveillance efforts. This capacity is indispensable for TB outbreak investigations and tracking transmission patterns, directly impacting community health and strategic TB control [24].

a. Solid media

- **Lowenstein–Jensen (LJ) and Ogawa media:** This is an egg-primarily conventional medium for the isolation and identification of different types of Mycobacterium spp, especially MTB, from different clinical samples like sputum and others sample collection (pleural fluid, wound swab, urine and others) based at the original system of Lowenstein that was in the end modified by Jensen, the medium consists of glycerol and egg, which provide the required protein and fatty acids [25].The coagulation of the egg albumin in the media of instruction also affords a stable floor for inoculation purposes. Malachite green was integrated into the medium to inhibit contaminating other microorganisms than the mycobacteria that could nevertheless be present in the specimen after decontamination. Explicitly state that the

incubation for LJ and Ogawa media is performed under aerobic conditions with 5-10% CO_2 to ensure complete procedural documentation and reproducibility of the culture method, as mycobacteria are generally obligate aerobes [26]. Container caps must be left loose for the primary week of incubation to permit the movement of the carbon dioxide as this could assist in stimulating growth of MTB [27].

- **Middle brook 7H10/7H11 agar:** This is obvious, nutrient-rich, agar-based totally sold media used in medical microbiology and bacteriology for the isolation, cultivation, and estimation of the DST of MTB and different mycobacteria; in which the 7H11 was enriched with casein hydrolysate to help fastidious or drug-resistant MTB strains, allowing colony visualization and identification typically within 3-4 weeks compared to 4-8 weeks for LJ media' to provide concrete performance metrics for procedural setup documentation [28].

b. Liquid culture systems

MTB can be rapidly detected, isolated and tested for drug susceptibility from clinical specimens using the fully automated BACTEC MGIT 960 and BACTEC 460TB liquid culture systems. These methods track mycobacterial metabolism in rich liquid media using radiometric CO_2 release detection (460TB) or fluorescent oxygen sensors (MGIT 960) [5,29]. Liquid culture techniques dramatically reduce detection time from several weeks to 7-14 days when compared to conventional solid media (LJ) or Middlebrooks agar, while maintaining excellent sensitivity and specificity [29]. The MGIT 960 has replaced the older radiometric 460TB due to its non-radioactive, automated, and high-throughput layout, making it more secure and extra appropriate for contemporary clinical microbiology laboratories. These systems were actually taken into consideration the gold standard of classical culture diagnosis and DST of TB, as encouraged with the aid of the WHO. When comparing these advance methods with solid media (LJ) [30], the BACTEC MGIT 960 and BACTEC 460TB were rapid, highly sensitive, automated detection of MTB with DST and associated with standardized monitoring. However, need for specialized infrastructure and trained personnel, risk of contamination, inability to assess colony morphology and the need for confirmatory identification tests [31].

5. Molecular diagnostic methods

Compared to culture conventional (LJ) methods, molecular diagnostic techniques for MTB, despite requiring specialized equipment and trained personnel, the rapid and accurate results provided by molecular methods often lead to overall cost savings by reducing patient hospitalization days, preventing disease progression, and limiting expensive empirical treatments for drug-resistant TB, thereby justifying the initial investment in infrastructure, especially in high-burden settings [32].

a. Nucleic acid amplification tests (NAATs)

- ***Xpert MTB/RIF and Xpert Ultra GeneXpert, Cepheid):*** The Xpert MTB/RIF and Xpert Ultra (GeneXpert, Cepheid) are both automated, cartridge-based molecular tests that can identify MTB. However, these tests were expensive, require constant power and temperature-controlled cartridges, and primarily detect MTB and rifampicin resistance, not a broader spectrum of drug resistances ultra system was more sensitive to samples with low bacterial content (such as pediatric or EPTB) and provides rapid on-site diagnosis. However, these tests were expensive, require constant power and temperature-controlled cartridges, and can only detect rifampicin resistance. By focusing on a the 81-bp rifampicin resistance-determining region (RRDR) of the rpoB gene the usage of 5 molecular beacon probes (A–E), Xpert MTB/RIF and Xpert Ultra pick out MTB and rifampicin

resistance, with ultra offering progressed sensitivity for low bacterial load samples [33].

- ***Truenat MTB:*** The Truenat MTB was a chip-primarily based, battery-operated real-time polymerase chain reaction (PCR) assay, which can pick out MTB DNA immediately from medical specimens in 1-2 hours. It offers a quick, point-of-care diagnosis in settings with limited sources, with high sensitivity and specificity. However, its obstacles encompass the need for skilled employees, reliance on solid reagents and equipment, and detection of only MTB (without drug resistance profiling until blended with Truenat MTB-RIF assays) [34,35].

b. Line probe assays (LPA)

LPAs are advanced molecular laboratory techniques that can identify MTB and detect mutations associated with drug resistance (e.g., isoniazid through katG/inhA and rifampicin with rpoB) directly from clinical specimens or culture isolates within 1-2 days (like isoniazid through katG/inhA and rifampicin with rpoB) immediately from clinical specimens or lifestyle isolates in 1-2 days. They provide short, touchy, and precise detection of MTB and multidrug resistance, however detect not able to resistance to all anti-TB medicines and require well-equipped laboratories and professional personnel [36].

C. PCR and real-time PCR

For MTB laboratory prognosis, PCR and real-time PCR were molecular techniques that expand MTB-specific DNA sequences (such as IS6110, 16S rRNA, or mpb64 genes) at once from medical specimens pulmonary and EPTB, enabling quick, sensitive, and unique detection within hours. Real-time PCR also offers quantitative dimension of bacterial load, excessive throughput, and reduced contamination hazard, but they require specialized system, skilled employees, and cannot offer entire DST without additional assays [37].

6. Immunologic tests

A. Antibody detection

Recognition of MTB antigen by antibodies of the humoral immune response was the basis of serological testing. The WHO does not recommend the use of commercial serological tests for the diagnosis of TB due to their low sensitivity and specificity, which can lead to misdiagnosis of MTB [38].

B. Antigen detection

The sandwich enzyme-linked immunosorbent assay method can be used to identify circulating MTB antigen in clinical specimens such as sputum, serum and urine. A special part of the cell membrane of MTB called lipoarabinomannan (LAM) can serve as a biomarker to identify TB [39]. The urine lateral flow LAM test was called FuGLAM. In adult patients, the sensitivity and specificity of Fuzilam were 70% and 93%, respectively, but in children with TB they were 51% and 87%. It works better and has greater diagnostic sensitivity in both adult and pediatric patients with HIV infection or CD4+ T cell counts less than 200 cells/μL [40].

C. Tuberculin skin test (TST)

TST was widely used for epidemiological screening and LTBI detection. It involves intradermal injection of purified protein derivative (PPD) to elicit a not on time-kind allergic reaction, with induration measured after 48-72 hours to signify prior sensitization to Mycobacterium tuberculosis [41]. However, its specificity and sensitivity were limited by Bacillus Calmette-Guérin (BCG) vaccination, exposure to non-TB mycobacteria, age, and immunological or molecular assay. Depending on the patient's risk factors, TST was considered positive if there was significant swelling (raised, hardened swelling) rather than just redness at the injection site [42].

≥5 mm period: Positive in immunocompromised patients, human immunodeficiency virus (HIV)-positive individuals, persons who have been recently exposed to TB or those with a previous chest X-ray suggestive of TB.

≥10 mm in length: Positive in children under 4 years of age, injection drug users, new immigrants from areas with high TB prevalence and other high-risk clinical situations.

≥15 mm duration: Positive in individuals without established TB risk factors [43].

D. Interferon-gamma release assays (IGRAs)

IGRA identifies host T-cell release of interferon-gamma in response to MTB-specific antigens (ESAT-6, CFP-10, TB 7.7) in blood samples. They provide a rapid, sensitive and specific method for LTBI unaffected by BCG vaccination, with results available within 24 hours. However, they are unable to differentiate between active and latent TB, require laboratory equipment and trained personnel, and may give false negative results in immunocompromised patients [44,45].

E. T-SPOT

After MTB-specific antigen stimulation, the T-spot test, was based on enzyme-linked immunospot (ELISPOT) technology, counts the number of cells producing interferon gamma. The T-spot test is currently commonly used to diagnose MTB infection. In addition to identifying MTB infection in children and HIV patients, T-SPOT is useful in screening for LTBI in immigrant populations, healthcare workers and students, as well as determining the risk of developing tuberculosis in chronic inflammatory diseases before anti-TNF therapy [46]. In addition, the T-SPOT is useful complementary test for further diagnosis of pulmonary tuberculosis. Despite the importance of the T-spot assay in identifying MTB infection, its primary drawback its inability to differentiate between active TB and LTBI. Due to its insufficient specificity, the WHO expert committee recommended the use of T-SPOT for the diagnosis of active pulmonary tuberculosis in low- and middle-income countries [47].

7. Histopathology and cytology

To diagnose of MTB, histopathology and cytology use tissue biopsies or microscopic analysis of cytological specimens to identify characteristic granulomatous inflammation with caseous necrosis, Langhans giant cells and AFB. These methods were less sensitive than culture or molecular methods, cannot distinguish between active and latent infection, require invasive sampling, require skilled interpretation, and may miss cases with low MTB bacterial load [48]. The diagnosis of MTB, especially in EPTB sites, can be supported by minimally invasive collection of cells or tissue from suspected TB lesions using FNAC, and AFB staining. However, sensitivity in paucibacillary samples was limited, and additional culture or molecular confirmation by PCR was often required [49].

8. DST of MTB

Using different culture classical techniques (LJ) or advance and molecular assays, DST of MTB to first-line drugs including (isoniazid, rifampicin, ethambutol, pyrazinamide) while the second-line drugs using in detection (fluoroquinolones, aminoglycosides, capreomycin) and in some cases resistance to newer panal third line anti-TB such as (linezolid, bed aquiline). Daylight saving time takes time, however and molecular advancing testing cannot cover for all drugs estimation [50].

9. Emerging and advanced technologies

- ***Whole-genome sequencing (WGS)***

  In the laboratory diagnosis of MTB, WGS involves sequencing the entire bacterial genome to identify species, strain types and mutations associated with resistance to first, second and third-line anti-TB drugs. It provides comprehensive, high-resolution data for epidemiology and personalized

care, but was currently expensive and time-consuming for routine use and requires sophisticated laboratory infrastructure and bioinformatics expertise [51].

- ***loop-mediated isothermal amplification (LAMP)***

  For MTB, loop-mediated isothermal amplification (LAMP) was a rapid, highly sensitive molecular technique that amplifies MTB-specific DNA at constant temperatures without the need for thermocycler. This allows direct on-site detection in resource-limited environments, but may not be able to detect drug resistance and requires careful primer design to prevent false positive results [52].

- ***Nano pore sequencing***

  The amplification of extremely-micro amounts (%-scale) of DNA/RNA was made feasible by means of using both mechanical lysis and enzymatic chemistry to penetrate the cell partitions. Pathogen detection with a sensitivity threshold as low as one hundred copies/mL was made feasible by this approach. Rifampicin (rpoB), isoniazid (katG, inhA), ethambutol (embB), streptomycin (rrs, rpsL), quinolone (gyrA), and pyrazinamide (p.C.) were the eight drug resistance genes protected by way of our targeted identity of 169 pathogenic microorganisms, together with MTB. The supplementary document contains facts on high-quality management, positive and negative controls [53].

In conclusion, laboratory diagnosis of MTB has transitioned to an integrated, multi-modal strategy combining conventional, molecular, immunological, and sequencing-based approaches to enhance diagnostic accuracy and timeliness. Smear microscopy and solid and liquid culture systems remain WHO-endorsed reference standards for bacteriological confirmation and phenotypic drug susceptibility testing, despite limitations related to turnaround time and reduced sensitivity in paucibacillary and EPTB. WHO-recommended rapid diagnostic tests (WRDs), including Xpert MTB/RIF Ultra and Truenat assays, enable early detection of TB and rifampicin resistance, supporting prompt treatment initiation. Immunological assays assist in identifying LTBI and high-risk populations, while histopathology contributes to extra pulmonary diagnosis. Advanced methods such as whole-genome sequencing offer comprehensive resistance profiling but are currently restricted to reference laboratories. Context-specific diagnostic integration remains central to effective TB control.

References

1. Tobin EH, Tristram D. Tuberculosis Overview. 2024; In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2025.
2. Forrellad MA, Klepp LI, Gioffré A, et al. Virulence factors of the Mycobacterium tuberculosis complex. Virulence. 2013; 4(1): 3-66. doi: 10.4161/viru.22329.
3. World Health Organization. Global Tuberculosis Report. 2024. URL: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024
4. Zaporojan N, Negrean RA, Hodișan R, et al. Evolution of laboratory diagnosis of tuberculosis. Clin Pract. 2024; 14(2): 388-416. doi: 10.3390/clinpract14020030.
5. Al-Hassani AL, Alnajjar AR, Mankhi AA. A study on drug resistance of Mycobacterium Tuberculosis isolated from a pulmonary Iraqi patient. Int J Pharm Pharm Res. 2016; 7 (4): 7-99.
6. Seung KJ, Keshavjee S, Rich ML. Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. Cold Spring Harb Perspect Med. 2015; 5(9): a017863. doi: 10.1101/cshperspect. a017863.
7. Hassan JS, Al-Tamimi BJ, Abdulrahman TR, et al. Resistance mechanisms to first line drug in Mycobacterium tuberculosis: A review. Iraqi J Med Sci. 2022; 20(1): 35-43. doi: 10.22578/IJMS.20.1.5.
8. Bouazzaoui A, Abdellatif AAH, Al-Allaf FA, et al. Strategies for vaccination: Conventional vaccine approaches versus new-generation strategies in combination with adjuvants. Pharmaceutics. 2021; 13(2): 140. doi: 10.3390/pharmaceutics13020140.
9. Dorman SE, Schumacher SG, Alland D, et al. Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. Lancet Infect Dis. 2018; 18(1): 76-84. doi: 10.1016/S1473-3099(17)30691-6.
10. Siddiqi S, Ahmed A, Asif S, et al. Direct drug susceptibility testing of Mycobacterium tuberculosis for rapid detection of multidrug resistance using the Bactec MGIT 960 system: a multicenter study. J Clin

Microbiol. 2012; 50(2): 435-40. doi: 10.1128/JCM.05188-11.
11. Dev Bhattarai M. Universal drug-susceptibility testing of first-line drugs to preserve their efficacy: An essential strategy to defeat tuberculosis. J Clin Tuberc Other Mycobact Dis. 2023; 33: 100394. doi: 10.1016/j.jctube.2023.100394.
12. Temur N, Eren EE, Celik I, et al. The recent development in the diagnosis of Mycobacterium tuberculosis. Smart Med. 2025; 4(2): e70007. doi: 10.1002/smmd.70007.
13. MacGregor-Fairlie M, Wilkinson S, Besra GS, et al. Tuberculosis diagnostics: overcoming ancient challenges with modern solutions. Emerg Top Life Sci. 2020; 4(4): 423-36. doi: 10.1042/ETLS20200335.
14. Ssengooba W, Kateete DP, Wajja A, et al. An early morning sputum sample is necessary for the diagnosis of pulmonary tuberculosis, even with more sensitive techniques: A prospective cohort study among adolescent TB-suspects in Uganda. Tuberc Res Treat. 2012; 2012: 970203. doi: 10.1155/2012/970203.
15. Shen F, Zubair M, Sergi C. Sputum analysis. 2025; StatPearls. Treasure Island (FL): StatPearls Publishing; 2025.
16. Hunter CJ, Ward R, Woltmann G, et al. The safety and success rate of sputum induction using a low output ultrasonic nebuliser. Respir Med. 1999; 93(5): 345-8. doi: 10.1016/s0954-6111(99)90317-7.
17. Roberts T, Chandna A, Watthanaworawit W, et al. Impact of delayed processing of positive blood cultures on organism detection: A prospective multi-centre study. BMC Infect Dis. 2022; 22(1): 517. doi: 10.1186/s12879-022-07504-1.
18. Lee JY. Diagnosis and treatment of extrapulmonary tuberculosis. Tuberc Respir Dis (Seoul). 2015; 78(2): 47-55. doi: 10.4046/trd.2015.78.2.47.
19. Surveillance Guidelines for Measles, Rubella and Congenital Rubella Syndrome in the WHO European Region. Geneva: World Health Organization; 2012.
20. Bayot ML, Mirza TM, Sharma S. Acid Fast Bacteria. 2023 Aug 7. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025.
21. Bradner L, Robbe-Austerman S, Beitz DC, et al. Chemical decontamination with N-Acetyl-L-cysteine-sodium hydroxide improves recovery of viable Mycobacterium avium subsp. Para tuberculosis organisms from cultured milk. J Clin Microbiol. 2013; 51(7): 2139-46. doi: 10.1128/JCM.00508-13.
22. Hänscheid T, Mahomed S, Oliveira L, et al. Fluorescent acid-fast stains for diagnosing mycobacteria and beyond: back to the future? Lancet Microbe. 2025; 101233. doi: 10.1016/j.lanmic.2025.101233.
23. Atiya N, Ong LT, Loh TC, et al. Diagnostic utility of aura mine O smear microscopy for detecting non-tuberculous mycobacteria versus Mycobacterium tuberculosis complex in adult clinical samples: a 5-year retrospective study (2018-2022). BMC Infect Dis. 2025; 25(1): 916. doi: 10.1186/s12879-025-11298-3.
24. Campelo TA, Cardoso de Sousa PR, Nogueira LL, et al. Revisiting the methods for detecting Mycobacterium tuberculosis: what has the new millennium brought thus far? Access Microbiol. 2021; 3(8): 000245. doi: 10.1099/acmi.0.000245.
25. Gil-Setas A, Torroba L, Fernandez JL, et al. Evaluation of the MB/BacT system compared with Middlebrook 7H11 and Lowenstein-Jensen media for detection and recovery of mycobacteria from clinical specimens. Clin Microbiol Infect. 2004; 10(3): 224-8. doi: 10.1111/j.1198-743x.2004.00733. x.
26. Kassaza K, Orikiriza P, Llosa A, et al. Lowenstein-Jensen selective medium for reducing contamination in Mycobacterium tuberculosis culture. J Clin Microbiol. 2014; 52(7): 2671-3. doi: 10.1128/JCM.00749-14. Epub 2014 Apr 30.
27. Realini L, De Ridder K, Palomino J, et al. Microaerophilic conditions promote growth of Mycobacterium genavense. J Clin Microbiol. 1998; 36(9): 2565-70. doi: 10.1128/JCM.36.9.2565-2570.1998.
28. Coban AY, Uzun M, Bozdogan B. Evaluation of agar-based medium with sheep sera for testing of drug susceptibility of Mycobacterium tuberculosis to Isoniazid, Rifampin, Ethambutol, and Streptomycin. J Clin Microbiol. 2013; 51(12): 4243-5. doi: 10.1128/JCM.01947-13.
29. Tortoli E, Benedetti M, Fontanelli A, et al. Evaluation of automated BACTEC MGIT 960 system for testing susceptibility of Mycobacterium tuberculosis to four major antituberculous drugs: comparison with the radiometric BACTEC 460TB method and the agar plate method of proportion. J Clin Microbiol. 2002; 40(2): 607-10. doi: 10.1128/JCM.40.2.607-610.2002.
30. Leitritz L, Schubert S, Bücherl B, et al. Evaluation of BACTEC MGIT 960 and BACTEC 460TB systems for recovery of mycobacteria from clinical specimens of a university hospital with low incidence of tuberculosis. J Clin Microbiol. 2001; 39(10): 3764-7. doi: 10.1128/JCM.39.10.3764-3767.2001.
31. Rodrigues C, Jani J, Shenai S, et al. Drug susceptibility testing of Mycobacterium tuberculosis against second-line drugs using the Bactec MGIT 960 System. Int J Tuberc Lung Dis. 2008; 12(12): 1449-55.
32. Kania K, Wójcik K, Drożdż K, et al. Utility of rapid molecular assays for detecting multidrug-resistant Mycobacterium tuberculosis in Extrapulmonary Samples. Diagnostics (Basel). 2025; 15(9): 1113. doi: 10.3390/diagnostics15091113.
33. Sava C, Sava M, Drăgan AM, et al. The use of Xpert MTB/RIF ultra-testing for early diagnosis of Tuberculosis: A retrospective study from a single-center database. Genes (Basel). 2023; 14(6): 1231. doi: 10.3390/genes14061231.
34. Inbaraj LR, Daniel J, Sathya Narayanan MK, et al. Truenat MTB assays for pulmonary tuberculosis and rifampicin resistance in adults and adolescents. Cochrane Database Syst Rev. 2025; 3(3): CD015543. doi: 10.1002/14651858.CD015543.pub2.

35. Nikam C, Jagannath M, Narayanan MM, et al. Rapid diagnosis of Mycobacterium tuberculosis with Truenat MTB: a near-care approach. PLoS One. 2013; 8(1): e51121. doi: 10.1371/journal.pone.0051121.
36. Farooqi JQ, Khan E, Alam SM, et al. Line probe assay for detection of rifampicin and isoniazid resistant tuberculosis in Pakistan. J Pak Med Assoc. 2012; 62(8): 767-72.
37. Angmo D, Bashir G, Jan A, A. Khan M and Yasin S B. Multiplex PCR targeting IS6110 and mpb64 Genes for Detecting Extra-Pulmonary Tuberculosis: A Cross-Sectional Study in a Tertiary Care Center, Kashmir, India. J Med Microbiol Inf Dis. 2023; 11(2): 78-85. doi: 10.61186/JoMMID.11.2.78.
38. Niki M, Suzukawa M, Akashi S, et al. Evaluation of humoral immunity to mycobacterium tuberculosis-specific antigens for correlation with clinical status and effective vaccine development. J Immunol Res. 2015; 2015: 527395. doi: 10.1155/2015/527395.
39. Pereira Arias-Bouda LM, Nguyen LN, Ho LM, Kuijper S, et al. Development of antigen detection assay for diagnosis of tuberculosis using sputum samples. J Clin Microbiol. 2000; 38(6): 2278-83. doi: 10.1128/JCM.38.6.2278-2283.2000.
40. Olbrich L, Khambati N, Bijker EM, et al. FujiLAM for the diagnosis of childhood tuberculosis: a systematic review. BMJ Paediatr Open. 2022; 6(1): e001447. doi: 10.1136/bmjpo-2022-001447.
41. Pahal P, Pollard EJ, Sharma S. PPD Skin Test. 2023 Apr 23; Stat Pearls. Treasure Island (FL): Stat Pearls. 2025.
42. Seddon JA, Paton J, Nademi Z, et al. The impact of BCG vaccination on tuberculin skin test responses in children is age dependent: evidence to be considered when screening children for tuberculosis infection. Thorax. 2016; 71(10): 932-9. doi: 10.1136/thoraxjnl-2015-207687.
43. Auguste P, Tsertsvadze A, Pink J, et al. Accurate diagnosis of latent tuberculosis in children, people who are immunocompromised or at risk from immunosuppression and recent arrivals from countries with a high incidence of tuberculosis: systematic review and economic evaluation. Health Technol Assess. 2016; 20(38): 1-678. doi: 10.3310/hta20380.
44. Pai M, Denkinger CM, Kik SV, et al. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. Clin Microbiol Rev. 2014; 27(1): 3-20. doi: 10.1128/CMR.00034-13.
45. Banaei N, Gaur RL, Pai M. Interferon gamma release assays for latent tuberculosis: what are the sources of variability? J Clin Microbiol. 2016; 54(4): 845-50. doi: 10.1128/JCM.02803-15.
46. Shams H, Weis SE, Klucar P, et al. Enzyme-linked immunospot and tuberculin skin testing to detect latent tuberculosis infection. Am J Respir Crit Care Med. 2005; 172(9): 1161-8. doi: 10.1164/rccm.200505-748OC.
47. Ma Y, Li R, Shen J, et al. Clinical effect of T-SPOT.TB test for the diagnosis of tuberculosis. BMC Infect Dis. 2019; 19(1): 993. doi: 10.1186/s12879-019-4597-8.
48. Öztomurcuk D, Terzi Ö, Demirci C, et al. Investigation of granulomatous inflammations in terms of tuberculosis diagnosis: A 5-year multi-center laboratory study. Turk Thorac J. 2022; 23(1): 11-6. doi: 10.5152/TurkThoracJ.2022.20314.
49. Li W, Sha W. Diagnosis of chest wall tuberculosis using fine needle aspiration: A single-center experience. Infect Drug Resist. 2023; 16: 2281-90. doi: 10.2147/IDR.S404804.
50. Mao X, Wang J, Xu J, et al. Current diagnosing strategies for Mycobacterium tuberculosis and its drug resistance: a review. J Appl Microbiol. 2025; 136(5): lxaf100. doi: 10.1093/jambio/lxaf100.
51. Bartolomeu-Gonçalves G, Souza JM, Fernandes BT, et al. Tuberculosis diagnosis: current, ongoing, and future approaches. Diseases. 2024; 12(9): 202. doi: 10.3390/diseases12090202.
52. Jang WS, Park S, Lee JM, et al. Development of the MTB/IC LAMP-MS assay for rapid detection of mycobacterium tuberculosis. Diagnostics (Basel). 2025; 15(8): 996. doi: 10.3390/diagnostics15080996.
53. Ren F, Ma J, Dang L, et al. Potential of Nano pore sequencing for tuberculosis diagnosis and drug resistance detection. BMC Infect Dis. 2024; 24(1): 1469. doi: 10.1186/s12879-024-10378-0.

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