NATIONAL TUBERCULOSIS (TB) GUIDELINES FOR IRELAND

Chapter 5: Laboratory Diagnosis of *Mycobacterium tuberculosis* disease

Please note that this document should be used in tandem with other Irish tuberculosis guidance

Readers should not rely solely on the information contained with these guidance outputs. Guidance information is not intended to be a substitute for advice from other relevant sources including and not limited to, the advice from a health professional. Clinical judgement and discretion will be required in the interpretation and application of this guidance document. This guidance document is regularly reviewed based upon emerging evidence at national

and international levels and national policy decisions. In tandem with this, the guidance will be formally reviewed on a three-year cycle.

For further information please contact: rgdu@hpsc.ie.

Working version: 1.0

Publication date: 22 July 2026

VERSION HISTORY

Version History		
Title of Guidance:		**National Tuberculosis (TB) Guidelines for Ireland, Chapter 5: Laboratory Diagnosis of *Mycobacterium tuberculosis* disease**
Approved by:		**Director of National Health Protection, Dr Éamonn O'Moore**
Version number:		**1.0**
Publication Date:		**22/07/2026**
Scheduled Review Date:		**21/07/2029**
Electronic Location:		**https://www.hpsc.ie/a-z/vaccinepreventable/tuberculosistb/guidance/**
Version	**Final Approval Date:**	**List section numbers and changes**
1.0	22/07/2026	*De novo* guideline development. First published.

TABLE OF CONTENTS

CHAPTER 5: LABORATORY DIAGNOSIS OF MYCOBACTERIUM TUBERCULOSIS DISEASE

Chapter developed by: Prof. Breida Boyle[1], Dr Margaret Fitzgibbon[1], Prof. Johannes Wagener[1] and Dr Mary Mansfield in collaboration with the Guideline Development Group for the National Tuberculosis (TB) Guidelines and the Research and Guideline Development Unit (RGDU).[2]

Significant expert input was also provided by the HSE Laboratory Services Reform Programme.

1. The National Irish Mycobacteria Reference Laboratory (IMRL), St James's Hospital, Dublin
2. HSE: Public Health, National Health Protection Office

SUMMARY OF RECOMMENDATIONS

- For patients with clinically suspected *Mycobacterium tuberculosis* disease (TB), in line with WHO recommendation, rapid molecular techniques (mWRDs) are the ***initial diagnostic test*** (first line test) to detect *Mycobacterium Tuberculosis* complex (MTBC) and rifampicin resistance, in order to minimise delays in starting appropriate treatment.
- Mycobacterial detection should be attempted in all cases of suspected pulmonary TB using WHO recommended diagnostics (see **Table 5.1**). The same diagnostic tests can be used to provide mycobacterial detection in cases of extrapulmonary TB (EPTB) where an appropriate specimen can be collected.
- Following appropriate laboratory biosafety risk assessment. diagnostic tests should be placed in a tiered laboratory TB network structure (**Figure 5.1**), e.g. Xpert® MTB/RIF Ultra assay may be performed at Containment Level 2 (CL2) (may also be referred as Biosafety Level 2-BSL2)
- *Mycobacterium tuberculosis* **culture** is classified as a biological risk group 3 agent, requires Containment Level 3 laboratory (may also be referred as Biosafety Level 3-BSL3) for culture, drug susceptibility testing and other laboratory examinations of mycobacterial cultures.
- Key considerations when developing algorithms to optimise TB diagnosis include local/national epidemiology, prevalence of multi-drug resistance TB, and HIV, in addition to resources, infrastructure and technical expertise in keeping with Striving to end Tuberculosis a Strategy for Ireland 2024-2030 and HSE Health region structure and Integrated Healthcare Areas.
- The IMRL, in conjunction with Public Health epidemiology, can provide supporting data for cluster recognition.

Introduction

The effective management of TB relies on the rapid diagnosis of TB, rapid detection of drug resistance and prompt initiation of an effective treatment regimen. Thus, there is a need for access to fast and accurate detection tests, and rapid and accurate drug susceptibility testing (DST) for all people with TB (1)

In 2024, the World Health Organization (WHO) published new guidelines on rapid diagnostics for TB detection and recommended that in **all settings**, rapid techniques be used as the initial diagnostic test to detect *M. tuberculosis* complex (MTBC) and rifampicin (RIF) resistance, to minimize delays in starting appropriate treatment (2, 3)

Note: Diagnosis of Mycobacterium tuberculosis infection (previously known as latent TB) is not included in this chapter.

5.1 General Considerations regarding diagnosis of TB

Mycobacterial confirmation should be attempted in all cases of pulmonary TB using WHO recommended diagnostics. The same diagnostic tests could be used to provide bacteriological confirmation in cases of extrapulmonary TB (EPTB) where an appropriate specimen can be collected (4)

All specimens should be collected and submitted in sterile, plastic, leak-proof, disposable, widemouthed, appropriately labelled, laboratory approved containers, without any fixative. Specimens should be transported to the laboratory as soon as possible and refrigerated until processed. Cerebrospinal Spinal Fluid should be processed immediately (4). The transportation of infectious substances is subject to international legislation, to which laboratories are required to comply (5).

All clinical material for TB diagnosis sent to the laboratory should be accompanied by a laboratory form including all relevant information (4) including clinician name and contact details.

5.2 Specific Considerations regarding diagnosis of TB

5.2.1 Pulmonary Material/Specimens

For optimal recovery of acid-fast bacilli on microscopy from sputum, at least 3 good quality specimens should be collected on consecutive days (6). Early morning specimens have the highest yield of acid-fast bacilli; however good diagnostic specimens can be collected at any time. In addition, induced sputum, bronchial lavage are suitable pulmonary specimens (4).

Gastric aspirates and washings may be processed for children and people unable to produce sputum (4). Repeated testing in additional specimens may be required depending on pre-test probability (2).

Stool is another WHO recommended specimen for the diagnosis of TB in children using molecular testing e.g., Xpert® MTB/RIF Ultra assay (4).

5.2.2 Extrapulmonary Material

Blood: Specific mycobacterial blood culture bottles or isolators should be provided by the laboratory to clinicians in advance of collection. Blood should only be processed where disseminated infection is suspected or where the host is immunocompromised (7).

Urine: Multiple early morning specimens over several days can aid in the diagnosis of renal TB, disseminated TB or TB in severely immunocompromised persons (8). However, urine is an inappropriate specimen for the diagnosis of pulmonary TB.

Specimens such as cerebrospinal fluid, body fluids (e.g., pleural, peritoneal, synovial and pericardial fluids), pus, bone marrow and tissue can all be processed for TB diagnosis where appropriate. Where tissue is collected for the diagnosis of TB, containers must be free of any additives or fixatives (e.g., formalin) (4).

5.3 Laboratory Services

5.3.1 Tiered pyramidal TB laboratory structure

The WHO recommends the placement of laboratory diagnostic tests in a tiered pyramidal TB laboratory structure with most laboratories at peripheral/intermediate level (Levels I and II) and one Central National Reference Laboratory (Level III) as shown in **Figure 5.1** (2).

In a similar way, the HSE Laboratory Services Reform Programme (9) recommends that laboratory services should be structured in three levels:

1. Laboratory Network (serving one hospital or a network of hospitals);
2. Health Region level and
3. National level

Each level has specific requirements for laboratory infrastructure and biosafety, defined by the activities and diagnostic tests performed in these laboratories (10). A laboratory biosafety risk assessment should be performed to identify and evaluate the risks associated with performing diagnostic tests at each level and implement risk control measures and decide on testing algorithms (11, 12).

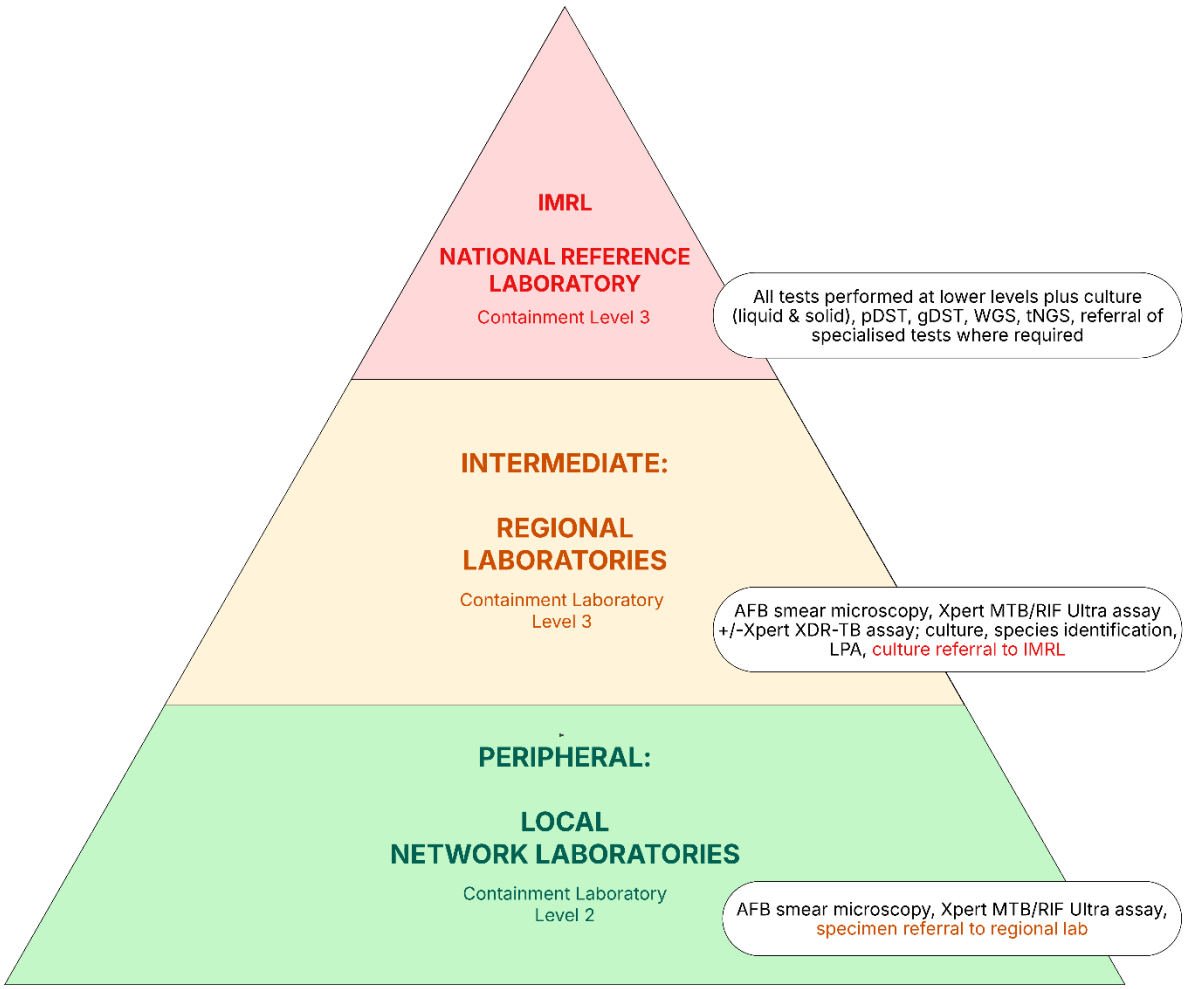

Figure 5.1: Organisation of a TB laboratory network. Adapted from WHO Guidelines 2025 (2).
Risk assessment required for assignment of containment laboratory level for diagnostic testing.

AFB: acid-fast bacilli; DST: drug susceptibility testing; gDST: genotypic drug susceptibility tests; IMRL, Irish Mycobacteria Reference Laboratory; LPA: line probe assay; pDST: phenotypic drug susceptibility tests; tNGS:, targeted next generation sequencing; WGS: whole genome sequencing.

5.4 Diagnostic Testing-Requirements for Biological Risk Assessment

In compliance with the Biological Agents Regulations (11) each laboratory should perform a local biological risk assessment for each test method performed onsite. Diagnostic specimens are regarded as containing biological risk group 2 agents and are handled in Containment level 2 laboratories. *Mycobacterium tuberculosis* **culture** is classified as a biological risk group 3 agent, that must be processed in a Containment Level 3 laboratory for culture, drug susceptibility testing and other laboratory examinations.

The Biological Agents Regulations are accompanied by the 2020 Biological Agents Code of Practice (10). Schedule 2 of the Code outlines the minimum containment measures for Containment laboratories. These schedules should be taken into account when performing laboratory biosafety risk assessments (11).

5.4.1 Transporting Biological Agents

Transport of specimens and cultures of mycobacteria by road should be in compliance with the International Carriage of Dangerous Goods by Road (ADR) (5).

5.5 Quality Assurance

National TB programmes depend on laboratories that deliver reliable and high-quality results, forming the backbone of effective tuberculosis control (4). The Quality Management System (QMS) encompasses the entire quality system followed within a laboratory. It allows for consistency of results to be maintained, transparency, reproducibility, traceability and accountability to be achieved, to ensure that accurate results are produced in a timely manner for quality patient care. Laboratory accreditation is based on an International Organization for Standardization (ISO) standard, which outlines the specific quality and competence requirements for medical laboratories. It is a requirement for all clinical laboratories to follow these standards to attain and maintain accreditation status. The Irish National Accreditation Board (INAB) is the accreditation body for Ireland. They are responsible for ensuring all processes and procedures are performed to the ISO 15189 standard for medical laboratory testing.

5.6 Laboratory Diagnostic tests and algorithms

Key considerations when developing algorithms to optimise TB diagnosis include local/national epidemiology, prevalence of multi-drug resistance TB (MDR-TB), and HIV, along with resources, infrastructure and technical expertise. The WHO and European Reference laboratory network for Tuberculosis recommend the following tests for the laboratory diagnosis of TB (2, 13).

- First line testing: Molecular WHO-recommended rapid diagnostic tests (mWRDs) such as Xpert® MTB/RIF Ultra assay etc (see **Table 5.1**)
- Smear microscopy
- Mycobacteriological confirmation using culture methods (lowest limit of detection and gold standard but requires time)
- Species identification
- Drug susceptibility testing using both phenotypic and genotypic methods (for prediction)
- Surveillance of TB strains using whole genome sequencing for lineage calling and relatedness analysis to identify clusters/outbreaks of TB.

Of note: Currently Lateral flow LAM assays are not available in European Union.

Laboratory diagnosis of TB varies significantly by method as shown in the algorithm in **Figure 5.2** (adapted from the WHO and ERLTB-Net diagnostic algorithms) (2, 13).

Rapid molecular tests (such as Xpert® MTB/RIF Ultra) give results within hours; smear microscopy turnaround time is under 24 hours while traditional culture methods for mycobacteriological confirmation may take days to weeks with subsequent drug susceptibility testing taking a further 2-4 weeks for full profile results.

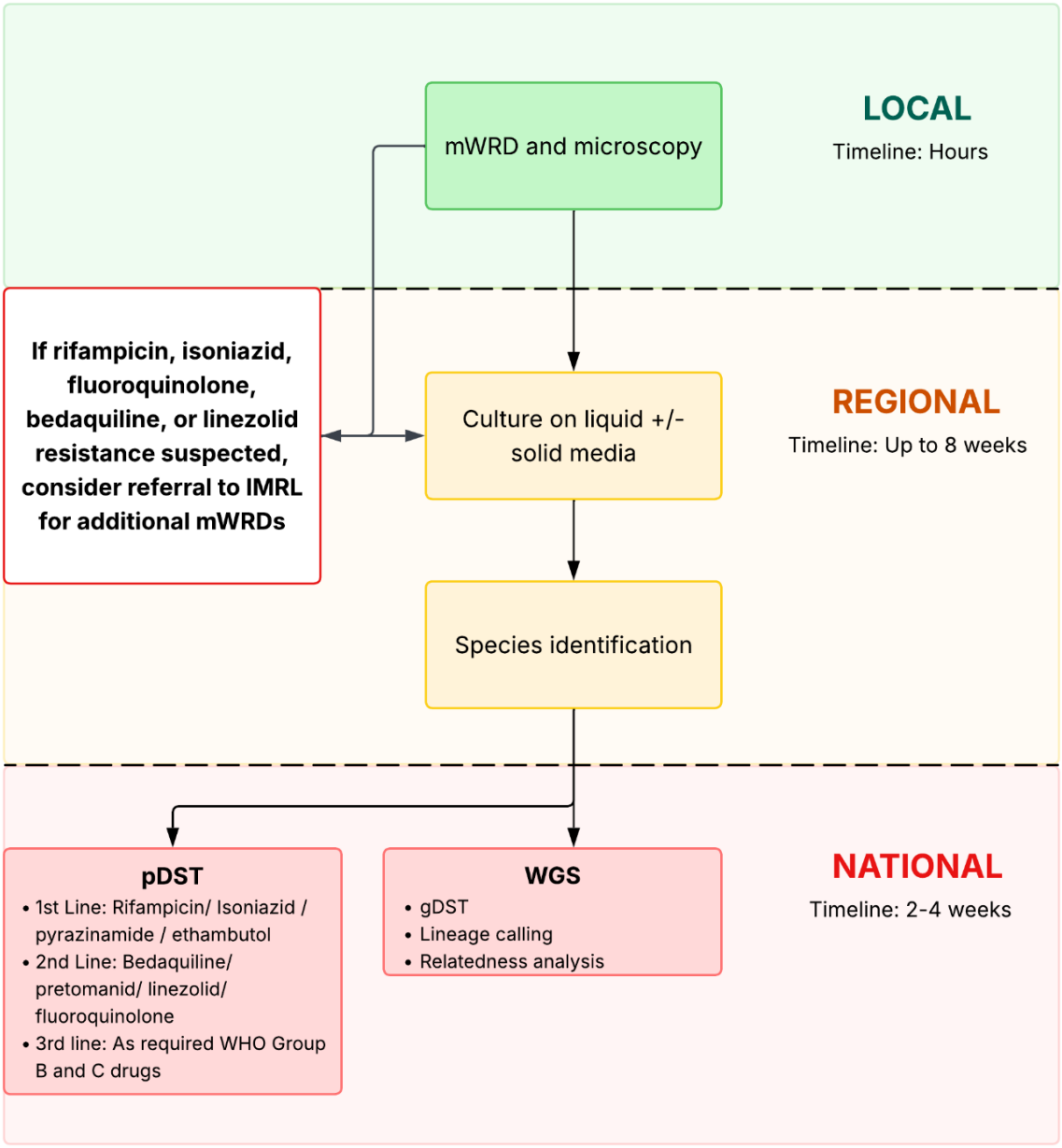

Figure 5.2: Diagnostic algorithm for tuberculosis (TB) and drug resistance detection Adapted from WHO 2025 & Saluzzo et al, 2026 (2, 13).

mWRD: WHO-recommended molecular rapid diagnostic tests, tNGS: Targeted Next generation sequencing, pDST: phenotypic Drug Susceptibility Testing, WGS: Whole genome sequencing, gDST: genotypic Drug Susceptibility Testing.

5.6.1 Initial molecular tests for the diagnosis of TB with drug resistance detection

In all settings, initial front-line testing using a molecular WHO-recommended rapid diagnostic tests (such as Xpert® MTB/RIF Ultra) should be performed on specimens for the detection of TB and rifampicin resistance.

The Xpert® MTB/RIF Ultra assay has a limit of detection (LOD) of 16 colony forming units [cfu]/mL respectively. At very low bacterial loads, Xpert® MTB/RIF Ultra assay can give a "trace" result, which may require further clinical information and consideration of the clinical context of TB and is not based on amplification of the *rpoB* target, therefore does not give results for rifampicin susceptibility or resistance (2). If the limit of detection is not reached due to low bacterial load the test cannot detect rifampicin resistance genes, therefore rifampicin resistance cannot be determined by this test under these conditions, this is reported as "rifampicin resistance indeterminate."

Additional assessment of isoniazid and fluoroquinolone resistance detection should be considered in certain cases and specimens referred to the Irish Mycobacteria Reference Laboratory (IMRL) for testing. Where there is an increased risk of bedaquiline and/or linezolid resistance, specimens should be referred to the IMRL for targeted next generation sequencing.

5.6.2 Smear microscopy

Smear microscopy using auramine -O or Ziehl-Neelsen staining may be used as a useful marker of TB infectiousness in conjunction with clinical features/risk assessment.

However, sputum-smear microscopy is a relatively insensitive test, with a LOD of 5 x 10^3 –10^4 bacilli/ml of sputum. Furthermore, sputum-smear microscopy cannot distinguish between viable and non-viable organisms or drug-susceptible strains from drug-resistant strains (2).

5.6.3 Culture

Mycobacterial culture (using liquid +/- solid media) should be performed on all specimens investigated for TB; as culture remains the current gold standard method for the mycobacteriological confirmation of TB. Mycobacteria are slow-growing organisms and may take days to weeks to yield a positive culture and up to 8 weeks (occasionally may be incubated for 12 weeks) for a negative result.

Culture is important in the diagnosis of paediatric and extrapulmonary TB from paucibacillary specimens, and in the differential diagnosis of NTM infection. Culture, with a LOD of 10^1-10^2 cfu/ml, ensures the availability of isolates for downstream phenotypic and genotypic testing, maximising the diagnostic yield for both *M. tuberculosis* complex and NTMs.

5.6.4 Species Identification

The culture process can result in the growth of many of the *Mycobacterium* species. As such, laboratory confirmation of TB requires testing of the recovered mycobacteria using a WHO recommended species identification test to definitively identify *M. tuberculosis* complex, as species identification is important before initiating phenotypic drug susceptibility testing (2).

5.6.5 Drug susceptibility testing

5.6.5.1 Phenotypic drug susceptibility testing

Phenotypic drug susceptibility testing (pDST) remains the reference standard for most anti-TB drugs; therefore, should be performed on all positive *M. tuberculosis* complex cultures to guide appropriate management of cases.

Positive cultures should be referred for pDST to the national reference laboratory (IMRL) without delay.

Where no resistance is suspected, pDST should be performed against standard TB regimen drugs (rifampicin isoniazid, ethambutol and pyrazinamide).

In drug-resistant TB cases (where a rifampicin and/or isoniazid and/or fluoroquinolone resistant mutation (s) are detected by Xpert® tests or other mWRDs)/ or patients with drug intolerance, pDST should be prioritised and performed against MDR-TB regimen drugs (bedaquiline, pretomanid, linezolid and fluoroquinolones) as recommended by WHO guidelines (2).

Follow-on pDST should be performed as required against WHO defined Group B (clofazimine, cycloserine) and Group C (delamanid, aminoglycosides, prothionamide) drugs, respectively (2).

5.6.5.2 Genotypic drug susceptibility testing

Genotypic drug susceptibility testing (gDST) performed after culture serves as a complimentary approach to pDST of *M. tuberculosis* complex. While gDST has advanced with the availability of rapid molecular tests for rifampicin, isoniazid and fluoroquinolones through

Xpert® (Cepheid, Sunnyvale, CA) and line probe assays (Bruker/Hain Lifescience, Nehren, Germany), the spectrum of drugs covered by these tests is limited and does not cover the new and repurposed drugs.

Whole genome sequencing should be performed on all positive MTC cultures for genotypic prediction of drug resistance. Results should support treatment modifications in both drug-susceptible and drug-resistant TB, in particular rifampicin-resistant, multidrug resistant, pre-extensively drug resistant and extensively drug-resistant TB cases which require comprehensive DST data (2).

5.6.6 Surveillance of TB strains

Whole genome sequencing (WGS) is recommended for use in the surveillance of TB and drug-resistant strains (2). WGS of *M. tuberculosis* complex for identification, lineage calling, drug resistance prediction and relatedness analysis is accredited to ISO15189 medical laboratory standards in the IMRL, thus, positive cultures should be referred without delay.

A key factor that influences the LOD of WGS is sample/DNA input, therefore WGS is performed on positive cultures only.

WGS results (species identification, lineage calling +/-cluster code) are reported to clinicians and public health nationwide through Computerised Infectious Disease Reporting (CIDR) / Outbreak, Case and Incident Management (OCIM) systems.

Table 5.1: WHO-recommended rapid molecular diagnostic tests for the initial detection of M. tuberculosis complex and drug resistance

Xpert® MTB/RIF Ultra (Cepheid)
Truenat™ (Molbio)
TB-LAMP (Eiken)
Moderate complexity automated NAATs for detection of TB and resistance to rifampicin and isoniazid Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott) BD MAX™ MDR-TB (Becton Dickinson) cobas® MTB and cobas MTB-RIF/INH (Roche) FluoroType® MTBDR and FluoroType® MTB (Hain Lifescience/Bruker)

Adapted from WHO 2025

REFERENCES

1. World Health Organization (WHO). The End TB Strategy. 2015. Available from: https://iris.who.int/server/api/core/bitstreams/d128ca35-55c7-4ec8-b2f3-987ca112a4a2/content.
2. World Health Organization (WHO). WHO Operational Handbook on Tuberculosis Module 3: Diagnosis – Rapid Diagnostics for Tuberculosis Detection. 2025. Available from: https://www.paho.org/sites/default/files/2024-03/2024-cde-who-operational-handbook-tb-module-3-rapid-tx-tb.pdf.
3. World Health Organization (WHO). WHO standard: universal access to rapid tuberculosis diagnostics. 2023. Available from: https://www.who.int/publications/i/item/9789240071315.
4. European Centre for Disease Control and Prevention (ECDC). Handbook on Tuberculosis laboratory diagnostic methods in the European Union. 2025. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/handbook-diagnostic-methods-tb.pdf.
5. Health and Safety Authority (HSA). ADR Regulations - Carriage of Dangerous Goods. 2025. Available from: https://www.hsa.ie/your_industry/adr_-_carriage_of_dangerous_goods_by_road/news_updates/adr_2025_-_summary_of_main_changes/.
6. David M. Lewinsohn MKL, Philip A. LoBue, David L. Cohn, Charles L. Daley, Ed Desmond, Joseph Keane, Deborah A. Lewinsohn, Ann M. Loeffler, Gerald H. Mazurek, Richard J. O'Brien, Madhukar Pai, Luca Richeldi, Max Salfinger, Thomas M. Shinnick, Timothy R. Sterling, David M. Warshauer, Gail L. Woods. ATS/CDC/IDSA Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children. 2017. Available from: https://www.idsociety.org/practice-guideline/diagnosis-of-tb-in-adults-and-children/#ExecutiveSummary.
7. Shrivas A, Singh S. Tuberculosis Diagnosis and Management: Recent Advances. Journal of Global Infectious Diseases. 2025;17(1):3-9.
8. Mortier E, Pouchot J, Girard L, Boussougant Y, Vinceneux P. Assessment of urine analysis for the diagnosis of tuberculosis. British Medical Journal. 1996;312(7022):27-8.
9. Laboratory Services Reform Programme. HSE Outline Strategic Plan for Laboratory Services (2026 – 2035). 2026. Available from: https://about.hse.ie/api/v2/download-file/file_based_publications/Strategic_Plan_for_Laboratory_Services_2026_-_2035.pdf/.

10. Health and Safety Authority (HSA). Biological Agents Code of Practice 2020. 2020. Available from: https://www.hsa.ie/eng/publications_and_forms/publications/biological_agents/biological_agents_code_of_practice_2020.

11. Health and Safety Authority (HSA). Managing Exposure to Biological Agents in Laboratories. 2023. Available from: https://www.hsa.ie/media/n4abvmwg/104223-health-and-safety-authority-managing-exposure-to-biological-agents-in-labs-aw.pdf.

12. World Health Organization (WHO). Laboratory biosafety manual, 4th edition. 2020. Available from: https://www.who.int/publications/i/item/9789240011311.

13. Saluzzo F, Cabibbe AM, Anthony R, Aubry A, Drobniewski F, Holicka Y, et al. Diagnostic algorithms for tuberculosis in Europe: insights from the European Reference Laboratory Network for Tuberculosis (ERLTB-Net). The Lancet Regional Health - Europe. 2026;60:101516.