

Directory of services

Name of the laboratory: Microbiology Laboratory at NITRD

Postal Address: Sri Aurobindo Marg, New Delhi-110058

Phone: 011-26854929, 011-26854922, Ext-1215

Email: sa-nitrd@nic.nic.in

1. Purpose and scope:

The laboratory of NITRD being a National Reference laboratory receives samples from various chest clinics, different Hospitals and Health units/States as well as research institutions for diagnosis of tuberculosis by microscopy, mycobacterial culture and Drug Susceptibility testing (DST), phenotypic/genotypic drug susceptibility testing for patient care and management. The manner in which the primary sample for diagnosis from the patient is collected and transported to the laboratory plays an important role in generating accurate results. This manual therefore is to assure that the quality of specimens received at NITRD for microscopy, culture and DST are of the required standard.

2. Abbreviations:

- NITRD-National Institute of Tuberculosis and Respiratory Diseases
- DTO - District tuberculosis Officer
- N.A. -Not applicable
- TB- Tuberculosis
- BAL- Broncho Alveolar Lavage
- MOTT- Mycobacteria Other Than Tuberculosis
- DST -Drug Susceptibility Testing
- WHO- World Health Organization
- MDR -Multi-Drug Resistance
- XDR- Extensive drug resistance
- NTM- Non Tuberculous Mycobacteria
- LPA -Line probe Assay
- TAT -Turnaround time
- LT- Lab Technician

3. Tasks, responsibilities and accountabilities:

Task	Responsible	Accountable
Proper Collection of sample	Patient supervised by the health worker/LT	Health worker/LT
Transportation of samples to NITRD lab	Health care facility, Research facility	DTO/ Head of facility
Receipt of sample	Lab personnel at NITRD	Laboratory manager
Safe handling of samples	Patient/ Health care worker/ Lab personnel at NITRD	DTO/ NITRD Biosafety Officer/ Section Incharge
Sample accessioning	Lab personnel at NITRD	Data Manager/QA Manager/ Section Incharge

4. Operational Hours:(Sample Receiving Time)

OPD Samples	9.00am – 12.00 noon	OPD Room no.16
Indoor Samples	9.00am – 12.00 noon	Microbiology Department
Extra-pulmonary TB samples	9.00am – 3.00 pm	Microbiology Department
Samples/ Culture from states	9.00am – 4.00 pm	Microbiology Department
Bacteriology culture samples	9.00am- 12.00 noon	Microbiology Department
Mycology samples	9.00am-12.00 noon	Microbiology Department
Serology samples	9.00am-12.00 noon	Microbiology Department

Type of Test	Turnaround time
ZN Smear Microscopy	24 hours
Fluorescent Stain	24 hours
Culture and isolation of Mycobacterium (Culture)	4-8 weeks
Culture and isolation of Mycobacterium (Liquid)	2-4 weeks (6 weeks/ 42 days)
Drug susceptibility testing (DST) by liquid culture after culture is positive	14 days
Drug susceptibility testing (DST) by solid culture	42 days
Drug susceptibility testing (DST) by line probe assay on smear positive specimens	5 days
Drug susceptibility testing (DST) by line probe assay on smear negative specimens(after culture positive)	5 days

5. **Examinations done at NITRD**-The list below gives the full name of the tests being performed in the laboratory

1. Microscopy

- a. Ziehl-Neelsen Smear Microscopy (ZN Microscopy)
- b. Fluorescence Microscopy (FM)

2. Culture and isolation of Mycobacterium (Culture)

- a. Löweinsten-Jensen solid Media Method (LJ Culture)
- b. Mycobacterial Growth Indicator Tube (MGIT) method

3. Drug susceptibility testing (DST)

- a. Mycobacterial Growth Indicator Tube (MGIT) liquid culture method for both 1st and 2nd line anti-tuberculosis drugs
- b. Genomic MDRTBplus test (HAIN test) also known as Line Probe assay (LPA) for both 1st and 2nd line anti-tuberculosis drugs
- c. Xpert MTB/Rif also known as CBNAAT or Gene Xpert.

4. Bacteriology culture

5. Mycology culture

6. Serology tests.

7. Urine and stool microscopy.

5.1. Guide for selection of Tests and their indications

1. Microscopy

- a. Ziehl-Neelsen Smear Microscopy (ZN Microscopy)
- b. Fluorescence Microscopy (FM) Indicated for the diagnosis of TB suspects, TB smear negatives by peripheral results (but clinically suggestive of TB) and Extra-pulmonary samples (often smear negative by peripheral results).

2. Culture and isolation of Mycobacterium (Culture)

- a. Löweinsten-Jensen solid Media Method (LJ Culture) Indicated for the follow up of the patients undergoing treatment for MDR tuberculosis follow-up test for MDR TB Treatment (culture conversion)
- b. Mycobacterial Growth Indicator Tube (MGIT) method Indicated for the follow up of the patients undergoing treatment for MDR tuberculosis
Follow-up test for MDR TB Treatment (culture conversion), as a prior test to DST and for the diagnosis of NTM (Non-TB *Mycobacterium*).

Drug susceptibility testing

Presently drug resistance detection using the following technologies is available for diagnosis of Drug Resistant TB through rapid molecular diagnostic testing:

- Line Probe Assay (LPA) for detection of MTB complex and resistance to first line drugs R, H and second-line drugs class FQ and class SLID; and
- Cartridge Based - Nucleic Acid Amplification Test (CBNAAT) Xpert MTB/Rif testing using the Gene-Xpert platform for now.

Growth-based phenotypic drug susceptibility testing: Culture though a highly sensitive and specific method for TB diagnosis, requires 2-8 weeks to yield results and hence does not help in early detection. However culture will be used for long-term follow-up of patients on Drug Resistant TB treatment and help detect early recurrence in both drug sensitive and drug-resistant TB. The growth-based phenotypic culture methods include automated Liquid culture systems e.g., BACTEC MGIT 960, and solid (Löwenstein Jensen) media.

Rapid molecular Drug Resistance Testing: Line Probe Assay (LPA) provides rapid diagnosis of R and H resistance as well as resistance to class FQ and class SLID. LPA can yield results in 72 hours. Nucleic Acid Amplification Test (NAAT) provides accurate and rapid diagnosis of TB by detecting M.Tb and R resistance conferring mutations. The test can be performed on both respiratory and non-respiratory specimens and yields results in 2 hours. Presently, under RNTCP, its use is recommended for diagnosis of DR-TB in presumptive DR-TB patients and TB in children, EP TB and in Key Population such as PLHIV, socially and clinically vulnerable groups and for active case finding efforts.

Drug resistance status is determined by either of the following methods:

Drug Resistance Tests (DRT) using molecular methods: This can be performed on sputum specimen (direct) or on culture isolates (indirect) for diagnostic purpose. The methods are PCR-based and cannot be used for determining response to treatment. The tests that belong to this group include:

- CBNAAT: can be performed on smear positive, smear negative and extra pulmonary specimen. The test detects TB and resistance to R; and

– Line Probe Assay (LPA): is performed on smear positive specimen. The test detects TB and resistance to R and H (FL- LPA) as well as class FQ and class SLI (SL-LPA)

Drug Susceptibility Test (DST): These are growth based tests and can be performed on L J culture isolates or in Liquid culture system Mycobacteria growth indicator tube (MGIT) for both pulmonary and EP specimen. Most commonly used method for testing is the economic variant of the proportion sensitivity. MGIT is preferred method for DST and both first and second-line anti-TB drugs can be tested.

Following drugs can be tested for susceptibility by liquid culture:

- first-line drugs: R, H, Z*
 - second-line drugs: Lfx, Mfx, Km, Cm, Am
 - other drugs: Lzd, Cfz*, Bdq*, Dlm* etc.,
- *when standardized, WHO endorsed and approved for use in programme

5.2 Indications of tests as per RNTCP protocol

INDICATION	TEST
MDR-TB suspects which are smear positive at diagnosis or have follow up smears positive during the course of treatment (CAT I, CAT II)	Line Probe Assay (LPA) if smear positive at NITRD Culture Isolation and then LPA if smear negative at NITRD
MDR-TB suspects which are smear negative at diagnosis	CBNAAT
Presumptive XDR suspects (confirmed MDR-TB patients and Mono Rif resistant patients)	Susceptibility testing to second line Antituberculosis drugs using liquid culture
Follow up of patients undergoing MDR/XDR treatment	Liquid culture- for critical months (3, 4, 5, 6, 18, 21, 24). Solid culture- for non-critical months

6.0 Sending the results back:

The results are entered in the standard RNTCP request form, scanned and mailed to the concerned districts. All OPD reports are sent to Window no. 5 and all indoor patients reports are sent to respective wards in the Institute.