# Guidance for accreditation of laboratories under RNTCP for Mycobacterial Culture &DST

(Applicable for mycobacteriology laboratories other than Intermediate Reference Laboratories (IRLs) applying for accreditation)

**The laboratories offering mycobacteriology services may process specimens either by solid or liquid methods and same should be indicated in their application forms.**

## Conditions for applying for accreditation

- The lab should have adequate infrastructure, equipment and staff i.e. is an existing, functioning mycobacteria culture and drug susceptibility laboratory
- Should be willing to undergo routine Quality Assurance & annual proficiency testing with RNTCP National Reference Laboratory(NRL)
- Should be willing to report on culture & DST activities to RNTCP NRL using standard culture and DST laboratory indicators
- Should be willing to offer culture &DST services to patients from RNTCP
- Should be able to develop the capacity to process at least 400 cultures per month by the year 2012.

## Process of Accreditation

### Procedures to be followed

- The applying lab should fill up the accreditation application form available in www.tbcindia.org/ **documents downloadable /format /accreditation of Medical College Labs for C&DST under RNTCP'** and send the electronic as well as print copy to Central TB Division(CTD) (ddgtb@rntcp.org;labcdst@rntcp.org)
- The form will be forwarded by CTD to the designated NRL for scrutiny
- The NRL along with members from CTD will undertake a pre-assessment visit to the applying lab to evaluate the infrastructure, manpower, procedures followed in the lab etc

### Proficiency testing

**There are two steps for this process**

**A. Retesting of strains identified from the applicant laboratory by the reference laboratory**

- This process actually assesses the laboratory performance in real time (their daily routine)

**B. Testing of Panel cultures sent out from the reference laboratories, by the applicant laboratory at periodic intervals**

- This is an actual test of performance like an exam conducted at periodic intervals

**For initial accreditation, all the applying laboratories should undergo the procedures of retesting as well as panel testing of the cultures. For renewal of accreditation, only panel testing is required.**

**A. Retesting**

- The reference lab will ask the applying laboratory to send a list of at least 100 recently done cultures with their sensitivity pattern[1]
- The reference lab will randomly select at least 10 cultures (consisting of various combinations of resistance pattern) from the list and ask the laboratory to send these cultures. These selected cultures should be freshly sub-cultured and send to the reference lab either in liquid or solid media
- The packing should be as per the transport guidelines for infectious material (triple packing) Transport guidelines-As Annexure III

**B. Panel testing**

- The NRL will send a panel of 20 strains to the applying laboratory
- The panel will consist of pan-sensitive, mono-resistant, MDR and poly resistant strains and some isolates in duplicate to check for reproducibility
- The lab should sub-culture these 20 strains and then set up DST
- DST is set up by the method that the laboratory routinely uses to perform them
- The test is also to be put up by the lab technician who routinely performs the DST test
- DST can also be set up by multiple methods if the lab wished to do so ( if the lab routinely uses different methods)
- The results of DST to be reported to NRL
- This exercise is usually done at annual intervals

Once the results of both retesting and panel testing are ready, the NRL checks for concordance between the two laboratories.

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[1] The 100 strains with various DST pattern is required to get a sufficient representation of all types of resistance ( including H mono, R mono , two, three, four drug and MDR

Concordance criteria as per international guidelines:

- H&R, >90%
- S >80%
- E>80%

**Accreditation is granted if the lab achieves the desired concordance for Isoniazid(H) and Rifampicin(R)**

**Accreditation**

- If the lab has achieved the desired concordance the central team will visit the lab(accreditation visit) for granting accreditation
- If the lab has not achieved the desired concordance, proficiency testing to be repeated and corrective actions also to be taken as per recommendations of the NRL
- The initial accreditation is valid for two years with panel testing every six months for the first two years and then annually

## Guidelines on manpower, infrastructure and Equipments

### Essential manpower

Each mycobacteriology laboratory should have at least the following personnel:

- **One Microbiologist( MSc/MD/PhD)**
- **Four lab technicians**
- **Three lab attendants**

**(**If needed, the training for the microbiologist and the lab technicians can be provided at one of the RNTCP National reference laboratories)

### Minimum space and equipments for culture and DST laboratory

(The sample layout of a mycobacteriology lab as recommended by RNTCP is at Annexure I.
**(**These specifications may be used while planning for new laboratories. However, slight modifications may also be required in the existing labs so that bio-safety measures are not compromised**)**

1. Adequate  space for washing and Sterilization
2. Space for storing  sterile items
3. Media Preparation & Inspissation room
4. Walk in Cold room
5. Culture Room and culture reading area
6. Walk-in incubator room/ space for keeping large-sized incubators to hold cultures
7. Space for keeping the deep freezer, BOD Incubator etc

***Technical specifications for Equipments & Lab consumables required for a lab is available in www.tbcindia.org/ documents downloadable/Technical Specifications***

**Annexures**

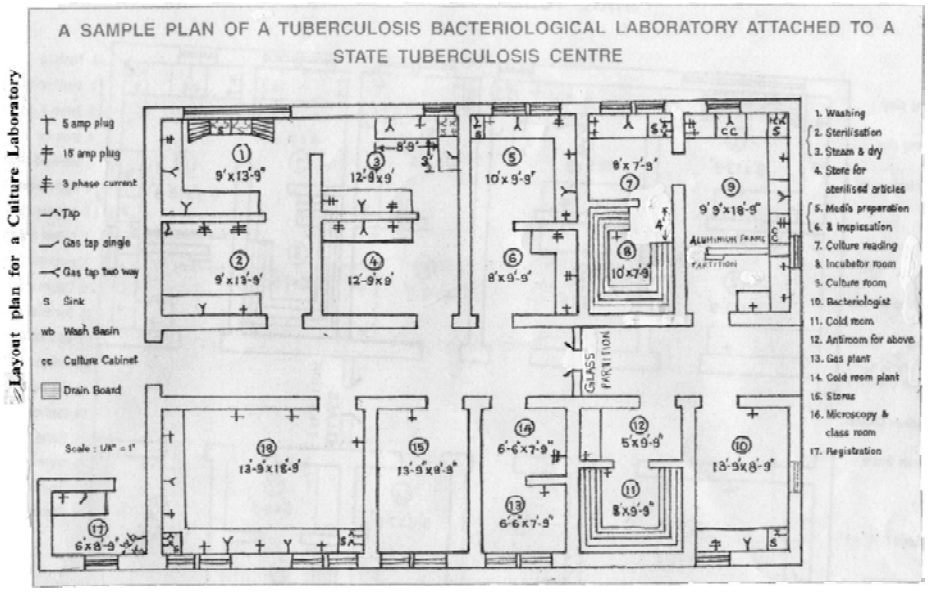
**I-Sample layout of a mycobacteriology lab recommended by RNTCP**

**II-Specifications for a walk in cold room**

**III-Guidelines for transportation of infectious materials**

**Annexure I**

## Sample layout of a mycobacteriology laboratory recommended by RNTCP




**1**.**Washing room**: It needs to have RCC re-inforced platforms on two sides with 2 or three large sinks, taps and drying platforms. Above the sinks and on the third wall preferably have a layer of cement shelves for holding trays of McCartney bottles. The washing area should have vitreous tiles on walls and floor with appropriate drainage, so as to facilitate washing. Small area to be identified for installing the bottle washer and another area against the wall to install the Distilled water plant. Electrical points and lighting to be fitted accordingly. All wash basins should have either foot or elbow operated taps. Washing area should have a Geyser, bottle/flask dryers and acid trough.

**2.Sterilisation room**: In the sterilization room, three three-phase electricity points with 15 amp plug points are needed to install autoclaves and hot-air oven. This room preferably should have large windows with ventilator fans as lot of steam and foul smell would be generated here. Platform with large sink required in this room too. Sterilization section should preferably use separate autoclaves for sterilization and disinfection of media and glassware

**3**. Store for sterile articles: Cement shelves along the walls, single entry which can be locked, room should be sealable for fumigation. The sterile store should be in proximity to Media preparation room.

**Main Lab unit**:
The remaining rooms can form the main lab unit and they should be in a "Restricted entry" area. For this, a glass partition may be provided in the corridor and have restricted entry in this area.

4. **Media Preparation room**: Work bench amenable to cleaning like enamel topped or granite topped cement platforms needed. Few normal plug points for use of homogenizer-stirrer and some storage area (almirahs, lockable shelves) for keeping the consumables are needed in this room. Good lighting. Platform with sink/ wash basin also required. Preferably, anti-static and anti-dust flooring such as Vinyl flooring is required for Media Preparation room It is ideal to have a refrigerator for storing the drugs, drug stock solutions, sterile malachite green solution and surface sterilized eggs.
Ideally, media should be prepared under a laminar flow cabinet and space for the same may be provided
The media preparation room should have an ante-room to limit external air flow and should be sealable for fumigation.

5. **Inspissation room**: Small room with RCC platforms to keep the inspissators (Normal Plug points required). This room will be kept warm (No A/C).

6. **Culture room**: As per WHO recommendations, the culture &DST for mycobacteria should be undertaken in a BSL -2 or 3 lab .There should be a large area for installing at least two bio-safety cabinets (BSC). Plug points to be fitted appropriately. The programme recommends Class II BSC. Depends on the type of BSC and maintenance, most of these cabinets require ducting outside and therefore space for that to be earmarked while planning the civil works. Adequate space and 15 amps plug point to be provided for keeping the centrifuge and also there should be enough space to keep the culture racks (centre table with washable top).The room should be air-conditioned.

**7. Culture reading area**: Work bench amenable to cleaning like enamel topped or granite topped cement platforms needed Good lighting, few movable table lights to help in reading the number of colonies in the bottles, Shelves and lockable almirahs. This area should be connected directly to walk-in incubator and culture room.

The Culture room and Culture reading area will be one unit with one entry point. An air-curtain (e.g. Almonard) may be installed at entry of this unit. Walk-in incubator will be accessible only from culture reading area. They must be sealable for fumigation.

**8.** Space also to be provided for keeping the deep freezers (-20/-80) and BOD Incubator

**9**. **Walk in cold room:** ( see the specifications at Annexure III)

- Approximate size: 6 feet x 7 feet x 9 feet high
- Able to maintain a temperature of 2 to 4 degrees centigrade at all times
- Have adjustable metal racks inside along the walls to hold the Universal bottle trays

**10. Walk-in incubator room**: Should be connected directly to culture reading room and indirectly to culture room.

- Approximate size: 8 feet x 10 feet x 9 feet
- Able to maintain temperature of 37 degrees centigrade at all times.
- Have adjustable metal racks inside along the walls to hold the Universal bottle trays

The walk in incubator rooms need a heater with a thermostat set at 37 degrees and a temperature indicator and maybe a room thermometer. It would automatically switch off at 38 degrees. There would be a small fan in the room which would be linked to the thermostat and would come on for few minutes if the room temperature reaches 38 degrees. The door to this room has to be made of very thick material to maintain the heat.
Alternatively, adequate space may be provided for keeping multiple (3-4) 37 degree incubators as Walk-in incubator rooms are only necessary if the lab is undertaking large number of solid cultures.

**Annexure II**

## Technical Specification for WIC Room (Walk in Cold Room)

Walk-In-Cooler should comply with the following specification

Panels & door: External Size of the Cold Room : 2.65m x 2.65m x 3.61 m(height)

Thickness of Panel: 60 mm

Insulation: CFC free Foamed in Place Polyurethane Foam (PUF), Prefabricated.

Thermal conductivity : 0.16 K –BTU/HR/DEG F/INCH of Panel Thickness

Co-efficient of Heat Transfer: 0.366 W/m C FOR 60 mm PUF PANEL THICKNESS

Density: 36-40 KG/CU.M

Internal & External Finish: 24 SWG pre-painted GI Sheet

Door Specification:

1. Size: 34" x 78" – One No.
2. Type: Flush Type Door
3. Posi seal door closer
4. Positive Cam lift Hardware
5. Vinyl Wiper Gasket with SS Bracket Bottom of Door
6. Safety Release Exit Device for Opening Door from Inside
7. Lock Arrangement from Outside
8. Metallic Cam lock
9. FRP Door Perimeter

Corners: Cove (Rounded) Corners

Temperature Indicator: Digital Type Temperature Indicator

Corner Panels: 12" x 12" L – Shaped

Panel Joints: With Double vinyl Gasket (Pre-fabricated with the panels to make leak proof joints)

Lamps: 40W, INCANDESCENT, MOISTURE PROOF – 01

Panel Design should have:

1. Walls/Corners have double bends on length for fixing sectional gaskets with return Top/Bottom of walls also has 'U' sect, Gasket. These PVC gaskets helps to accept fit of gaps between the panels and provides Air tight joints without using Silicon sealant.

2. Panel doors should be of flush type with Posi-seal door closure, brushed chrome latch strap type. Cam lift hinges.

3. Door Frame/leaf perimeter is of fiberglass plastic (FRP). FRP resists rust, scratches Dents, impacts and distortion.

4. Corners and Floors should have radius to impeded bacterial growth.

5. Metallic Cam locks designed to withstand 550lbs and uprooting 750lbs maximum.

Floor Design: 100 mm EPS to be provided by supplier and concrete and stone over and above EPS will be provided by Customer

Refrigeration system

Total refrigeration capacity: 10000 BTUH

No. of refrigeration systems: ONE

Condensing Unit

1. Compressor Type : Hermetic
2. Compressor Make : Any reputed firm (eg., Kirloskar – Copeland)
3. No of Compressor : 01 (One Nos.)
4. No of Circuit : 01 (One Nos.)
5. Compressor Power : 1400 W x (Maximum)
6. Compressor Setting : thur Thermostat
7. Total Power Consumption : 1900 W
8. Fan Diameter : 405 mm
9. Condensing Fan Motor : 1/10 H.P., 900 RPM
10. No of Condensing Fan : One
11. Condensing Coil Material : H" inner Grooved Copper tubes with Slit Aluminium
12. Row Depth : 2
13. Fins Per Inch : 8 FPI
14. Condensing Coil Face Area : 0.37 Sq. mt.
15. Size of Condensing Unit : 850 L x 335 W x 540 H mm
16. Refrigeration Capacity : 10000 BTUH at 4 Deg C Room Temperature
17. Power Supply : 230 V/1 Ph/50 Hz
18. Air Flow : 1600 CFM
19. Weight : 61 KGS (Maximum)
20. Refrigerant : R-22
21. Refrigerant Connections : Flared
22. Suction Line : 5/8 Inch
23. Discharge Line : 3/8 Inch

Evaporating Unit

1. No of Evaporators : 01
2. Air Flow Rate : 1600 CFM
3. Size (L x W x H) : 1245 x 305 x 380 mm
4. Refrigerant : R-22
5. No of Fans : Two
6. Fan Motor : 1/10 HP, 1200 RPM – 2 Nos.
7. Fan Diameter : 300 mm
8. Cooling Coil : 3 Row , 8 FPI
9. Weight : 25 KG
10. Power Supply : 230 V/ 1 PH / 50 Hz
11. Mounting : Ceiling Mounted
12. Piping : Copper Piping and Cabling as required between condensing and evaporating unit
13. Length of Copper Piping : 15 Feet
14. Operation : Through Independent Refrigeration Circuit of Condensing Units.

Qualification for the tender

1) The supplier company must have supplied, installed and commissioned at least Ten cold room in the last three years in the respective state
2) The supplier company must provide at least 25 customer satisfaction certificate for the equipment supplied and installed all over India
3) The supplier company must have a local service in the city of IRL for proper service for the equipment.
4) The supplier company should take care of after sales service and AMC, which has to be provided on chargeable basis after completion of warranty.
5) The suppler must provide factory made product to be assembled at site for insulation and the refrigeration system should be preassemble, pre tested and charged with Gas only to be fitted at site on the insulation system
6) The supplier should have ISO 9001 certification for last 5 years

Approximate Budget is Rs. 3, 00, 000/- Inclusive of all charges, taxes & duties

**Annexure III**

## Transport Guidelines for infectious materials (as per WHO)

**Packing Instruction**

The packaging shall be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport, including transshipment between transport units and between transport units and warehouses as well as any removal from a pallet or overpack for subsequent manual or mechanical handling. Packaging shall be constructed and closed to prevent any loss of contents that might be caused under normal conditions of transport by vibration or by changes in temperature, humidity or pressure.

The packaging shall consist of three components:

***(a) a primary receptacle,***
***(b) a secondary packaging, and***
***(c) an outer packaging (The outer packaging must be rigid).***

Primary receptacles shall be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging.

Secondary packaging shall be secured in outer packaging with suitable cushioning material. Any leakage of the contents shall not compromise the integrity of the cushioning material or of the outer packaging.

For transport, the biohazard mark shall be displayed on the external surface of the outer packaging.

For liquid substances

(a) The primary receptacle(s) shall be leak-proof; and must not contain more than 1 litre;

(b) The secondary packaging shall be leak-proof;

(c) If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them;

(d) Absorbent material shall be placed between the primary receptacle(s) and the secondary packaging. The absorbent material shall be in quantity sufficient to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging;

(e) The primary receptacle or the secondary packaging shall be capable of withstanding, and without leakage

(f) The outer package must not contain more than 4 litres. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold.

For solid substances

(a) The primary receptacle(s) shall be sift-proof; and must not exceed the outer packaging mass limit;

(b) The secondary packaging shall be sift-proof;

(c) If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them.

(d) Except for packages containing body parts, organs or whole bodies, the outer package must not contain more than 4 kg. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold;

(e) If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport, then packaging suitable for liquids, including absorbent materials, must be used.

Refrigerated or frozen specimens: Ice, dry ice and liquid nitrogen

(a) When dry ice or liquid nitrogen is used to keep specimens cold, the ice or dry ice shall be placed outside the secondary packaging or in the outer packaging or an overpack. Interior supports shall be provided to secure the secondary packaging in the original position after the ice or dry ice has dissipated. If ice is used, the outside packaging or overpack shall be leak-proof. If carbon dioxide, solid (dry ice) is used, the packaging shall be designed and constructed to permit the release of carbon dioxide gas to prevent a build-up of pressure that could rupture the packaging and shall be marked "Carbon dioxide, solid" or "Dry ice".

(b) The primary receptacle and the secondary packaging shall maintain their integrity at the temperature of the refrigerant used as well as the temperatures and the pressures which could result if refrigeration were lost.

When packages are placed in an overpack, the package markings required by this packing instructions shall either be clearly visible or be reproduced on the outside of the overpack.