



Revised National TB Control Programme
Manual of Standard Operating Procedures (SOPs)

Culture of *Mycobacterium tuberculosis* and Drug
Susceptibility Testing on solid Medium



सत्यमेव जयते

Central TB Division

Directorate General of Health Services

Ministry of Health and Family Welfare, Nirman Bhavan,

New Delhi 110011

IRL <<name, address>>	Standard Operating Procedures (SOPs) INTERMEDIATE REFERENCE LABORATORY for Tuberculosis
-----------------------------	---

Manual of Standard Operating Procedures (SOPs)

INTERMEDIATE REFERENCE LABORATORY for Tuberculosis

<< State name, place, full address >>

Culture of *Mycobacterium tuberculosis* and Drug Susceptibility Testing on solid Medium

Revised National Tuberculosis Control Programme
Central TB Division,
(Ministry of Health & Family Welfare)
New Delhi

Version No. 01.01

Date: 01/04/2009

IRL <<name, address>>	Standard Operating Procedures (SOPs) INTERMEDIATE REFERENCE LABORATORY for Tuberculosis
-----------------------------	---

Scope

This SOPs document has been specially compiled for the needs of the RNTCP laboratory network performing Culture & Drug susceptibility testing for *Mycobacterium tuberculosis* and intended for the use of Intermediate Reference Laboratories, located in various states of the country. The drafting, editing and reviewing comments were provided by the NRLs in India. It is also intended as a companion to the NRL training manuals.

<u>Scope- Place of implementation</u>
Microbiologist-IRL, Director-STDC/IRL, <<state name, address>>, <<Address and name of State TB cell>>
<<NRL in charge of the IRL, address>.>

Acknowledgements:

The Central TB Division acknowledges the technical contributions of National Reference Laboratories, WHO-India, and WHO-SEARO in the preparation of this generic SOP document.

IRL <<name, address>>	Standard Operating Procedures (SOPs) INTERMEDIATE REFERENCE LABORATORY for Tuberculosis
------------------------------------	--

Reviewed by:		
Sl. No.	Name & Signature	Designation & date
		Microbiologist

Date of SOPs implementation:

Head of Laboratory:

Laboratory staff have read the SOPs and are been trained to use, appropriately

Sl. No.	Name & Signature	Designation & date

IRL <<name, address>>	Standard Operating Procedures (SOPs) INTERMEDIATE REFERENCE LABORATORY for Tuberculosis
-----------------------------	--

Overview of the Standard operating procedures

Standard operating procedures (SOPs) describe the activities performed in the tuberculosis laboratory to:

- provide uniformity, consistency and reliability in each of the activities performed in the laboratory;
- reduce systematic errors;
- provide training and guidance for new staff

Standard operating procedures should be drawn up by technical staff in the laboratory, revised by microbiologist and approved by the Director of the laboratory.

The lay out of the SOPs is given below:

Layout – standard operating procedure

The header on the top of all pages will identify:

- the laboratory;
- the number relating to each procedure;
- Date of approved version
- Authors

Title: Descriptive title of the SOP

1. Objective and Scope: The aim of the procedure being described -expressed clearly and concisely. Name the operating unit that will apply the procedure, and the field of application of the procedure.

2. Definitions and abbreviations: The meaning of the principal terms used in the procedure, along with abbreviations used in procedure.

3. Procedure: Each SOP is drawn up clearly, without ambiguity, so that it can be understood by staff with and without experience. Each step for performing the activity that is regulated by the procedure is described in detail. Flow diagrams may be aid and complement the description.

4. Safety conditions: Reflects the safety measures and conditions to be kept in mind for the correct execution of the SOP.

5. Documentation: The form and register in which the data and measurements involved in the procedures are recorded.

RNTCP has developed these SOPs for smooth functioning of Intermediate reference TB laboratories. These are generic to some extent, and the microbiologists are encouraged to adapt them to their needs and follow to improve the quality of diagnostic services.

IRL <<name, address>>	Standard Operating Procedures (SOPs) INTERMEDIATE REFERENCE LABORATORY for Tuberculosis
------------------------------------	--

CONTENTS

<u>SOP Number</u>	<u>SOP TITLE</u>
1.0.	General description of IRL & Infection Control Measures
2.0.	Bio-safety & precautions in TB Laboratory
2.1.	Fumigation for rooms : Formaldehyde & Potassium Permanganate Method
3.0.	Culture Media preparation
3.1.	Preparation of plain egg-based Lowenstein Jensen (LJ) medium
3.2.	Preparation of LJ Medium with anti-TB drugs
3.3.	Preparation of Para Nitro Benzoic acid (PNB) medium
4.0.	Sample processing
4.1.	Specimen collection, transport, and handling
4.2.	Specimen processing for culture
5.0.	Identification for <i>M .tuberculosis</i> (PNB method)
5.1.	Identification of <i>M .tuberculosis</i>
6.0.	Long term Preservation & recovery of the <i>Mycobacterial</i> cultures
7.0.	Drug Susceptibility Testing (DST)
7.1.	Drug susceptibility testing (DST) on LJ medium by 1% Proportion method - standard economic variant
8.0.	Transportation of <i>Mycobacterium tuberculosis</i> containing specimens and cultures
9.0.	Maintenance of Instruments
9.1.	Maintenance and use of a BSC, Class II
9.2.	Maintenance and use of an autoclave
9.3.	Maintenance and use of a centrifuge

IRL <<name, address>>	Standard Operating Procedures (SOPs) INTERMEDIATE REFERENCE LABORATORY for Tuberculosis
-----------------------------	---

<u>SOP Number</u>	<u>SOP TITLE</u>
9.4.	Maintenance and use of a incubator
9.5.	Maintenance and use of a refrigerator
9.6.	Maintenance and use of a balance
9.7.	Maintenance and use of a freezer
9.8.	Maintenance and use of a pH meter
10.0.	Washing glassware
	Performance Indicators for C & DST laboratories
	<u>Flow diagrams of procedures</u>
	<u>Annexure-format of registers</u>
	References

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/4/2009

Procedure No. 01.01

Author(s): Lab- microbiologist & lab staff

1.0. General description of IRL & Infection Control Measures

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>
Date: 1/4/2009

Procedure No. 01.01
Author(s): Lab- microbiologist & lab staff

TITLE: General description of Intermediate Reference Laboratory and infection control measures

1. Objective and scope:

1. To describe roles & organisation of the IRL, <<name and address>>
2. To describe containment and infection control measures of the laboratory
3. To detail the flow of routine specimens for culture and Drug Susceptibility Testing in the laboratory.

2. Definitions and abbreviations:

IRL-Intermediate reference laboratory, a state level TB laboratory providing C&DST facilities and supervision for EQA of smear microscopy

3. Procedure:

3.1 Laboratory organisation:

THE ROLES & RESPONSIBILITIES OF THE Intermediate TB REFERENCE LAB ARE:

- (1) To train the Lab technicians for TB diagnosis and update/refresh their technical skills
- (2) Provide quality assured Culture and Drug sensitivity Testing (DST) facilities to first line anti-TB drugs, namely Streptomycin, Isoniazid, Rifmapicin and Ethambutol.
- (3) To assist & guide state level National TB control Programme on all laboratory aspects
- (4) Supervision of TB Lab network in all district level laboratories for quality assurance (External quality assessment: on-site evaluation, Panel testing, and Random blinded rechecking).

Organisation:

TB LABORATORY ORGANISATION



Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

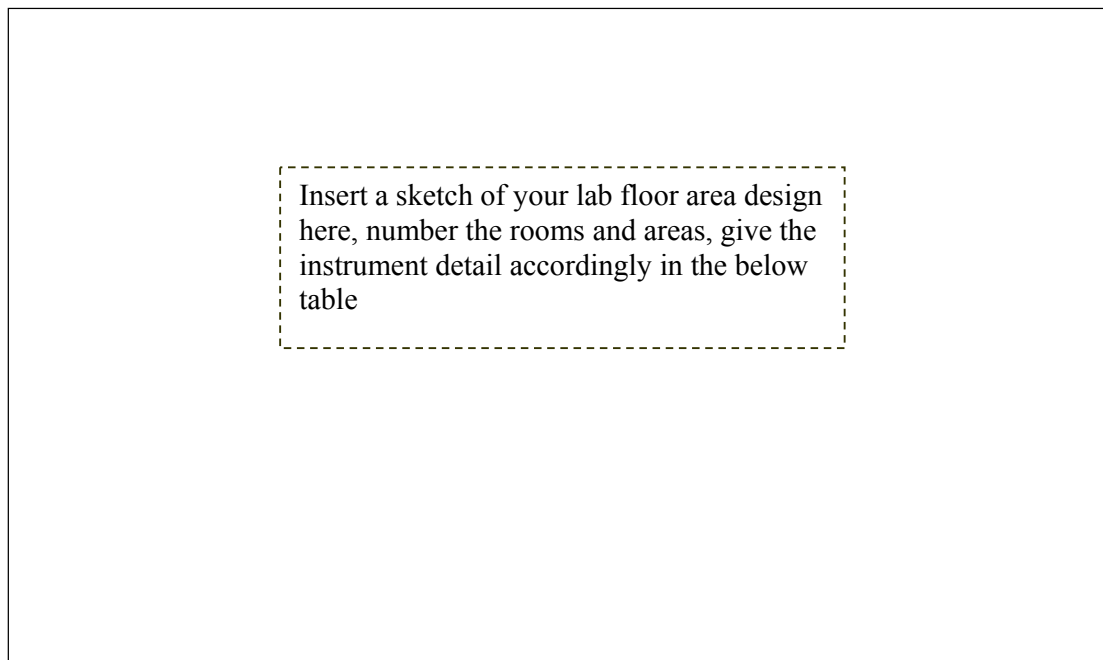
Procedure No. 01.01

Date: 1/4/2009

Author(s): Lab- microbiologist & lab staff

Laboratory facility: Lab facility sketch is given below

Fig 1: The schematic sketch of lab floor plan



The organisation in terms of rooms and equipment:

<<Describe the room & instruments organisation for the above diagram>>

Room No.	Work	Instruments	Access
1	Specimen receipt Microscopy, reading and reporting	Microscopes, staining sink, work bench for recording and specimen receipt, computer for data entry	Separate access, away from containment room
2	Media preparation room	Inspissator, weighing balance, Sink, and clean-air bench.	Nearer to culture room, separate
3	Culture room (directional air flow)	2 BSC (class II with ducting to out), work bench, centrifuge, A/C, one hand-wash sink. Refrigerator, Incubator	Through anteroom
4	Sterilization room	Autoclave with exhaust, workbenches, big washing sink, and disinfection containers, hot air oven	Direct Access to outside the lab, separated from culture room and media preparation room
6	Anteroom/Change-over partition	Cupboard for coveralls, N95 masks, shoes for inside work, hand-wash sink.	Culture room
5 & 7	Passage ways		

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/4/2009

Procedure No. 01.01

Author(s): Lab- microbiologist & lab staff

3.1.2. Major Safety requirements:

1. In any kind of Major Spill/breakage of tubes in the containment room intimate all co-workers to get out of room. Keep the Bio-safety cabinets ON for 1 hour, before you enter with N95 mask, gloves, & clean using spill kit with 5% phenol; allow contact time of 30 min. Dispose the same as the infectious material. Make entries in spill register.
2. Every day, in the morning, the containment room should be mopped with disinfectant. Do not use the same mop for use outside the containment room or bench surfaces.

3.1.3. Personnel:

1. Laboratory technicians must have specific training for handling *Mycobacterium tuberculosis* complex strains and need to be supervised by the Laboratory microbiologist.
2. Infection control measures, clinical symptoms of tuberculosis and nature of causative agent, and containment of laboratory infections should be known to the Laboratory technician.
3. Laboratory technicians must have full working knowledge of microbiology, sterile technique and work experience in handling *Mycobacterium tuberculosis*.
4. The health of Laboratory technician should be monitored on a regular basis. Any unexplained episodes of weight loss, fever, or chronic cough in an individual should be notified to the Lab In-charge.

3.2. INFECTION CONTROL ASPECTS

A three tiered infection control measures are under taken with regard to laboratory bio-safety and infection control.

3.2.1. Administrative Controls:

The following Administrative TB control measures are used, and required, to reduce the risk for exposure to TB infectious aerosols, in the laboratory settings.

Administrative controls consist of the following activities:

- Laboratory microbiologist is the responsible officer for TB infection control measures. The laboratory microbiologist in turn can assign these responsibilities to other senior laboratory personnel, in case of her/his absence.
- Written TB infection control measures for lab are available, and LTs are trained/oriented in ensuring airborne precautions
- Written bio-safety document is available
- Orientations/trainings/refreshers activities for laboratory activities are performed, periodically, once in a year.
- Identification of suspected TB patient, segregation as far as possible, and prompt reporting is practised for smear microscopy
- Standard operating procedures for the laboratory operation from sputum collection to disposal, to minimize the aerosol generation, were developed and LTs were trained

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 01.01

Date: 1/4/2009

Author(s): Lab- microbiologist & lab staff

- Emphasis is placed on timely availability of laboratory results & reporting
- Screening, monitoring and evaluating LTs at risk for TB disease or who might be exposed to *M. tuberculosis* is emphasized.
- Appropriate signs, check-lists are posted in laboratory, especially the discarding bins/buckets/containers.
- Monthly analysis & abstracting of results and assessment of laboratory procedures is carried out.
- Shipping of cultures to reference laboratory need to follow bio-safe-triple packaged containers.

Laboratory activity-wise list of administrative controls and bio-safe practises is listed below:

Activity	Administrative controls	Bio-safe Practice and procedure
Preparing specimens for centrifugation and AFB culture	Train personnel in safety procedures.	Conduct all work in the BSC on a tissue-wad moistened with a tuberculocidal agent; use aerosol-containing safety cups for centrifugation.
Centrifugation of specimens with live TB bacteria	Bio-safe centrifuge & biocontainment devices.	Use aerosol-containing safety cups for centrifugation; open only in the BSC.
Inoculating cultures from specimens	Use BSC and follow biosafety practices and procedures.	Follow aseptic techniques; autoclave all wastes from the BSC
Handling unopened primary-isolation culture bottles/Mccartney bottles	Treat all cultures as potentially infectious	Carry all the materials in trays and in designated racks; and not individually in hands. Label the racks and trays.
Staining smear of material from culture	Biosafety cabinet class II B	Prepare slides in a BSC. Before removal from BSC, heat-fix to kill tubercle bacilli. Do not carry individual slides in hands, carry as a whole on a slide-carrying tray
Manipulating grown cultures of <i>M. tuberculosis</i> complex species on solid medium	Label culture bottles as containing <i>M. tuberculosis</i> complex; and screw-caps tightened. Biosafety cabinets Class II B.	Open all culture bottles only in BSC. Irradiate inoculation loops before and after use, in the flame.
Transferring suspensions of bacilli	Ensure BSC is certified annually using calibrated instruments by qualified person; maintain directional air flow and room air changes; Adhere to spill protocol for management of accidents.	Vortex and sonicate suspensions in BSC in closed tubes that are opened only in BSC. Use aerosol-containing centrifuge cups and open only in BSC. Manage waste safely.
Disposing of cultures of <i>M. tuberculosis</i> complex	Identify material with proper disposal labels and autoclave prior to disposal.	Discard liquid waste into a tuberculocidal 5% phenolic disinfectant solution; transfer to autoclave carefully for sterilization.
Shipping cultures or specimens of <i>M. tuberculosis</i> complex	Provide approved and safe shipping containers	Ship in triple - packaged container. Follow SOP and regulations for transport of diagnostic specimens and infectious substances.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 01.01

Date: 1/4/2009

Author(s): Lab- microbiologist & lab staff

3.2.2. Environmental controls (facility requirements) adhered at IRL:

Facility wise, the environmental control measures are listed below:

- Laboratory is either placed at the blind-end of building and/ physically Isolated from the common lab/hospital environments
- The entry to lab is restricted to trained laboratory personnel
- The containment room where culture and DST is carried out is sealable for decontamination, including formaldehyde fumigation
- Bio-safety cabinets, ducted to outside, while switched ON, would maintain an inward air flow into the culture and DST facilities
- Access to the culture and DST rooms is always through an anteroom
- Hand-wash sink is provided in the culture & DST room with effective disinfectant
- Autoclave (steam sterilization facility) is provided within the laboratory facility.
- Biological safety cabinets (BSC) class II B, ducted to outside, is provided
- Bio-safe centrifuge, with aerosol-seal buckets is provided

3.2.3. Personal Protection measures:

Following protection measures to be followed in the lab by lab staff

- (a) For sputum collection & smear microscopy:
 - a. Proper cough hygiene needs to be explained to the patients
 - b. LT would maintain at least one arm length distance and upwind when a patient is collecting a sputum sample.
 - c. Wear lab coats while performing the lab work.
- (b) For culture and DST activities:
 - a. All personnel working in culture lab, need to wear separate clothing, not the common lab coat. Separate foot-wear to be used.
 - b. While performing culture and DST activities and while reading and recording the results laboratory technicians should exercise maximum caution. Wherever available N95 masks should be worn with appropriately nose-fit. However, mere wearing of masks lead to false safety. Safety practises are more important.
 - c. Remove lab coat before leaving the lab
 - d. Decontaminate lab coat before laundering or disposal.
 - e. In case of accidents and spillages, LT should strictly adhere to the SOP 2.01 given at page 14.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/4/2009

Procedure No. 01.01

Author(s): Lab- microbiologist & lab staff

3.3. FLOW OF SPECIMENS FOR CULTURE & DST

1. Specimen receipt
 - a. Entry made into **Specimen registers** (every patient gets a unique consecutive Lab number),
 - b. **Culture card** for each specimen initiated (a patient with a unique number will have two specimen cards with suffix a (spot), & b (early morning)). Culture card system is optional.
 - c. Visual observation by the LT, and for unsatisfactory specimens, sputum collected again.
2. Specimen goes into the culture room
 - a. Sputum processing for the ZN sputum smear in designated place/table nearer to window, sterile wooden applicators would be used. Heat fixed slides goes into staining area; results entered into RNTCP **Lab register**.
 - b. Sputum stored in the refrigerator (if not without CPC), when not processed for Culture.
 - c. Go to step 4 when media is ready.
3. LJ Media is prepared in the media room, in the dust-free and clean, media preparation room as per SOP 08.01 (all bench, surfaces need to be disinfected),
 - a. details entered into **Media register**;
 - b. entries made in **Egg stock register**; Eggs, country type, should not be more than one day old when purchased and would be exhausted in a weeks time.
 - c. [if drug media is prepared-SOP 11.01, details entered of drug stock preparation and usage, in **Drug stock register**]
 - d. Each batch is **sterility** checked, entries made into media register
 - e. All the materials used in media preparation are cleaned and sterilized. Entries made in the **Autoclave register**. Manual is strictly followed for washing and sterilization.
4. The sputum is processed for culture (in BSC no.2), as per the SOP 4.2, detailed entries made into **culture cards**.
Each specimen is inoculated into two LJ drug free media slopes. (Thus, a patient would have 4 slopes).
5. (a) Cultures transferred to 37°C incubator/walk-in incubator room. All slopes strictly arranged, in the racks, as per dates of inoculation, and placed in the incubator. All racks would have a label tag on date of inoculation and person responsible.
(b) Cards are kept in a box, for Reading and reporting <In a convenient place, near the incubator>
6. The tubes/culture bottles are observed for growth as per reading and reporting schedule and entries made into the **Primary culture register**.
7. Identity is tested initially on PNB, along with DST.
8. Biochemical confirmatory test for *M.tb* identity are: Niacin and 68°C catalase test. Record in the register.
9. Finalized results of primary culture & identity entered into culture cards.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 01.01

Date: 1/4/2009

Author(s): Lab- microbiologist & lab staff

10. If DST is started, the card is entered with relevant entries, and 1% Proportion method is followed as per SOP 7.01. Details entered into the **DST register**; reading and entry done as per the schedule given in the SOP.
11. Fully filled, culture cards are autoclaved and sent back to the specimen receipt room for final reporting.
12. Cultures preserved in cryo-vials and transferred to -70°C or -20°C, rest discarded and sent back to autoclave room and entries made in the autoclave register.
13. Three registers are digitized / computerized. (a) Specimen register (b) primary culture register and (c) DST register.
14. Lab microbiologist would assign personnel for the work as per a regular roster:
 - a. Two personnel for Specimen receipt & Microscopy, along with reading and reporting. (personnel at 'c' would share the work at 'a')
 - b. Two personnel for Culture and DST, along with reading and reporting.
 - c. Weekly rotational basis, two personnel for Media preparation and Sterilization sections, and maintenance of section supplies.
15. Regular entries are made into hard copy registers, as well as three electronic registers by the concerned personnel after completion of the work, without fail.

4.0. Documentation:

Details of the forms and registers to be used for data entries are listed in the section 3.3.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/4/2009

Procedure No. 02.00

Author(s): Lab- microbiologist & lab staff

2.0 Bio-safety precautions in Tuberculosis Laboratory

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 02.00

Date: 1/4/2009

Author(s): Lab- microbiologist & lab staff

TITLE: Bio-safety precautions in Tuberculosis Laboratory

1. Objective & Scope:

1. To describe general laboratory safety precautions meant to be followed in the TB laboratory.
2. To indicate specific bio-safety practises & bio-safety signage for TB laboratory

2. Definitions and abbreviations:

Bio-safety: Safety to the personnel and environment needed while handling biologically infectious/hazardous substances, such as tuberculosis bacilli.

N-95 respirtor: A disposable particular respirator that has the ability to filter out 95% of particles greater than 0.3 microns of diameter.

3. Procedures:

3.1.1 Description:

TB Laboratory has all the major facility requirements for handling *Mycobacterium tuberculosis* safely, and involves minimum risk to the laboratory personnel if they take proper precautions, and employ proper techniques described in these SOPs. Laboratory safety involves all the procedures and methods one needs to follow to minimise the risks of laboratory acquired infections. Use of Laboratory is limited to trained TB lab personnel.

Do:

1. Ensure that laboratory floor (containment lab) is cleaned with disinfectant every day. The same mop should not be used for mopping outside the lab, or bench surfaces.
2. Ensure that the instruments surfaces are wiped out with 70% alcohol solution, regularly
3. Use double pairs of gloves while working inside bio-safety cabinet. Remove the outer pair after completion of work inside the bio-safety cabinet and discard into biohazard bag inside the bio-safety cabinet. Apply disinfectant to hands after removing the inner pair of gloves.
4. Ensure that any materials taken out of culture & DST facility are to be disinfected and autoclaved.
5. Ensure that infectious materials are placed away from the regular reagents.
6. **Important:** Wash hands thoroughly with disinfectant and tap water before starting work, after work, and before leaving the containment facility. Wash again while leaving the anteroom.

Don't:

1. Eating, drinking, smoking, applying cosmetics, use of mobile phones, or applying contact lenses in the TB laboratory.
2. Don't allow unauthorized personnel to enter the TB Laboratory.
3. Mouth pipetting.
4. Crowding of lab with material that is not required inside.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/4/2009

Procedure No. 02.00

Author(s): Lab- microbiologist & lab staff

3.1.2 Entry & Exit requirements for Culture and Drug susceptibility testing facilities

1. Enter the anteroom, change the lab coat to the one required that is placed inside. Change the street shoes to the required ones.
2. Wash hands thoroughly with a disinfectant and let dry.
3. Optional: Wear the gloves, and tuck inside the coat sleeves, it is preferred to wear double pair of gloves.
4. Firmly close the outer door of anteroom,
5. Open and inner door and make an entry.
6. Close the door firmly behind you.
7. The same procedures are followed in reverse order while exiting the facility

3.1.3 Biological safety Cabinets

All procedures requiring manipulation of TB cultures must be done within the Bio safety cabinet.

1. Switch ON the safety cabinets for at least 30 min before use. Note that the reading on the mini gauge pressure is satisfactory.
2. Wear double pair of gloves, every time you work inside the cabinet.
3. Biosafety cabinets need to be cleaned with 5% Phenlic solution before work.
4. Keep disposal bin/vessel with 5% phenolic disinfect inside the cabinet at right side corner.
5. Wipes of Gauge-cloth soaked in 5% phenolic, should be readily available inside the cabinet.
6. Arrange all un-infected material required towards left side.
7. All the processed samples need to be arranged right side.
8. Don't process more than 8 specimens at a time, inside the cabinet.
9. After completion of work, wipe off the surface with 5% phenolic solution, and discard all wipes in biohazard bags, or in disposal container meant for infectious materials.
10. Discard off the outer glove, too, inside the bio-safety cabinet.
11. Wipe off inner glove with disinfectant before touching anything else in the lab.

3.1.4 Waste disposal and handling:

All infectious waste should be discarded in the bio-safety disposal bin. All infectious solid waste-wipes, swabs, plastic, paper towels, guaze pads, gloves, etc., should be placed inside the double autoclave bags, sealed with autoclave tape and sterilized at 121°c for 30 min in the autoclave.

Liquid waste, in the steel discarding bins, should be disinfected in 5% phenol for at least 1 hour, before sealing the caps and autoclaved at 121° c for 30 min.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 02.00

Date: 1/4/2009

Author(s): Lab- microbiologist & lab staff

Place all autoclaving material inside the steel/iron mesh tray of autoclave. Don't place things directly on the chamber of autoclave.

All reusable material such as glass ware should be autoclaved in the autoclave steel trays/Iron mesh for 121°C for 30 min before washing and repacked for sterilization.

3.1.5 ACCIDENTS & SPILLAGES:

Any major accidents in the laboratory should be entered in the register along with remedial measures taken before undertaking further work.

Laboratory personnel who are accidentally exposed to an infectious TB aerosol or solution should report the incident as soon as possible to the Laboratory Microbiologist/ Director. The Laboratory Director will see that necessary treatment or health monitoring is organized without delay.

3.1.5.1 Spills inside biological safety cabinet

A Bio-safety Cabinet is designed to contain spills and associated aerosols which are released during work within the cabinet. A spill of a TB material should be attended to immediately.

Decontamination of the work zone is done by direct application of 5% phenol disinfectant solution along with a thorough wipe down procedure. Formaldehyde gas decontamination may be required to treat inaccessible sections of the cabinet interior following a spill.

Follow as given below:

- 1) All workers using the Bio-safety Cabinets should keep absorbent materials (gauge cloth/adsorbent sheet) and 5% phenol within the cabinet.
- 2) Alert all people in lab of immediate area of in the event of spill.
- 3) Spread 5% phenol soaked wipe immediately, while the biological safety cabinet continues to operate. Wait for 15-20 mins.
- 4) Wear double pair of gloves during decontamination procedure.
- 5) Contain the spill and decontaminate.
- 6) Use paper towels to wipe up the spill, working from the edges into the center.
- 7) Decontaminate equipment. Items that are not readily or easily surface decontaminated should be carefully placed into autoclave bags and removed for further treatment (e.g., decontamination by autoclaving)
- 8) Contaminated gloves and clothes (sleeves are most likely to be contaminated and, remove and decontaminate the lab coat by autoclaving or soaking in decontaminant).
- 9) Individuals involved in the spill and clean-up should remove protective clothing (disposing and decontaminating), wash their hands and face with an appropriate detergent soap, and report to the Lab in-charge.

3.1.5.2 Spills outside containment room in the biological safety cabinet

Spills on equipment (such as vortex, centrifuge, incubator, refrigerator etc.), laboratory benches, walls, or floors:

- 1) Immediately indicate to all personnel working in the lab, and evacuate for 1 hour to allow dissipation of aerosols created by the spill (negative air pressure system would clear the aerosols).
- 2) Leave the biological safety cabinet operating and cultures inside cabinet.
- 3) Leave the containment facility following exit procedures.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 02.00

Date: 1/4/2009

Author(s): Lab- microbiologist & lab staff

- 4) Close laboratory doors and post warning signs to prevent others from entering the laboratory.
- 5) Thoroughly wash hands and other apparently contaminated areas with soap and water. Put on clean disposable gloves.
- 6) If personal clothing is contaminated, remove all outer clothing and place it in the autoclave or container for autoclaving. Put on clean garments.
- 7) Wear the N95 mask, fresh lab coat and double pair gloves, and reenter
- 8) Upon returning to the laboratory to start decontamination Cover the spill area with paper towels soaked in 5% Phenol solution or 1:10 dilution of 20% bleach (freshly prepared), or 70% ethanol solution. Do not pour decontamination solution directly onto the spill in order to avoid additional release of aerosols.
- 9) Let stand for 20 minutes then wipe up with paper towels.
- 10) Wipe up the spill with the soaked paper towels and place the used towels in an autoclave bag and autoclave.
- 11) Place gloves and paper towels in autoclave bag and autoclave.
- 12) Spill is inside the centrifuge bucket/tube: Always use the aerosol containment cups for centrifuging. Always open the centrifuge buckets inside the bio-safety cabinet. Autoclave the buckets.
- 13) Wash hands and other apparently contaminated areas again with soap and water.
- 14) Remove all protective equipment immediately upon leaving the work area and as soon as possible if overtly contaminated. De-Contaminate and DISPOSED.

Table: Aerosol generating activities which require bio-safety measure in the Mycobacteriology Labs:

	Activity
1	Preparing specimens for centrifugation and AFB culture
2	Centrifugation of specimens
3	Inoculating cultures from specimen sediment
4	Handling unopened primary- isolation plates or tubes
5	Staining smear of material from culture
6	Manipulating cultures (suspension preparation, vortex, and transferring) of <i>M. tuberculosis</i> complex on solid medium
7	Transferring large volumes of cultures or suspensions of bacilli
8	Disposing of cultures of <i>M. tuberculosis</i> complex
9	Conducting research (isolation of lipids, DNA and other macromolecules) on <i>M. tuberculosis</i> complex species
10	Shipping cultures or specimens of <i>M. tuberculosis</i> complex

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/4/2009

Procedure No. 02.00

Author(s): Lab- microbiologist & lab staff

3.2. Bio-safety procedures & Bio-safety Signage:

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment facility in TB labs. However, given the constraints, Bio-safety level 2 plus is advised in the laboratories performing culture and DST on solid media:

3.2.1 Biosafety Level 1 (BL1)

BL1 Standard Microbiological Practices

- Access to the laboratory is limited or restricted to the lab personnel.
- Work surfaces are decontaminated at least once a day and after work with infectious materials is finished, and after any spill of viable material is cleaned with disinfectants that are effective against the agents of concern.
- All contaminated liquid or solid wastes are decontaminated before disposal
- Mechanical pipetting devices are used; mouth pipetting is prohibited.
- Policies for the safe handling of sharps are followed. The needle and syringe should be promptly placed in a puncture-resistant container and autoclaved
- Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.
- Persons wash their hands:
 - (i) after handling materials involving TB organisms
 - (ii) before exiting the laboratory
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall worn all times in the laboratory
- A biohazard sign must be posted at the entrance to the laboratory whenever infectious agents are present. The sign must include the name of the agent(s) in use and the name and the phone number of the Microbiologist and director. Please see last page of this document for an example of an appropriate hazard warning sign.
- Gloves should be worn if the skin on the hands is broken or if a rash is present.
- Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.
- Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
- Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.
- Each laboratory contains a sink for hand washing. Foot, knee, or automatically operated sinks are recommended.
- If the laboratory has windows that open, they are fitted with fly screens.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/4/2009

Procedure No. 02.00

Author(s): Lab- microbiologist & lab staff

3.2.2 Bio-safety Level 2 (BL2)

BL2 Standard Microbiological Practices

- **All procedures for BL1 Standard Microbiological Practices, AND**
- Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

BL2 Special Practices

- **All BL1 Special Practices, AND**
- The microbiologist or the Director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections.
- The microbiologist or the Director establishes policies and procedures whereby only persons who have been advised of the potential hazard.
- A hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent and the biosafety level, lists the name and telephone number of the Microbiologist or other responsible person(s), and indicates the special requirement(s) for entering and exiting the laboratory (e.g., immunization, personal protective equipment). Please see last page of this document for an example of an appropriate hazard warning sign.
- Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.
- All wastes from laboratories are appropriately decontaminated before disposal.
- Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Broken glassware should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved,
- Spills and accidents that result in overt exposures to organisms are immediately reported to the lab In-charge or director.
- A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
- The Microbiologist ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
- Properly maintained biological safety cabinets (Class I or II), preferably Class II, or other appropriate personal protective or physical containment devices are used whenever:
 - (1.) Procedures with a high potential for creating aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures.
 - (2.) Centrifuge safety cups are used for all centrifugations involving *Mycobacterium tuberculosis* and are opened only in a biological safety cabinet.
- A properly maintained biological safety cabinet (Class II), will have a current, annual certification that under normal operating circumstances the unit performs to Manufacturer's specification.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 02.00

Date: 1/4/2009

Author(s): Lab- microbiologist & lab staff

BL2 Laboratory Facilities

- **All BL1 Laboratory Facility Requirements, AND**
- Provide lockable doors for facilities that house restricted agents.
- Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- An autoclave for decontaminating laboratory wastes is available.
- Entry to the Culture and DST containment room should be through an anteroom.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 02.00

Date: 1/4/2009

Author(s): Lab- microbiologist & lab staff

BIOHAZARD-Signage

Room: _____

DATE POSTED: _____



BIOHAZARD

Mycobacterium tuberculosis

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

Entry By: Lab Staff

Infectious Agents: Clinical specimens containing Mycobacterium tuberculosis
M.tuberuclosis Culture, and other Mtb material

Mode of transmission: Aerosol of Mycobacterium tuberculosis

Bio-safety Level 2

Risk Group 3

Special Lab Requirements:

In Case of Emergency Contact:

MICROBIOLOGIST Name: _____

Work Phone # _____

Home Phone # _____

Mobile #

Sr Lab person Name: _____

Work Phone # _____

Mobile Phone # _____

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/4/2009

Procedure No. 02.01

Author(s): Lab- microbiologist & lab staff

TITLE: Fumigation Procedure: Formaldehyde & Potassium Permanganate Method

1. Objective and Scope:

To Fumigate the Laboratory Rooms, Bio-safety cabinets and incubators as decontamination measure of the work environment. This document contains the procedures that are required for fumigation of contamination rooms and bio-safety cabinets in TB Laboratory.

2. Definitions and abbreviations:

Fumigation: a process of decontamination of the environment/laboratories through exposure to the formaldehyde vapours for specific periods.

3. Procedure:

The procedures, from start to finish, would take a minimum of 24 hours. Formaldehyde fumigation is a simple and easy procedure to perform but is inherently hazardous if practiced without appropriate precautions. Formaldehyde vapour is an extremely effective biocidal agent. It acts as an alkylating agent, inactivating micro-organisms by reacting with carboxyl, amino, hydroxyl and sulphhydryl groups of proteins as well as amino groups of nucleic acid bases. Microbiological safety cabinets, if they have been used for hazardous micro-organisms, must be fumigated in the following circumstances:

- after a major spillage or a spillage where inaccessible surfaces have been contaminated;
- before any maintenance work on the cabinet where access to potentially contaminated parts is necessary (including filter and pre-filter changes); and
- When there are any changes in the nature of the work that result in significantly different risks.

3.1 Requirements:

- (a) Formalin (40% Formaldehyde solution), commercial solution
- (b) Potassium permanganate (KMnO₄) solid
- (c) Wide mouthed Containers or metallic Bowl

3.2 Fumigation procedure:

1. Indicate to all that fumigation needs to be conducted and ensure that no one is remaining back in the laboratory.
2. For decontaminating of bio-safety cabinet,
 - a. Switch-off the cabinet fans
 - b. Take 35ml of Formalin in a wide mouthed bowl and place the vaporiser in the cabinet.
 - c. Add 10grams of Potassium permanganate crystals, to the formalin. (Initially, bobbles starts appearing in the formalin, and soon a violet froth is seen)
 - d. Vacate the place.
3. For de-contaminating the media room and culture room,
 - a. Pour 140ml of Formalin in a wide mouth bowl,
 - b. Add 40 grms of Potassium permanganate to formalin solution

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/4/2009

Procedure No. 02.01

Author(s): Lab- microbiologist & lab staff

- c. Vacate the place, immediately.
4. Close the door fully and seal the sides with sealing tape to ensure that there are no leaks.
5. Post a notice on the front of the door indicating that fumigation is in progress.
6. Notice to be posted: (in large type on the door):
WARNING, DO NOT ENTER, FORMALDEHYDE GAS
DECONTAMINATION TAKING PLACE
7. Next day morning, allow the place to purge for at least the time calculated as necessary to remove the formaldehyde.
8. For bio-safety cabinet, run the cabinet for at least a further 15-20 minutes to remove the last traces of formaldehyde.
9. Neutralise the left out traces of formaldehyde by placing appropriate quantities of a dilute (28%) Ammonia solution. Generally by opening the ammonia solution bottle for 1hr.

4. Safety Conditions:

Formaldehyde fumigation is a simple and easy procedure to perform but is inherently hazardous if practised without appropriate precautions.

Material Safety Data Sheets for hazardous chemicals (formalin) should be consulted.

Check levels of residual formaldehyde in the room with suitable air monitoring equipment, if available (formaldameter or air sampling tubes). Only enter the place if the level of formaldehyde is below 2ppm and wear a laboratory coat, and gloves. Check the room and all surfaces for formaldehyde residues and clean up as necessary.

Only allow other staff to enter the room when formaldehyde levels are below 0.5 ppm. Levels should be as low as practicable before staff re-enter.

5. Documentation:

A record must be kept of all fumigations of rooms/laboratories including date, personnel involved and the results of air monitoring on completion of the procedure.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 03.01

Date: 1/4/2009

Author(s): Microbiologist & lab staff

3.0 Culture Media preparation

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 03.01

Date: 1/4/2009

Author(s): Microbiologist & lab staff

TITLE: Preparation of Lowenstein-Jensen (LJ) Medium

1. Objective and Scope

To prepare LJ medium for culture of *Mycobacterium tuberculosis* at IRL.

2. Definitions and abbreviations

Medium: Laboratory preparation for artificial culturing of bacterium.

LJ medium: Lowenstein-Jensen egg-based solid medium specific for Tuberculosis bacterial culture.

3. Procedure

Media preparation must be done in the media preparation room. Care must be taken to ensure that the room is absolutely kept clean, free from dirt or dust and that the benches are swabbed daily with 5% Lysol/ phenol solution or 70% alcohol. During at all stages of media preparation, the door must be kept closed, to avoid contamination of the media. All equipments, sterile glassware etc., as listed, must be available before commencing media preparation. Equipment and glassware are to be sterilized prior to the day of media preparation, labeled indicating the date of sterilization and stored in a clean place. Equipment must be re-sterilized, if more than one week elapses between date of sterilization and date of use.

STRICT ADHERENCE TO ASEPTIC TECHNIQUES MUST BE OBSERVED AT ALL TIMES, i.e., flaming of the mouths of flasks etc., before and after removal of the stopper, holding the stopper with the little finger and palm to avoid contamination and by working as near to the flame as possible.

Weighing and measuring should be accurate.

All used glassware must be sent for cleaning as soon as possible.

3.1.1 Material required:

Note: One batch of LJ medium is 1600 ml of solution.

1. Warring Blender (mixer) with Sterile Jar marked of 1000 ml capacity, along with sterile lid.
2. Sterile funnel with double layer' of gauze fixed over mouth.
3. 1 x 1lit stainless steel jars.
4. 2 x 1 litres sterile round, flat bottomed flask.
5. 500 ml methylated spirit.
6. Sterile McCartney bottles (28 ml universal containers) (~300 per batch of medium).
7. Sterile Mineral salt solution, 600 ml with malachite green.
8. **Fresh hen's eggs** (24 to 28 eggs per batch of 1000 ml fluid). Quality of media depends on the freshness of the hen's eggs.
 - a. Eggs should be obtained always from a reliable source. The eggs should not be more than a day or two old, at the time of purchase.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 03.01

Date: 1/4/2009

Author(s): Microbiologist & lab staff

- b. The eggs required for a week should be indented in one lot, soaked in water, scrubbed gently and washed in running water, followed by dematerialized water and placed in a drain board for draining.
- c. Cracked and broken eggs should be rejected. Soak the eggs in Methylated spirit for 10 min. Wipe off, with sterile gauze and store in sterile container till used.

9. Inspissator.

10. Mineral SALT SOLUTION

Ingredients:

Potassium dihydrogen phosphate anhydrous (KH ₂ PO ₄) A.R.	2.4 g
Magnesium sulphate (MgSO ₄ .7H ₂ O), A.R.	0.24 g
Magnesium citrate	0.6 g
Asparagine	3.6 g
Glycerol (reagent grade)	12 ml
Malachite green, 2% solution*	20 ml

**Malachite green solution 2%*

Malachite green dye	2.0 g
Distilled water	100 ml

Dissolve the dye in distilled water by grinding the dye with water using a mortar and pestle. Filter and store in refrigerator

Dissolve the ingredients in order in about 300 ml distilled water by heating. Add glycerol, malachite green solution and make up 600 ml with distilled water. Autoclave at 121° C for 30 minutes to sterilize. Cool to room temperature. This solution keeps indefinitely and may be stored in the refrigerator.

11. Inspissator, thermostatically maintained at 85°c.

12. Clean bench surfaces and a Bunsen burner

3.2.1 Preparation of complete medium:

1. Arrange Mineral salt solution, eggs in a sterile container, egg breaking flask, 500ml measuring cylinder, blender container, and funnel with gauze inside the bio-safety cabinet, at a corner.
2. Individually, break the eggs by means of sharp rap on the side of the sterile 1 lit steel jar.
3. Transfer egg yolk and white carefully into the jar. Take care that there are no stale eggs.
4. Break 24 eggs, at each time. Transfer approximately 500ml egg fluid from steel jar to blender container; place the Lid on the top.
5. Place the container with egg solution on the blender, and switch on the blender mains; Give 1 to ½ min breaks (once or twice) which is sufficient to homogenize the egg fluid. Do not give long breaks, froth would be formed in the solution which would affect medium slopes! Do not allow fluid to touch the lid.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 03.01

Date: 1/4/2009

Author(s): Microbiologist & lab staff

6. Filter the homogenized egg solution into sterile 500ml measuring cylinder through gauze covered (2 layers) glass funnel.
7. Transfer filtered homogenized egg solution 2 liters round bottom flask.
8. Repeat filtration, of another 500ml egg solution, and transfer to round bottom flask.
9. Add 600ml of mineral salt solution total 1 lit of homogenized egg solution in the round bottom flask. Mix thoroughly by gentle agitation for 5 min, till uniform pale green color is obtained.

3.2.2 Media pouring/dispensing:

1. Arrange the sterile Mc-Cartney bottles in the racks.
2. Open the cap, pour 5-8ml of LJ medium into each bottle
3. Reject any chipped, cracked or dirty bottles
4. Recap the bottles tightly.

3.2.3 Inspissations:

1. Inspissator should be switched on sufficiently early so that required temperature is reached. Leave the inspissator racks inside the inspissator.
2. The media bottles are sloped on the inspissator racks, and transferred to inspissator.
3. Slopes are left in the inspissator at 85°C for 85 min.
4. The maintenance of the temperature by the thermostat needs to be inspected at the time of loading, once in the middle, and at the end. Ensure that fan is working inside the inspissator.
5. Switch OFF the inspissator after the work.
6. Check tubes at the back side of the slope. If large number of transparent 'holes' in the opaque pale green background, it indicates the over-inspissation (more than adequate temperature for coagulation of egg medium). If the bubble holes are there on the surface of the slope, it indicates insufficient temperature for inspissation.

3.2.4 Sterility Test:

1. All the slopes of medium prepared in a day are incubated for 48 hours at 37°C.
2. From a batch of medium 10 slopes are randomly selected and incubated at 37°C for 14 days.
3. If bacterial and fungal contamination is noted, the entire batch is to be rejected.

3.2.5 Quality control:

The quality of drug-free culture media can be tested most simply by using a rapid growing *Mycobacterium* such as *M. fortuitum*. If no growth is observed within five days after inoculation, the medium does not have the properties required.

3.2.6 Storing:

Media slopes are packed in a saran-wrap, packed in boxes or iron meshes/racks, labeled with date of preparation, batch number and stored at the 4°C refrigerator. Although media stored at refrigerator can be used for 2 months, stock only one month's requirement, at a time.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 03.01

Date: 1/4/2009

Author(s): Microbiologist & lab staff

3.3 Results

3.3.0 Physical characteristics of the media

3.3.1 Color

Media-containing tubes from the same batch showing different shades of green color may be due to poor homogenization or to the presence of material residues in the tubes. A very dark tone of green can be caused by an excess of Malachite green or to a very low (acidic) pH. Yellowish media can indicate poor quality Malachite green or a very high (alkaline) pH.

3.3.2 Texture

If the medium disintegrates easily, the inspissation temperature might have been too low. This can be detected by tapping on one's hand one or two tubes randomly chosen from the inspissated batch. Tubes containing media of poor texture are not suitable for culture inoculation.

3.3.3 Homogeneity

If bubbles in the medium appear during inspissation, it is possible that the medium might have been subjected to excessive temperature, thus losing quality. The presence of clumps in the media indicates poor homogenization.

3.3.4 Sensitivity

Serious problems affecting the quality of culture medium can be detected by seeding a 1/10.000 dilution of a suspension of *Mycobacterium tuberculosis* equivalent to that of a bacterial suspension containing 1 mg/ml of tubercle bacilli. Five tubes of a previous batch of medium and 5 tubes of the new batch of medium are inoculated with 0.2 ml of the diluted suspension. If the number of colonies obtained on the recently prepared or purchased batch is significantly lower than on reference batch of medium, the sensitivity of the new medium, whether prepared or purchased, is not adequate.

4. Safety Conditions

Practice good aseptic technique in the media preparation room.

5. Documentation

The following registers are to be maintained:

- a) Media register
- b) Egg stock register, and
- c) Inspissation register

See the appendix for the formats for these registers. Enter data meticulously after end of the work.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 03.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

Title: Preparation of Lowenstein-Jensen (LJ) Medium with anti-TB drugs (Streptomycin, Isoniazid, Rifampicin and Ethambutol)

1. Objective and Scope:

To prepare LJ medium with anti-TB drugs- streptomycin, isoniazid, rifampicin and ethambutol- for drug sensitivity testing of *Mycobacterium tuberculosis* strains. This document contains procedure for preparation of LJ medium with anti-TB drugs at IRL.

2. Definition and abbreviations:

Medium: Laboratory preparation for artificial culturing of bacterium.

LJ medium: Lowenstein-Jensen solid egg based medium specific for tuberculosis bacterial culture.

3. Procedure:

Media preparation must be done in clean, dirt free work benches in the media preparation room. Care must be taken to ensure that the room is absolutely kept clean, free from dirt or dust and that the benches are swabbed daily with 5% Lysol/ phenol solution or 70% alcohol. During at all stages of media preparation, the door must be kept closed, to avoid contamination of the media. All equipments, sterile glassware etc., as listed, must be available before commencing media preparation. Equipment and glassware are to be sterilized prior to the day of media preparation, labeled indicating the date of sterilization and stored in a clean place. Equipment must be re-sterilized, if more than one week elapses between date of sterilization and date of use.

STRICT ADHERENCE TO ASEPTIC TECHNIQUES MUST BE OBSERVED AT ALL TIMES, i.e., flaming of the mouths of flasks etc., before and after removal of the stopper, holding the stopper with the little finger and palm to avoid contamination and by working as near to the flame as possible.

Weighing and measuring should be accurate.

All used glassware must be sent for cleaning as soon as possible.

3.1.1 Material required:

Note: One batch of LJ medium is 1600 ml of solution.

1. Warring Blender (mixer) with Sterile Jar marked of 1000 ml capacity, along with sterile lid.
2. Sterile funnel with double layer' of gauze fixed over funnel mouth.
3. 1 x 1lit stainless steel jars.
4. 4 x 1 litres sterile round, flat bottomed flask.
5. 2 x 100ml conical flask
6. 500 ml methylated spirit.
7. Sterile McCartney (28 ml universal containers) bottles (~300 per batch of medium).
8. Sterile Mineral salt solution, 600 ml with malachite green.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 03.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

9. **Fresh hen's eggs** (24 to 28 eggs per batch of 1000 ml fluid). Quality of media depends on the freshness of the hen's eggs.
 - a. Eggs should be obtained always from a reliable source. The eggs should not be more than a day or two old, at the time of purchase.
 - b. The eggs required for a week should be indented in one lot, soaked in water, scrubbed gently and washed in running water, followed by dematerialized water and placed in a drain board for draining.
 - c. Cracked and broken eggs should be rejected. Soak the eggs in Methylated spirit for 10 min. Wipe off, with sterile gauze and store in sterile container till used.
10. Inspissator at 85°C.

11. Mineral SALT SOLUTION

Ingredients:

Potassium dihydrogen phosphate anhydrous (KH ₂ PO ₄) A.R.	2.4 g
Magnesium sulphate (MgSO ₄ .7H ₂ O), A.R.	0.24 g
Magnesium citrate	0.6 g
Asparagine	3.6 g
Glycerol (reagent grade)	12 ml
Malachite green, 2% solution*	20 ml

**Malachite green solution 2%*

Malachite green dye	2.0 g
Distilled water	100 ml

Dissolve the dye in distilled water by grinding the dye with water using a mortar and pestle. Filter and store in refrigerator

Dissolve the ingredients in order in about 300 ml distilled water by heating. Add glycerol, malachite green solution and make up 600 ml with distilled water. Autoclave at 121° C for 30 minutes to sterilize. Cool to room temperature. This solution keeps indefinitely and may be stored in the refrigerator.

12. Inspissator thermostatically maintained at 85c.
13. dihydro streptomycin sulphate- Sigma, Cat No. 7253
14. Isoniazid- Sigma, Cat No. I - 3377
15. Ethambutol dihydrochloride- Sigma, Cat No. E-4630
16. Rifampicin- Sigma, Cat No. R3501

Work is to be performed on a clean workbench nearer to the flame

3.1.2 Preparation of Medium:

1. Arrange Mineral salt solution, eggs in a sterile container, egg breaking flask, 500ml measuring cylinder, blender container, and funnel with gauze.
2. Individually, break the eggs by means of sharp rap on the side of the sterile 1 lit steel jar.
3. Transfer egg yolk and white carefully into the jar. Ensure that there are no stale eggs.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

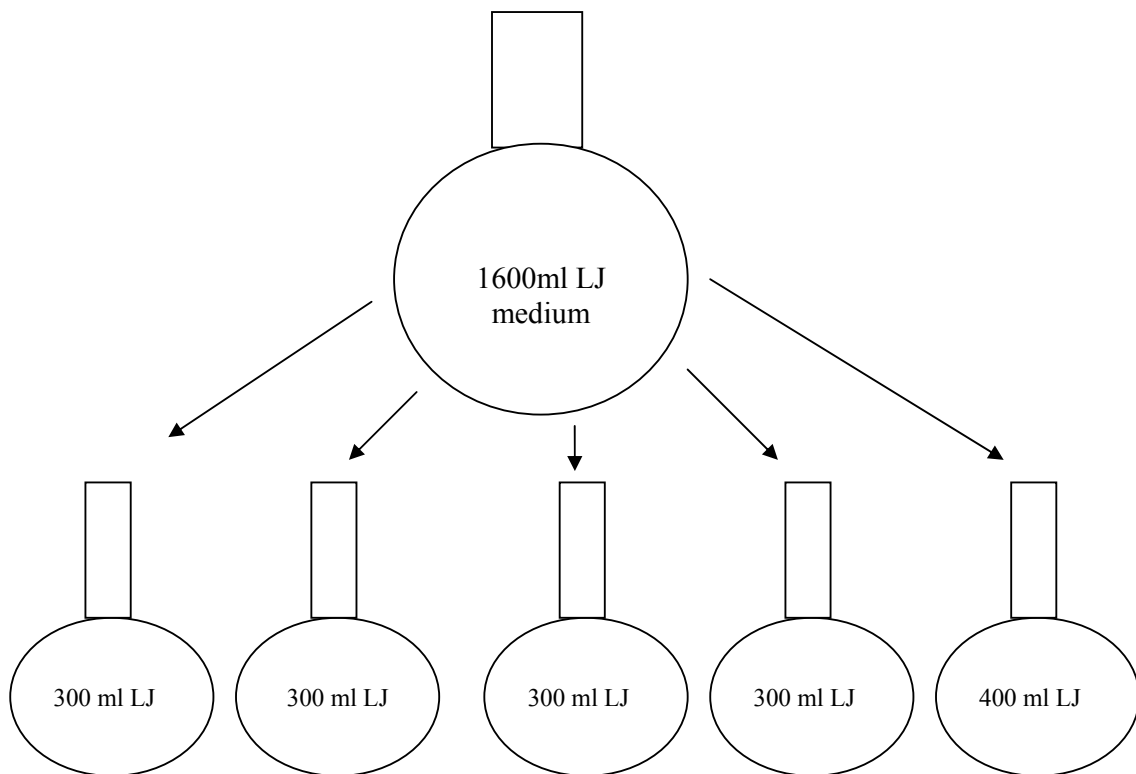
Procedure No. 03.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

4. Break 24 eggs, and transfer approximately 500ml egg fluid from steel jar to blender container; place the Lid on the top.
5. Take out the container from safety cabinet; place on the blender, and switch on the blender mains; Give 1 to ½ min breaks (once or twice) which is sufficient to homogenize the egg fluid. Do not give long breaks, froth would be formed in the solution which would affect medium slopes. Do not allow fluid to touch the lid.
6. Filter the homogenized egg solution into sterile 500ml measuring cylinder through sterile gauze covered glass funnel.
7. Transfer filtered homogenized egg solution in 2 liters round bottom flask.
8. Repeat filtration, of another 500ml egg solution, and transfer to round bottom flask.
9. Add 600ml of mineral salt solution total 1 lit of homogenized egg solution in the round bottom flask. Mix thoroughly by gentle agitation for 5 min, till uniform pale green color is obtained.

Distribute the 300ml of 1600ml LJ medium into 4 of one lit room bottom flasks, separately. Drug medium (300ml each with 4 drugs=1200ml) would be made from the same, and rest of medium (400ml) would be used for preparation of drug medium as shown below.



Medium is distributed into separate room bottom flasks

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 03.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

3.1.2 Concentrations: Drug containing media for economic variant proportion

method:

Only one concentration per drug is used. The final concentrations in LJ medium are as follows:

Isoniazid	0.2 µg/ml
Ethambutol	2 µg/ml
Streptomycin (dihydrostreptomycin sulfate)	4 µg/ml
Rifampicin	40 µg/ml

Prepare the drug solutions and add to LJ medium separately, as shown below:

1. Dihydro Sterptomycin sulphate (S):

Drug potency = 1g to 800mg substance. Potency factor = 0.80

Preferred substance Sigma S-7253

Working solution preparation:

Weigh out 25 mg / Potency of dihydro streptomycin sulphate powder (Potency correction: Weight required divided by potency factor, i.e., 20mg/0.80) and dissolve in 50 ml SDW to obtain 400µg/ml of stock solution.) Sterilize by filtering through a 0.22µ membrane filter.

Do not store this solution.

Addition to LJ plain medium:

Add 1ml per 100ml LJ medium prepared. If you are preparing 400ml of LJ with Streptomycin, you need to add 4ml to obtain final concentration of 4µg/ml LJ medium.

2. Isoniazid (H):

Drug potency = 1g to 1g substance. Potency Factor = 1, preferred substance: Sigma I-3377

Stock solution preparation

Weigh out 20mg of Isoniazid powder in 40ml of sterile distilled water to obtain a concentration of 500µg/ml Isoniazid solution. Label with date of preparation, as 'H Stock solution'.

Working solution:

Prepare the working solution 1 ml of stock solution (500µg/ml) + 24ml of sterile distilled water (=25ml of 20µg/ml). Sterilize by filtering through a 0.22 µ membrane filter. **Do not store this solution.**

Addition to LJ plain medium:

Add 1ml per 100ml LJ medium prepared. If you are preparing 400ml of LJ with INH, you need to add 4ml to obtain final concentration of 0.2 µg/ml isoniazid LJ medium.

3. Rifampicin (R):

Drug potency = 1g to 950mg substance. Potency factor = 0.95

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 03.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

Preferred substance Sigma R3501

Stock solution preparation:

Weigh out 42.1 mg of rifampicin (Potency correction: Weight required divided by potency factor, i.e., 40mg/0.95) dissolve in 5 ml of absolute methanol, followed by addition of 5 ml of 99% ethanol to get 4000µg/ml of stock solution. **Do not store this solution**

Addition to LJ plain medium:

Add 1ml per 100ml LJ medium prepared. If you are preparing 400ml of LJ with RIF, you need to add 4ml to obtain final concentration of 40µg/ml LJ medium.

4. Ethambutol (E):

Drug potency = 1g to 750mg substance. Potency factor = 0.75. Preferred substance E4630

Stock solution preparation:

Weigh out 26.7 mg of Ethambutol hydrochloride (Potency correction: weight required divided by potency factor, i.e., 20mg/0.75) and dissolve in 100ml of sterile distilled water to get 200µg/ml of drug stock solution. Sterilize by filtering through a membrane filter. **Do not store this solution.**

Addition to LJ medium:

Add 1ml per 100ml LJ medium prepared. If you are preparing 400ml of LJ with Ethambutol, you need to add 4ml to obtain final concentration of 2µg/ml LJ medium.

3.1.3 Media pouring and dispensing:

1. Label the bottles for specific drugs separately; arrange the sterile McCartney bottles on a clean work-bench.
2. Open the cap, pour 5-8ml of LJ medium into each bottle
3. Reject any chipped, cracked or dirty bottles
4. Recap the bottles tightly.

3.1.4 Inspissation:

1. Inspissator should be switched on sufficiently early so that required temperature is reached. Leave the inspissator racks inside the inspissator.
2. The media bottles are sloped on the inspissator racks, and transferred to inspissator.
3. Slopes are left in the inspissator at 85°C for 85 min.
4. The maintenance of the temperature by the thermostat needs to be inspected at the time of loading, once in the middle, and at the end. Ensure that fan is working inside the inspissator.
5. Switch OFF the inspissator after the work.
6. Check tubes at the back side of the slope. If large number of transparent 'holes' in the opaque pale green back-ground, it indicates the over-inspissation (more than adequate temperature for coagulation of egg medium). If the bubble holes are there on the surface of the slope, it indicates insufficient temperature for inspissation.

3.1.5 Sterility Test:

1. All the slopes of medium prepared in a day are incubated for 48 hours at 37°C.
2. From a batch of medium 5 slopes for each drug are randomly selected and incubated at 37°C for 14 days.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 03.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

3. If bacterial and fungal contamination is noted, the entire batch is to be rejected.

3.1.6 Internal quality control

Quality control of drug susceptibility tests is best performed by titrating the standard strain H37Rv of *M. tuberculosis* for each newly produced batch of drug susceptibility testing media. An alternative method of quality control consists of including in each series of testing the standard strain H37Rv as well as two clinical isolates of *M. tuberculosis* with moderate levels of drug resistance to some of the drugs, which together express all four drug resistance markers.

3.1.7 Storing of drug containing medium:

Media slopes are packed in a saran-wrap, packed in boxes, labeled with date of preparation, batch number and stored at the 4°C refrigerator. Although media stored at refrigerator can be used for 1 month, stock only one month's requirement, at a time.

3.2.0 Results:

Physical characteristics of the media

3.2.1 Color

Media-containing tubes from the same batch showing different shades of green color may be due to poor homogenization or to the presence of material residues in the tubes. A very dark tone of green can be caused by an excess of Malachite green or to a very low (acidic) pH. Yellowish media can indicate poor quality Malachite green or a very high (alkaline) pH. Rifampicin containing medium would be bit pale green compared to other drug media or plain media.

3.2.2. Texture

If the medium disintegrates easily, the inspissation temperature might have been too low. This can be detected by tapping on one's hand one or two tubes randomly chosen from the inspissated batch. Tubes containing media of poor texture are not suitable for culture inoculation.

3.2.3 Homogeneity

If bubbles in the medium appear during inspissation, it is possible that the medium might have been subjected to excessive temperature, thus losing quality. The presence of clumps in the media indicates poor homogenization.

4. Safety conditions

Practice good aseptic technique in the media preparation room.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 03.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

5. Documentation:

The following registers are to be maintained:

- a) Media register
- b) Drug stock register
- c) Egg stock register, and
- d) Inspissation register

See the appendix for the format for these registers. Enter data meticulously after end of work.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 04.00

Date: 1/04/2009

Author(s): Microbiologist & lab staff

4.0 Sample processing

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 04.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

TITLE: Specimen collection, transport and handling

1. Objective and Scope:

To describe the specimen collection instructions, transport to TB laboratory and subsequent handling of specimens by laboratory technician for culture of *Mycobacterium tuberculosis*. This document contains procedure for clinical specimens containing *Mycobacterium tuberculosis* from human for processing at IRL.

2. Definitions and abbreviations:

Sputum: mucous expectorate obtained from the lungs

Specimen: body fluid or substance, such as sputum, used for medical examination

3. Procedure:

An adequate specimen is essential for the success for culture of *Mycobacterium tuberculosis*. Specimens are to be collected with the utmost care and promptly transported to the laboratory. Although *M. tuberculosis* is capable of causing disease in almost any organ of the body, more than 85% of tuberculosis disease in high prevalence countries is pulmonary. Therefore, sputum is the specimen of choice in the investigation of tuberculosis and should always be collected. If extra-pulmonary disease is suspected, sputum should be collected in addition to any extra-pulmonary specimens.

3.1 Requirements:

3.1.1 Specimen collection containers

Containers must be rigid to avoid crushing in transit and must possess a water-tight wide-mouthed screw top to prevent leakage and contamination.

1. Wide-mouthed (at least 35mm in diameter) so that the patient can expectorate easily inside the container without contaminating the outside
2. Volume capacity of 50ml for the disposable 'falcon' tubes and 28ml for standard universal containers.
3. Made of translucent material in order to observe specimen volume and quality without opening the container
4. Made of single-use combustible material to facilitate disposal
5. Screw-capped to obtain an airtight seal and to reduce the risk of leakage during transport
6. The container should be sterile
7. Easily-labelled walls that will allow permanent identification

An alternative container is the 28ml Universal bottle (McCartney bottle), which is a heavy glass, screw-capped bottle with a wide neck. This container is reusable after thorough cleaning and sterilisation.

3.1.2 Transport container

Specimens must be packaged to withstand leakage of contents, shocks, pressure changes and other conditions. Postal or air transport should be in approved, robust, leak-proof primary containers which are packed into secondary containers made of

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 04.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

metal, wood or strong cardboard with enough absorbent material so that if they are damaged or leak the fluids will be absorbed.

3.1.3 Sputum specimens

A good sputum specimen consists of

1. recently-discharged material from the bronchial tree, with minimum amounts of oral or nasal material.
2. Satisfactory quality implies the presence of mucoid or mucopurulent material and is of greater significance than volume.
3. Ideally, a sputum specimen should have a volume of 3- 5ml, although smaller quantities are acceptable if the quality is satisfactory.
4. It is best to obtain sputum early in the morning before the patient has eaten or taken medication
5. Although one sputum specimen sample is sufficient, two are preferred. Some patients shed mycobacteria irregularly and in small numbers; for these patients the chance of obtaining a positive culture result will be improved if more specimens are cultured.

Collecting a good sputum specimen requires that the patient be given clear instructions.

3.1.4 Other specimens

Because *M. tuberculosis* may infect almost any organ in the body, the laboratory should expect to receive a variety of extra-pulmonary specimens, eg. body fluids, tissues, pus and urine. These specimens may be divided into two groups, namely:

- aseptically collected specimens, usually free from other micro-organisms
- specimens known to contain contaminating normal flora or specimens not collected aseptically

3.1.4.1 Aseptically collected fluids

Body fluids (spinal, pleural, pericardial, synovial, ascitic, blood, pus, bone-marrow) should be aseptically collected in a sterile container by the physician or OPD nurse using aspiration techniques or surgical procedures. For fluids that may clot, sterile potassium oxalate (0.01-0.02ml of 10% neutral oxalate per ml fluid) or heparin (0.2mg per ml) should be added. Specimens should be transported to the laboratory as quickly as possible.

3.1.4.2 Aseptically collected tissues

Aseptically collected tissue specimens should be placed in sterile containers *without fixatives or preservatives*. If the specimen is to be sent by mail it should be protected from drying by adding sterile saline and packing the container in dry ice or maintaining a temperature of 4-15°C. Specimens should be transported to the laboratory as quickly as possible.

3.2.4.3 Specimens expected to be contaminated

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 04.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

Urine is the most commonly encountered extra-pulmonary specimen that requires processing before culture. To minimise excessive contamination of urine specimens the external genitalia should be washed before the specimens are collected and the urine should be immediately processed or refrigerated. Three early mornings, voided midstream specimen should be collected.

3.2 Transport of specimen to IRL:

Specimens should be transported to the laboratory as soon as possible after collection. If a delay is unavoidable the specimens should be refrigerated to inhibit the growth of unwanted micro-organisms. If sputum specimens can be kept refrigerated they could be sent to the laboratory once a week; **extra-pulmonary specimens, however, should be submitted as soon as possible after collection.**

If specimens have to be transported at ambient temperatures, chemical preservation may be used. The following method provide reasonable results:

Mixing the fresh specimen with an equal volume of 1% cetyl pyridinium chloride in 2% sodium chloride. Tubercle bacilli will survive for up to a week, while the growth of unwanted organisms will be restricted

However, speedy transportation is essential for good results.

Request forms should be located separately from specimen containers. With each transport box an accompanying list must be prepared which identifies the specimens and the patients from whom the specimens were collected. Before dispatch from the health centre the following must be verified:

1. that the number of specimen containers in the box corresponds to that on the accompanying list
2. that the identification number on each specimen container corresponds to the identification number on the accompanying list
3. that the accompanying list contains the necessary data for each patient
4. that the date of dispatch and the particulars of the health centre are on the accompanying list

3.3 Specimen handling

3.3.1 Receipt of incoming specimens

Specimens should be received in the office area of the laboratory, preferably at a separate specimen delivery counter. Delivery boxes should be opened in the biosafety cabinet and the following procedures applied:

1. Wear disposable gloves during receipt and inspection of incoming specimens
2. Inspect the delivery box for signs of leakage. If mass leakage is evident discard the box by autoclaving or burning
3. Disinfect the outside of the delivery box using adsorbent cotton or paper towels saturated with a suitable disinfectant (eg. 5% phenol, or 70% Methanol)
4. Open the delivery box carefully and check for cracked or broken specimen containers. Autoclave or burn these without processing and request another specimen

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>

Procedure No. 04.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

5. Check that specimens have been adequately labelled with individual identification numbers and that these correspond with the numbers on the accompanying list
6. Disinfect the inside of the delivery box, discard gloves and wash hands after handling specimen containers

4. Safety conditions:

Aerosols containing tubercle bacilli may be formed when the patient produces a sputum specimen. Patients should, therefore, produce specimens either outside in the open air or away from other people and not in confined spaces such as toilets, laboratories, waiting room or reception room. Lab technician should know correct way of collecting the sputum & instruct the patient.

4.1 Specimen rejection policy

Specimen is liable for rejection for Culture & DST if:

1. Specimen is unlabeled or mislabeled.
2. Specimen without request form.
3. Specimen name and request form does not match.
4. Container is full up to the lid, because of pooling of specimens.
5. Specimen breakage or leakage.
6. Specimen not collected in an appropriate container.

The specimens should not be rejected without proper reasons.

5. Documentation:

Formats for specimen registration are as per RNTCP annexure.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 04.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

TITLE: Isolation of *Mycobacterium tuberculosis*: Sodium Hydroxide (NaOH) method by Modified Petroff's procedure

1. Objective and Scope:

To isolate *Mycobacterium tuberculosis* from human sputum specimens for-diagnosis, speciation and drug susceptibility testing.

2. Definitions and abbreviations:

3. Procedure:

M. tuberculosis grows slowly, taking three to six weeks or longer to give visible colonies and requires specific media for isolation. Because of the long incubation time required, cultures are usually made in McCartney bottles (or standard 28ml metal stopped bottles) and to prevent drying of the cultures. Clinical specimens submitted to the tuberculosis culture are contaminated to varying degrees by more rapidly growing unwanted normal flora. Most specimens must, therefore, be subjected to a harsh digestion and decontamination procedure that liquefies the organic debris and eliminates contaminants. However care should be taken so that the procedures avoid killing of tubercle bacilli to obtain a good recovery.

The clinical specimens submitted for culture in mycobacteriology are of 2 types. (1) specimens like sputum, urine, pus from sinuses contaminated with other organisms; (2) specimens like CSF, lymph node aspirates, biopsy materials etc., are collected under sterile conditions avoiding contamination.

Quality sputum specimen consists of recently discharged material from the bronchial tree, with minimum amounts of oral or nasal material. Satisfactory quality is indicated by mucoid or mucopurulent material. Ideally, a sputum specimen should have a volume of 4-5ml, although smaller quantities are acceptable if the quality is satisfactory.

Specimens should be **transported** to the laboratory as soon as possible after collection. If delay is unavoidable, the specimens should be refrigerated at 4°C to inhibit the growth of unwanted micro-organisms. If refrigeration is not possible and a delay of more than 3 days is anticipated, a suitable preservative viz., an equal volume of a mixture of 1% Cetyl Pyridinium chloride (CPC) along 2 % sodium chloride solution is recommended.

Always digest/decontaminate the whole specimen, i.e., do not attempt to select portions of the specimen as is done for direct microscopy. Specimens other than sputum require even more care during processing because of the low numbers of tubercle bacilli present in positive specimens.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 04.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

3.1 Material required

1. Incubator, 37°C.
2. Aerosol containment Centrifuge, capable of speed up to at least 3000 x g, fitted with rotor to take at least 8 Mc Cartney bottles.
3. Pan balance.
4. Bio-safety cabinet.
5. One Enamel bin containing 5% phenol solution.
6. Two 5 mm wire loops (Nichrome wire of 22 SWG).
7. One Enamel bin with lid for disposable waste.
8. Wire baskets or racks to hold 16 Mc Cartney tubes.
9. Wire racks for holding 100 universal containers.
10. Stock of clean, sterile Mc Cartney Tubes.
11. Stock of LJ slopes.
12. Sterile 4% NaOH solution: Weigh 10 grams of Sodium Hydroxide pellets and dissolve in 250ml of water in a conical flask (500 ml capacity); sterilize in solution cycle at 15 PSI for 20 min.
13. Sterile distilled water in 1lit Corning bottles or conical flask 500 ml.
14. Two Sterile 250ml conical flasks.
15. Grease marker pencils.
16. Timer.

Specimens are to be checked against the accompanying lab cards and smear prepared.

3.2 Sputum Processing

1. Transfer 4 to 5 ml of sputum to McCartney tubes and add double the volume of sterile 4% NaOH solution from the 250ml conical flask containing 4% NaOH solution, aseptically.
2. Tighten the caps of the McCartney tubes and mix it well. Invert each bottle to ensure that NaOH solution contacts all the sides and inner portion of caps.
3. The bottles are to be placed in shaker & kept in 37°C incubator for 15 minutes.
4. At the end of 15 minutes, McCartney tubes are to be removed from the incubator and add 15ml of sterile distilled water (in the 250ml conical flask).
5. Mix it well and centrifuge at 3000 x g for 15 minutes.
6. McCartney tubes are to be carefully removed from the centrifuge without shaking. The supernatant fluid should be discarded slowly into a container with 5% phenol solution.

Note: Avoid splashing, minimize aerosols and whole process should be carried out in a bio-safety cabinet.

- 6a. To the sediment, add 50ml sterile distilled water, and wash the pellet. Decant sup.
7. From the sediment, inoculate two slopes of LJ medium (using a sterile cool 5 mm inoculation loop made up of Nichrome wire (22 SWG). Individually wrapped, disposable 10mm loops can be used, if available.
8. Label the caps with the lab serial number of the specimen and name them as 1 and 2. Use one loopful of sediment for each inoculation.
9. Incubate all the LJ media slopes at 37°C.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 04.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

10. Check for growth weekly for eight weeks.

3.2.1 Sterility tests should be done on nutrient agar, nutrient broth and LJ slopes for the reagents used to treat the specimens, once before and once after the treatment of specimen. (see the flow chart)

3.3 Results:

For preliminary identification of tubercle bacilli the following characteristics apply:

- Tubercle bacilli do not grow in primary culture in less than one week and usually take three to four weeks to give visible growth
- The colonies are buff coloured (never yellow) and rough, having sometimes appearance of bread crumbs or cauliflower. However, morphology of the colonies vary if specimen are very old, or specimens obtained from patients while on treatment,
- They do not emulsify in the saline used for making smears but give a granular suspension
- Microscopically they are frequently arranged in serpentine cords of varying length or show distinct linear clumping. Individual cells are between 3µm and 4µm in length

3.4 Examination schedule

All cultures should be examined 72 hours after inoculation to check that liquid has completely evaporated, to tighten caps in order to prevent drying out of media and to detect contaminants.

Thereafter, cultures are to be examined weekly, or if this is not operationally feasible, on at least three occasions, viz

- after one week to detect rapidly growing mycobacteria which may be mistaken for *M. tuberculosis*
- after three to four weeks to detect positive cultures of *M. tuberculosis* as well as other slow-growing mycobacteria which may be either harmless saprophytes or potential pathogens
- after eight weeks to detect very slow-growing mycobacteria, including *M. tuberculosis*, before judging the culture to be negative

As a general rule, contamination rates of 2%-3% are expected in laboratories that receive fresh specimens. Should contaminated cultures be found during the examination, those where the surface has been completely contaminated or where medium has been liquefied or discoloured should be sterilised and discarded.

Certain contaminating organisms produce acid from constituents of the medium and the lowering of pH unbinds some of the malachite green from the egg (indicated by the medium changing to dark green). Tubercle bacilli will not grow under these conditions and cultures should be discarded.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 04.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

Cultures with partial contamination should be retained until the eighth week. Late contamination does not exclude the presence of *M. tuberculosis*; it is therefore advisable to prepare a smear from the surface of the medium. Should microscopy indicate the presence of acid-fast bacilli, an attempt could be made to re-decontaminate and re-inoculate the culture.

3.5 Reading of cultures

Mondays are designated as culture reading days for the purpose of rack labeling and subsequent examination. The label should show the Lab Numbers of first and last specimen in a rack, date of inoculation and date of 4th and 8th week reading.

Note: Culture reading can be done a day later if Monday happens to be a holiday.

Typical colonies of *M. tuberculosis* are rough, crumbly, waxy, non-pigmented (cream coloured) and slow- growers, ie. only appearing two to three weeks after inoculation.

With doubtful cultures or when less experienced staff read cultures, the acid-fastness should be confirmed by Ziehl-Neelsen (ZN) staining. A very small amount of growth is removed from the culture using a loop and gently rubbed into one drop of sterile saline on a slide. At this point the ease with which the organisms emulsify in the liquid should be noted: Tubercle bacilli do not form smooth suspensions, unlike some other mycobacteria. The smear is allowed to dry, fixed by heat and stained by the ZN method.

3.6 Recording results of Cultures

Reading for primary culture and for DST	Report
No growth	Negative
1-100 colonies	Positive (actual number of colonies)
>100 discrete colonies	Positive (2+)
Confluent growth	Positive (3+)
Contaminated	Contaminated
< 20 colonies of only NTM colonies in one or both slopes	No growth
> 20 colonies of only NTM colonies in both slopes	Negative for <i>M. tb.</i>

Important steps for culture reading:

1. Actual examination of the primary cultures & recording of results, keeping positive & doubtful cultures for sensitivity & identification tests respectively in the rack, discarding contaminated cultures and rearranging, discarding 8th week negative slopes and keeping cultures of microscopy positive specimens for 12th week reading, if negative at 8 weeks.
2. Checking all positive cultures and cultures given for identification tests.
3. Keeping back in the incubator cultures required for 8th and 12th week reading.
4. Sending cards of specimens contaminated and negative at 8 weeks and 12 weeks for reporting after autoclaving the cards.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 04.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

5. Keeping the cards of positive cultures for sensitivity and identification tests in the respective compartments of filing box.

The laboratory cards of cultures sent for sensitivity or identification tests and cards of cultures which are at different stages of identification can be kept in the appropriate compartments in a box for easy handling. The compartments required are:

1. Positives for sensitivity & identification tests.
2. A waiting for sensitivity & identification results.
3. Cultures for identification tests only (if repetition is required or for doubtful cultures)
4. Cultures for sensitivity tests only (for cultures proved to be *M.tuberculosis* by identification tests or requiring repetition).
5. Primary culture for further incubation.
6. Cards for finalisation & reporting (cultures for which all tests are completed).

3.7 Quality indicators:

3.7.1 Periodic monitoring routine

Depending on the workload of the prevalence of bacteriological positive cases, a monthly, quarterly or semi-annual analysis of the laboratory register allows the detection of systematic errors.

These analyses are key to the quality control of culture and are used to categorize adult pulmonary tuberculosis patients with bacteriological confirmation, in one of the following categories:

- a. smear positive and culture positive
- b. smear positive and culture not done
- c. smear negative and culture positive
- d. smear positive and culture negative
- e. smear positive and culture contaminated
- f. smear not done and culture positive

With the total of cases classified in each one of these categories, it is possible to calculate the following indicators:

$$\text{Contribution of culture to diagnosis} = \frac{c}{a + b + c + d + e + f} \times 100$$

Culture being more sensitive than smear microscopy is expected to contribute at least 20% to the bacteriological confirmation of adult pulmonary tuberculosis cases.

$$\text{Percentage of smear positive and culture negative cases} = \frac{d}{a + b + c + d + e + f} \times 100$$

This percentage should be very low, typically around 2-3 %. Exceptionally, patients are found with persistent smear negative and culture negative diagnostic specimens. Usually

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 04.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

these are undisclosed treatment control specimens cases. Higher percentages could be the result of too harsh decontamination procedures.

3.7.2 Other useful indicator:

Percent contamination

This indicator is calculated as the percentage of contaminated tubes among all inoculated tubes. It should not exceed 3-4% if the modified Petroff decontamination method is used.

3.8 Alarm signals on culture results:

Indicators of culture performance	Normal value (%)	Much higher: investigate*	Much lower: investigate*
Contribution of culture to bacteriological diagnosis of tuberculosis	20	A	B and C
Percentage of smear positive/culture negative specimens	2-3	C and D	Not a problem
Percentage of contaminated tubes	3-4	E	F

* Investigative steps are listed in the following table

	Probable cause & investigative steps
A	<ul style="list-style-type: none"> ▪ smear microscopy reading errors: "false negatives" ▪ a high percentage of incipient pulmonary TB and pediatric TB cases are being tested (not a problem) ▪ very strict selection of TB suspects examined by culture, examination of highly suspicious TB cases (not a problem)
B	<ul style="list-style-type: none"> ▪ inadequate use of culture: patients that are not TB suspects are being examined rather than incipient TB cases
C	<ul style="list-style-type: none"> ▪ excessive delay between specimen collection and specimen processing ▪ too harsh specimen decontamination procedures (excessive concentration and/or too much contact time with the decontaminant) ▪ low relative centrifugal force or over-heating of centrifuge ▪ low culture media sensibility (lack of homogeneity, over-heating during inspissation, too much Malaquite green, too acidic a pH) ▪ incubation at too high or too variable temperatures
D	<ul style="list-style-type: none"> ▪ smear microscopy reading errors: "false positives"
E	<ul style="list-style-type: none"> ▪ un-refrigerated storage of specimens ▪ excessive delay between collection and processing of the specimens ▪ low decontaminant concentration ▪ too short time of decontaminnt contact with the specimen ▪ deficiency in the sterilization procedure ▪ careless use of the Bunsen burner, heavy people movement in the work area, generation of air drafts by fans or by air conditioning systems, etc...

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 04.02

Date: 1/04/2009

Author(s): Microbiologist & lab staff

Probable cause & investigative steps	
F	<ul style="list-style-type: none">▪ too high a concentration of decontaminant▪ too long a time of contact of the specimen with the decontaminant▪ too high concentration of Malaquite green in culture medium

Analysis of the delay in the delivery of reports

More than the 95% of the results should be reported within 62 days after the processing of the specimen with the conventional method. Make sure that positive cultures are reported within 48 hrs following growth detection. Failing this, identify the stage (s) in which this delay occurs i.e. the reading of cultures, the entering of results, the writing of reports, the delivery of the result etc... Take immediate remedial action.

4. Safety Conditions:

1. All the work should be carried out in the bio-safety cabinets.
2. Care should be taken that aerosols are not generated or minimized.
3. Wear N95 masks and practise the Lab bio-safety practises strictly.
4. In case of difficulties, notify the laboratory in-charge of difficulties.

5. Documentation:

5.1 Recording in Lab Card

1. Lab card is stamped with date of culture, method, volume and the initials of the technician performing the primary culture to be entered.
2. The completed Lab cards are filed in serial order.
3. The specimens which are not cultured are marked in remarks column with the reason.

5.2 Primary culture register should be maintained showing details of processing including sterility tests and dates for recording the results. Format for primary culture register is given in the appendix.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 05.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

5.0 Identification for *M. tuberculosis* (PNB method)

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 05.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

TITLE: Identification of *M.tuberculosis*

1. Objectives and Scope

The process describes the differentiation of tubercle bacilli from other mycobacteria and the phenotypic identification of *M. tuberculosis*.

The vast majority of strains phenotypically identified as *M. tuberculosis* are genuine *M. tuberculosis* strains. However, phenotypic traits may not always differentiate *M. africanum* strains from the other main pathogen in humans, i.e. *M. tuberculosis*. This has no major impact on patient management and follow-up. For precise epidemiological studies in a geographical region where *M. africanum* or *M. bovis* are frequent or for a study focused on patients from such regions, a link should be established for reference purposes with a laboratory carrying out molecular identification of tubercle bacilli.

2. Definitions and abbreviations :

PNB p-nitro benzoate.

3. Procedure

3.1 Principle of procedure

The identification process comprises the phenotypic identification of cultures of acid-fast bacilli grown on solid medium based on the combination of observation of colony morphology, inability to grow on a culture medium containing p-nitrobenzoate and results of two biochemical tests specific for thermolabile catalase and nitrate reductase.

3.2 Samples:

- Cultures grown on solid medium. These could be primary cultures if growth is abundant
- Sub-culture, e.g. control tubes used for drug susceptibility testing.

Identification has to be carried out on pure cultures only.

3.3 Equipment and materials: N/A

3.4 Reagents and solutions: N/A

3.5 Process of identification of *M. tuberculosis*

3.5.1. Observation of colonies: morphology and growth rate

The following characteristics of isolated colonies may yield a tentative identification of *M. tuberculosis* :

- Formation of visible colonies between 10 to 28 days after incubation at 37°C,
- Greyish white or buff, dry, cauliflower-like colonies with no late production of pigment (up to 28 days),

3.5.2 Interpretation of the growth test on PNB

Growth is inhibited on Löwenstein Jensen medium containing PNB (500 µg/ml)

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 05.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

3.5.3 Interpretation of the catalase test

The catalase activity is not thermostable

3.5.4 Interpretation of the nitrate reductase test

The nitrate reductase test is positive

3.6 Interpretation and identification

3.6.1 Identification of *M. tuberculosis*

If all the above criteria are met, the culture is identified as *M. tuberculosis*.

3.6.2 Identification of other *M. tuberculosis* complex members

Most tubercle bacilli form colonies at about 10 days, but growth may take up to 2 to 3 weeks.

Most tubercle bacilli form non pigmented colonies, greyish white or buff in colour

Tubercle bacilli and few other mycobacterial species are inhibited by PNB

Most tubercle bacilli do not have a thermostable catalase

Acid-fast bacilli which share the above characteristics but do not match other characteristics of *M. tuberculosis* (cauliflower type of colony and/or nitrate reductase positive) may be other members of the *M. tuberculosis* complex.

Follow the chart for the identification of *M. bovis*. Colonies of *M. bovis* are dysgonic, small, flat and smooth.

If more precise identification is required, strains should be submitted to molecular tests and referred to laboratories which perform these tests.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

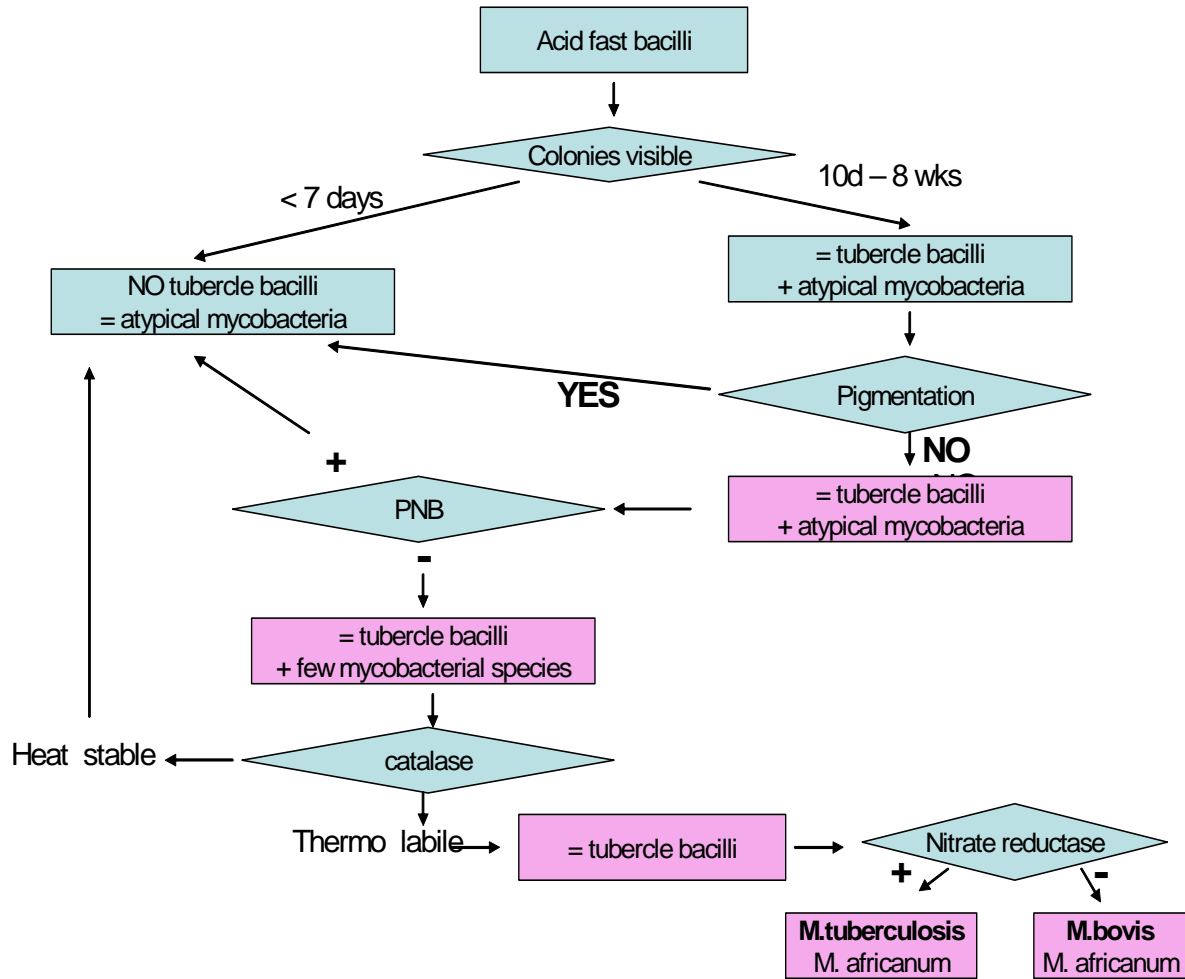
Lab: IRL <<name & address>>

Procedure No. 05.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

Chart of identification



Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 06.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

6.0 Long term Preservation & recovery of the *Mycobacterial* cultures

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 06.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

TITLE: Long term Preservation & recovery of the cultures

1. Objective and Scope:

To describe the procedures required for the preservation of culture by (a) 10% skim milk solution (b) 25% glycerol containing Middle brook 7H9 medium.

2. Definitions and abbreviations:

MB 7H9 medium: Middle brook 7H9 broth medium, enriched with OADC supplement, used specifically for the isolation of TB bacilli.

OADC: Oleic acid, Bovine serum albumin, Dextrose and Catalase, used as supplement for MB 7H9 medium

Glycerol: Trihydric Alcohol, used for cryo- preservation, or storage at lower temperatures.

3. Procedure

It is often required in the laboratory to preserve the stains isolated for long terms as probable drug sensitive and probable drug resistant cultures for quality control purposes. Such strains can be laboratory repository for further operational research studies.

3.1 Storage of *Mycobacterium tuberculosis* in 10% Skim milk solution

3.1.1 Materials required:

1. 10% skim milk power solution: Reconstitute skim milk powder in distilled water (100 grams per litre).
2. Cryovials & storage box
3. McCartney bottles
4. -20°C freezer

3.3.2 Method

a) Preservation

1. Dispense 1.6 ml of 10% skim milk solution into 1 oz. McCartney bottles containing 10 glass beads.
2. Autoclave at 15 lbs. and 121°C. for about 10 minutes. Tighten caps after cooling
3. *Medium can be frozen at this stage.*
4. Add bacterial growth from 1 slope of L-J medium with 3+ growth.
5. Mix on Vortex mixer for about 30 seconds.
6. Allow foam to subside for 2 hours.
7. Label the cryovial with culture lab number and date
8. Pipette 0.5ml of bacterial suspension into each cryovial (Nalgene 2.0 ml).
9. Freeze at -20 °C until needed.

b) Recovery

1. Thaw cryovial at room temperature.
2. Inoculate loopful of thawed cultures (2 or 3 drops) onto a slant of medium and incubate at appropriate temperature.
3. Vials can be re-frozen 2 or 3 times.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 06.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

3.2 Storage of *Mycobacterium tuberculosis* in Middle brook 7H9 medium with 25% glycerol

3.2.1 Materials Required:

1. Middle brook 7H9 medium with OADC as enrichment
2. Glycerol solution (50%), sterilized.
3. Cryovials & storage box
4. McCartney bottles
5. -20c freezer

3.2.2 Method

a) Preservation

1. Add equal volumes of Middle brook 7H9 with OADC enrichment and Glycerol (50%) solution to required quantity. (1 ml of storage medium is required for each culture)
2. Aliquot 1ml of storage medium to 1 oz. McCartney bottle with 10 glass beads.
3. Add a loopful of 3+ growth, mix or vortex for 30 seconds.
4. Allow to settle for 1 hours
5. Label the cryovial with culture lab number and date
6. Pipette 0.5ml of bacterial suspension into each labelled cryovial.
7. Freeze at -20c until needed.

b) Recovery

1. Thaw cryovial at room temperature.
2. Inoculate loopful of thawed cultures (2 or 3 drops) onto a slant of medium and incubate at appropriate temperature.
3. Vials can be re-frozen 2 or 3 times.

4. Safety conditions:

Handle all operation in the bio-safety cabinet, wearing gloves and N95 mask.

5. Documentation:

Data entries need to be made in the formatted registers.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 07.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

7.0 Drug Susceptibility Testing (DST)

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 07.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

TITLE: Drug sensitivity testing (DST) on LJ medium by standard economic variant of 1% Proportion method

1. Objective and Scope:

To perform, Drug sensitivity testing (DST) of *Mycobacterium tuberculosis* cultures on LJ medium. Method of DST is standard economic variant of 1% Proportion method for Drugs-Streptomycin, Isoniazid, Rifampicin and Ethambutol. The scope of document is for performing DST of *Mycobacterium tuberculosis* cultures on LJ medium at IRL.

2. Definitions and abbreviations:

LJ medium: Lowenstein-Jensen egg based solid medium used for culture of TB bacilli

DST: Drug sensitivity Testing: *In vitro* method of detecting 'Resistance' or 'Susceptibility' of bacterial isolate to a particular anti-bacterial drug.

MIC: Minimum inhibitory concentration

CFU: colony forming units

3. Procedure:

Drug resistance is the result of selection, by exposure to a drug, of a small number of pre-existing mutants in a population of tubercle bacilli. Resistance is defined as sufficient decrease in sensitivity of a test stain concerned, compared to a wild strain that has never come into contact with the drug. Proficiency in susceptibility tests demands an understanding of:

- The origin of drug resistance
- The variation in stability of drugs subjected to different conditions of filtration, heat or storage
- The alteration in the activity of certain drugs when incorporated into different kinds of media
- The type of susceptibility test performed
- The reading and reporting of test results
- The criteria of resistance

There are three general methods used for determining drug susceptibility of mycobacteria on slide LJ medium: the absolute concentration method (MIC method), the resistance ratio method, and the proportion method. When properly standardized and performed, all three methods have been shown to be equally satisfactory.

The Absolute Concentration Method:

This method uses a standardized inoculum grown on drug-free media and media containing graded concentrations of the drug(s) to be tested. Several concentrations of each drug are tested, and resistance is expressed in terms of the lowest concentration of the drug that inhibits growth; i.e., minimal inhibitory concentration (MIC). This method is greatly affected by inoculum size and the viability of the organisms.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 07.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

The Resistance Ratio Method:

It compares the resistance of unknown strains of tubercle bacilli with that of a standard laboratory strain. Parallel sets of media, containing twofold dilutions of the drug, are inoculated with a standard inoculum prepared from both the unknown and standard strains of tubercle bacilli. Resistance is expressed as the ratio of the minimal inhibitory concentration (MIC) of the test strain divided by the MIC for the standard strain in the same set.

3.1 Proportion Method:

All strains of tuberculosis contain some subpopulation of bacilli that are resistant to anti-TB drugs. However, in resistant strains, the proportion of such bacilli is considerably higher than the sensitive strains. The proportion method calculates the proportion of resistant bacilli present in a strain. Two appropriate dilution of the bacilli, 10^{-2} and 10^{-4} dilutions (undiluted = 10^6 to 10^8 CFU/ml), are inoculated on drug-containing and drug-free media, in order to obtain countable colonies on both media. The ratio of number of colonies observed on the drug-containing media to drug-free medium indicates proportion of resistant bacilli present in the strain. Below a certain proportion (critical proportion = 1%), the strain is classified as sensitive; above, as resistant.

The proportion method is currently the method of choice, in the majority of laboratories in the world, and the standard economic version of bacterial suspension and interpretation of results for proportion method would be adopted in lab and the method is given below.

Drug Concentrations added to LJ Media and critical proportion for Interpretation for economic variant of Proportion Method are:

Drug	Media Concentration	Critical proportion to determine resistance
Streptomycin(dihydro-streptomycin sulfate)	4 µg/ml	1%
Isoniazid	0.2 µg/ml	1%
Rifampicin	40 µg/ml	1%
Ethambutol	2 µg/ml	1%

One set of media bottles for testing one culture consist of five LJ slope, one for neat, two for 10^{-2} and two for 10^{-4} ; eight LJ drug containing slopes, two each for drugs H, R, E & S (one each for 10^{-2} and 10^{-4} suspensions) and one for PNB slope, total 14 LJ slopes are required.

3.1.1 Materials required

1. Drug-free LJ media bottles -5 per test culture
2. Drug media- Streptomycin (4ug/ml), Isoniazid(0.2ug/ml), Rifampicin(40ug/ml), Ethambutol(2ug/ml)- two bottles each for test culture

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 07.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

3. Calibrated inoculation loops: 3mm internal diameter loop made with 24 SWG nichrome wire. This delivers 0.01 ml of inoculum. Delivery volume must be verified by weighing 10 loopfuls of distilled water deposited on a filter paper.
4. Bijou bottles (5 per culture)
5. McCartney bottles with 1ml sterile distilled water and six 5mm glass beads, sterile. (1 per culture). And empty McCartney bottle, sterile.
6. Bijou bottles with one ml distilled water, sterile(2 per culture)
7. Sterile distilled water – 10ml per culture
8. Positive cultures, picked up within 1-2 weeks of declaration as positive.
9. Control cultures- *M. tuberculosis* H37Rv
10. McFarland standard No. 1:

Preparation of McFarland Nephelometer Barium chloride Standards (Paik, G. 1980)

- (a) Prepare 1% aqueous barium chloride (100 mg of Barium chloride (anhydrous) in 10 ml of sterile distilled water (SDW).
 - (b) Prepare 10ml of 1% sulphuric acid solution (99ml of distilled water and 1ml of concentrated sulphuric acid)
 - (c) Add 0.1 ml of 1% Barium Chloride solution to 9.9 ml of 1% Sulphuric acid solution to obtain the McFarland standard, which matches with 1 mg/ ml of *M. tuberculosis*.
 - (d) Seal the tubes (wrap with parafilm) and label as No. 1 McFarland standard tube with date of preparation.
 - (e) Once prepared, standard can be stored & used for up to 4 months.
11. Wire meshes to hold McCartney bottles and bijou bottles.

3.1.2 Inoculum preparation

1. With a 3mm wire loop, a representative sample of approximately 4-5 mg (loop full) is taken from the primary culture and placed on the side wall of a McCartney bottle containing 1 ml SDW and 6 glass beads of diameter 3 mm.
2. Emulsify the bacterial inoculum, (with a loop of water, if required), on to the side wall of McCartney bottle in round rotatory movements with inoculation loop, till the bacterial mass is emulsified, (this is visible by reduction in the clumpy hydrophobic to aqueous hydrophilic nature of suspension).
3. Let the emulsified suspension be fully dissolved in the 1ml of Sterile Distilled Water (SDW).
4. Vortex the bottle for 20–30 seconds;
5. 4 ml of distilled water is added slowly.
6. Allow the coarse particles to settle down (leave it on stand for approximately 5 min).

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 07.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

7. Decant the Mycobacterium solution carefully into another clear, sterile McCartney bottle.
8. Match the opacity/turbidity of inoculum with McFarland standard no.1, against a black background. This is the neat bacterial suspension, standardized at 1 mg/ml, equalling to 10^7 to 10^8 CFU/ml. Make sure that no clumps are taken.
9. If required, the opacity of the bacterial suspension is then adjusted by the addition of distilled water to obtain a concentration of 1 mg/ml of tubercle bacilli by matching with McFarland's standard 1.
10. Make further two log dilutions to achieve 10^{-2} and 10^{-4} dilutions as given below:
 - a. The dilution 10^{-2} is produced by discharging two loopfuls of the neat bacterial suspension, into a small tube containing 2 ml of distilled water, and shaking.
 - b. Similarly, the dilution 10^{-4} is produced by discharging two loopfuls of the dilution 10^{-2} into a small tube containing 2 ml of distilled water, and shaking.

Neat: 1 ml SDW with six 3 mm glass beads + 1 loop-full (3 mm loop) of culture

↓
Vortexed for 20 – 30 seconds

↓
Add 4 ml of SDW to the above

↓
Adjusted turbidity with McFarland std.1 with SDW

S2-10⁻² two loop-full of **neat** + 2ml of SDW, vortex

↓
S4-10⁻⁴ two loop-full of **S2** + 2ml of SDW

Precautions:

- Avoid touching the media while picking the colonies,
- Cool down the loop sufficiently before picking the colonies,
- Try to take loop-full of colonies in one sweep, by touching all colonies on the LJ slope.
- Avoid touching the water of condensation while scrapping the colonies

3.1.3 Drug susceptibility testing:

Label with media slopes with lab number of culture, and serially arrange in the wire mesh.

1. Heat the loop to red hot (incandescence) in flame for each dilution separately, ensure the loop is cooled by touching the insides of medium slope, before using the loop.
2. Inoculate a loop-full (using 3mm calibrated loop) of each dilution on to media slopes. Should inoculate uniform suspension in to all slopes.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 07.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

Inoculums	Drug-free media	Strep (4ug/ml) media	INH (0.2 ug/ml) media	Rif (40ug/ml) media	ETB (2ug/ml) media	PNB (500ug/ml) media
Neat (~10 ⁸ CFU/ml)	1					1
10 ⁻²	2	1	1	1	1	
10 ⁻⁴	2	1	1	1	1	
Total bottles	5	2	2	2	2	

- Precaution:** Avoid touching the water of condensation while inoculation, and do not tilt the LJ slope too much while inoculating.

3.1.4 Incubation and Reading

Incubate the inoculated slopes at 37°C. Read the growth at 28 days and again at 42 days.

Record growth as Confluent growth = 3+; More than 100 colonies = 2+; Record actual number of colonies = 1-100 cols.

When the number of colonies on a given dilution is less than 5, count the number of colonies with the next larger inoculum, or estimate if more than 100. Make no attempt to estimate the number of colonies if the growth is 3+.

3.2 Interpretation of the test

- First reading is taken at 28th day after inoculation.
- Count the colonies only on the slopes seeded with the inoculum that has produced exact readable counts or actual counts (up to 100 colonies on the slope). This inoculum may be the same for the control slopes and the drug-containing slopes, or it may be the low inoculum (10⁻⁴ dilution) for the control slopes and the high inoculum (10⁻² dilution) for the drug-containing slopes.
- The average number of colonies obtained for the drug-containing slopes indicates the number of resistant bacilli contained in the inoculum.
- Dividing the number of colonies in drug containing slopes by that in drug free slopes gives the proportion of resistant bacilli existing in the strain. Below a certain value – the critical proportion – the strain is classified as sensitive; above that value, it is classified as resistant. The proportions are reported as percentages.
- If, according to the criteria indicated below, the result of the reading made on the 28th day is “resistant”, no further reading of the test for that drug is required: the strain is classified as resistant. If the result at the 28th day is “sensitive”, a second reading is made on the 42nd day only for the sensitive strain. The final definitive results for all the four drugs should be reported on 42nd day. If the strain is resistant for all the four drugs on 28th day, then the report can be given on the same day. Otherwise, incomplete reports should not be given before 42nd day.
- In case growth on the control media is poor even after six weeks (i.e., few or no colonies on the 10⁻⁴ bacterial dilution), the test should be repeated.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & address>>

Procedure No. 07.01

Date: 1/04/2009

Author(s): Microbiologist & lab staff

3.3 Criteria of Resistance

Any strain with 1% (the critical proportion) of bacilli resistant to any of the four drugs – Rifampicin, Isoniazid, Ethambutol, and streptomycin – is classified as resistant to that drug. For calculating the proportion of resistant bacilli, the highest count obtained on the drugfree and on the drug-containing medium should be taken (*regardless of whether both counts are obtained on the 28th day, both on the 42nd day, or one on the 28th day and the other on the 42nd day.*)

Calculation of proportions – An illustration

Suspension	Dug-free medium		Drug concentration (mg/l)	
			INH 0.2	Rif 40
Neat	3+	(50000)	2+	26
S ₂	2+, 2+	(5000)	62	1
S ₄	46, 54	50	3	0
	Result		Resistant	Sensitive

3.4 Recording:

Recording is made in the register as per the form given in the annexure.

3.5 Internal Quality Control:

For each batch of testing, a culture of H37Rv (or a known all sensitive strain) along with two strains of *M. tuberculosis* with moderate levels of drug resistance to some of the drugs, which together express all four drug resistance markers, should be tested and validated.

4. Safety Conditions:

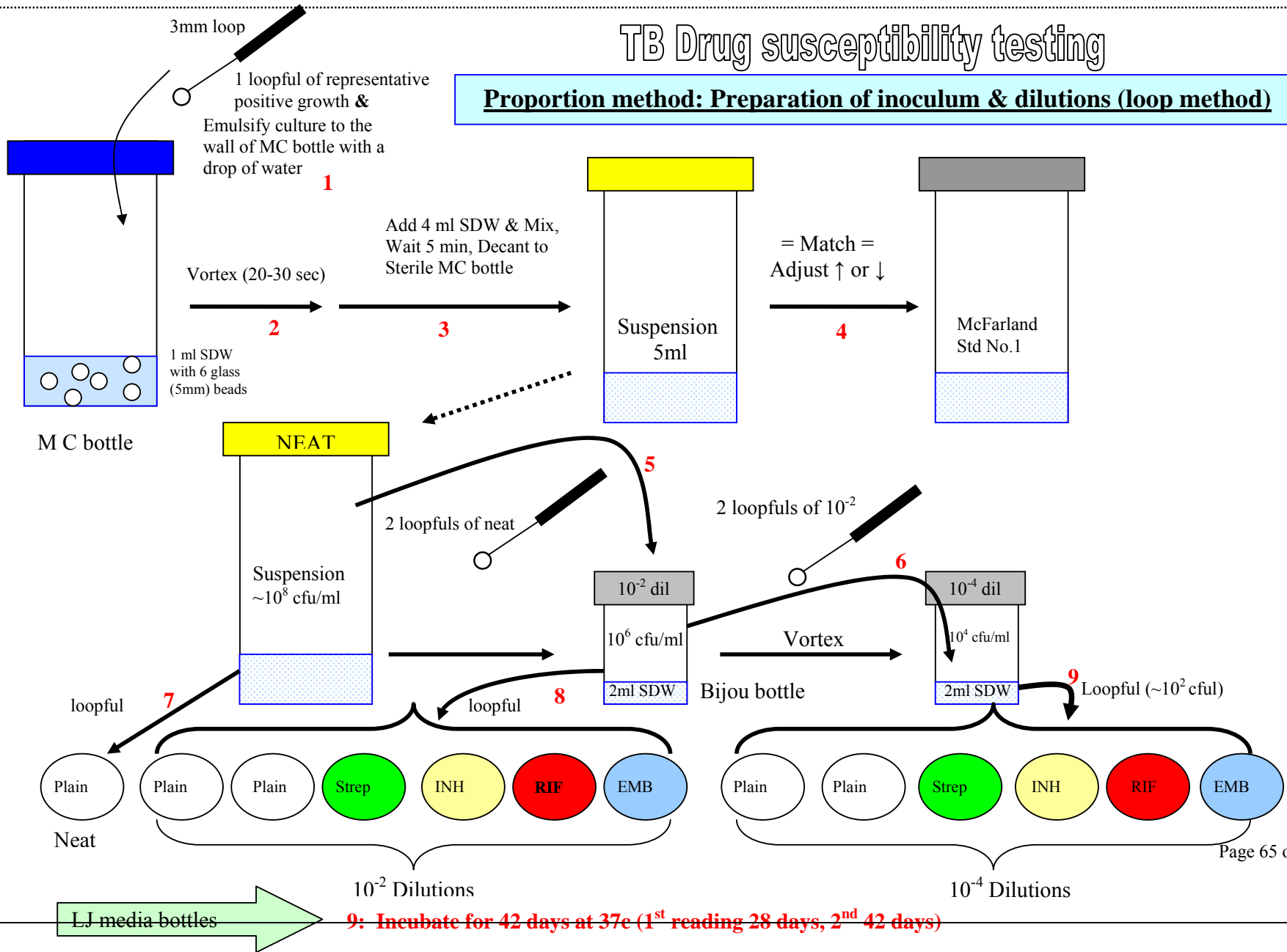
1. All the work should be carried out in the bio-safety cabinets.
2. Care should be taken that aerosols are not generated or minimized.
3. Wear N95 masks and practise the Lab bio-safety practises strictly.
4. In case of difficulties, notify the laboratory in-charge of difficulties.

5. Documentation:

Entries need to be made in the Drug susceptibility testing and Identity register. Format for the register is provided as the annexure.

TB Drug susceptibility testing

Proportion method: Preparation of inoculum & dilutions (loop method)



Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 08.00

Author(s): Lab-Microbiologist & lab staff

8.0 Transport of Mycobacterium tuberculosis containing specimens and cultures

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 08.00

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Title: Transport of *Mycobacterium tuberculosis* containing specimens and cultures

1. **Objective and Scope:** To transport *Mycobacterium tuberculosis* containing specimens, body fluids, tissues and pure cultures taking into consideration the requirements for bio-safety.

2. **Definitions and abbreviations:**

Triple Packaging system: The choice of preference for safe transportation of biologically infectious agents. Bio-hazard symbol: Standard signage for the biologically infectious agents.

3. **Procedure:**

3.1. General Principles:

Mycobacterium tuberculosis containing specimens, body fluids, tissues and pure cultures are infectious substances and biological hazardous material as improper handling or leakage/breakage of them during the transportation could potentially cause exposure to these materials with severe consequences. Special care need to be taken such complying with the transportation regulations. Protection is achieved through rigorous packaging requirements and hazard communication. Packages must be designed to withstand rough handling and other forces experienced in transportation, such as changes in air pressure, temperature, vibration, stacking, and moisture. Hazard communication includes shipping papers, labels, markings on the outside of packaging, and other information necessary to enable transport workers to correctly identify the material.

Laboratory personnel must ship infectious substances according to applicable transport regulations. Compliance with the rules will:

Reduce the likelihood that packages will be damaged and leak, and thereby

- A. Reduce the exposures resulting in possible infections
- B. Improve the efficiency of package delivery.

3.2. The basic triple packaging system

The triple packaging system, the choice for the transport of infectious and potentially infectious substances, is shown in Figure.

This packaging system consists of three layers: the primary receptacle, the secondary packaging and the outer packaging.

- ◆ The primary receptacle containing the specimen must be watertight, leak-proof and appropriately labelled as to content. The primary receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage or leakage.
- ◆ A second watertight, leak-proof packaging is used to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in a single secondary packaging. Volume and/or weight limits for packaged infectious substances are also critical, such that excess chance of breakage is not there.
- ◆ The third layer protects the secondary packaging from physical damage while in transit. Specimen data forms, letters and other types of information that identify or describe the specimen and identify the shipper and receiver,

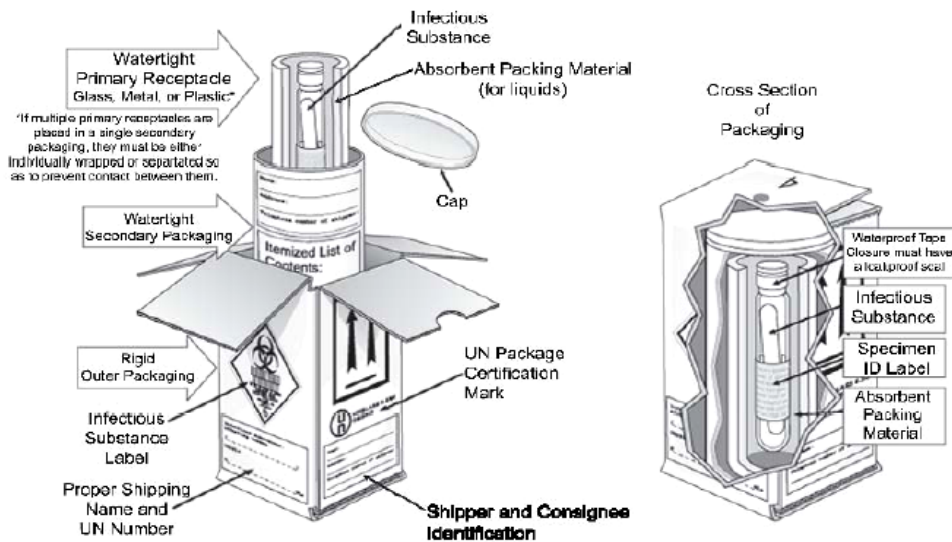
Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>
 Date: 1/04/2009

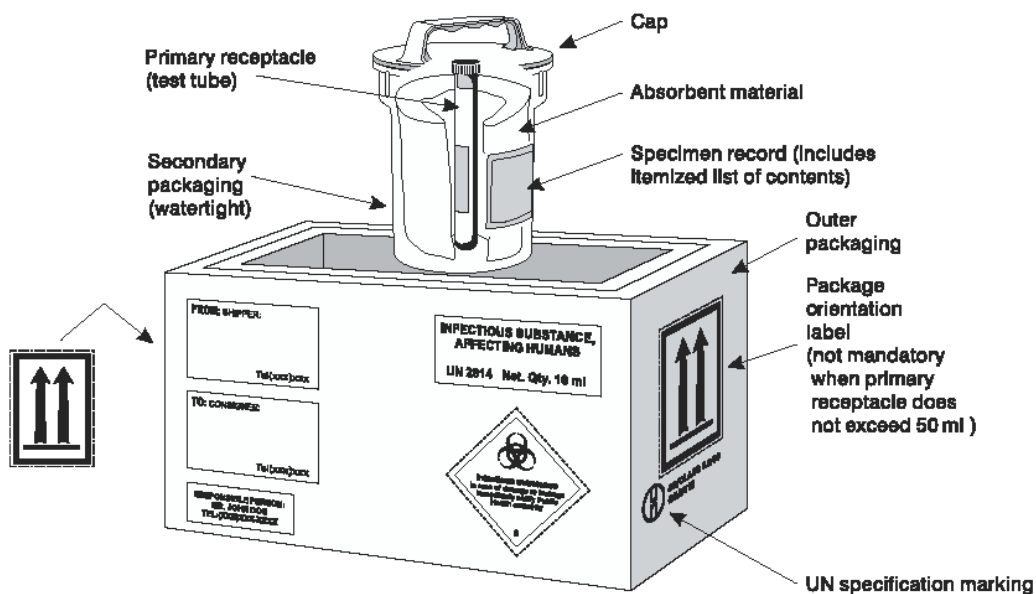
Procedure No. 08.00
 Author(s): Lab-Microbiologist & lab staff

and any other documentation required must also be provided according to latest regulations.

Each surface of the external dimension of the packaging must be 100 mm (3.9 inches) or more. The completed package must pass specific performance tests, including a drop test and a water-spray test, and must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of not less than 95 kPa (0.95 bar, 14 psi). The completed package must also be capable of withstanding, without leakage, temperatures in the range of -40 °C to +55 °C (-40 °F to 131 °F). The completed package must be marked "Infectious substances" and labelled with a Bio-Hazard label.



Another example of packing: (Reproduced from reference 9)



Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 08.00

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

3.3 Receipt of the package:

Upon receipt of the package,

1. Wear gloves and protective clothing
2. Carefully take package in to the Bio-safety cabinet
3. Open the box and observe carefully, for any leakage or spill
4. Act accordingly, in case of leakage or spill
5. Else, carefully, take out the contents into appropriate holder/strand.
6. Note down the details and any identity numbers in your lab register
7. Wipe off the surface of specimen bottles/ cultures with appropriate disinfectant or 70% ethanol or methanol.
8. Proceed further for primary culture, or sub-culture or DST.
9. Autoclave the any recyclable material packaging material, and discard the rest.

3.4 Spill clean-up procedure

In the event of a spill of infectious or potentially infectious material, the following spill clean-up procedure should be used.

1. Wear gloves and protective clothing, including face and eye protection if indicated.
2. Cover the spill with cloth or paper towels to contain it.
3. Pour an appropriate disinfectant over the paper towels and the immediately surrounding area (5% phenolic solution is appropriate).
4. Apply disinfectant concentrically beginning at the outer margin of the spill area, working toward the centre.
5. After the appropriate amount of time (e.g. 30 min), clear away the materials. If there is broken glass or other sharps involved, use a dustpan or a piece of stiff cardboard to collect the material and deposit it into a puncture-resistant container for disposal.
6. Clean and disinfect the area of the spillage (if necessary, repeat steps 2–5).
7. Dispose of contaminated materials into a leak-proof, puncture-resistant waste disposal container.
8. After successful disinfection, inform the competent authority that the site has now been decontaminated

4. Safety Conditions:

Treat all body fluids and transport specimens as potentially infectious and follow the bio-safety requirements.

5. Documentation:

It is necessary to maintain the inventory of receipt/despatch for the potentially infectious agents, especially the pure cultures, received from or despatched to other TB labs.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 09.00

Author(s): Lab-Microbiologist & lab staff

9.0 Maintenance of Instruments

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 09.01

Author(s): Lab-Microbiologist & lab staff

TITLE: Maintenance and Use of Biological Safety Cabinet (BSC) Class II

1. Objectives and scope.

To describe optimal operation of the biological safety cabinet and preventive maintenance. The biological safety cabinet is a fragile and precision piece of equipment intended for protecting the user (TB laboratory worker) from airborne aerosols that may cause infection. IRLs are provided with BSC class II units.

2. Definitions and abbreviations

BSC: Biological Safety Cabinet

HEPA: High Efficiency Particulate Air

UPS: Uninterrupted Power Supply

UV: Ultraviolet light; for this application it should be UV-C (short wave, germicidal)

GMT: Good Microbiological Techniques. Working methods applied to eliminate or minimize exposure to pathogens via e.g. aerosols, splashes, and accidental inoculation.

3. Procedure

3.1. Principle

A Class I BSC is an open fronted ventilated cabinet with a unidirectional inward airflow away from the operator. Exhaust air is hard-ducted through HEPA filters. Filters belong to either class H13 which removes particles equal to or greater than 0.3 μm with an efficiency of 99.95% or class H14 which removes particles equal to or greater than 0.3 μm with an efficiency of 99.995 at the outlet to the environment. Class I BSC provides protection for the worker and the environment but does not provide protection to the product (specimens, strain cultures) against contamination.

A Class II BSC, additionally provides protection against contamination of the product (specimens, strain cultures) by re-circulating part of the HEPA (H14) filtered air in a laminar vertical flow inside the cabinet so that a "curtain" of clean air descends across the whole working surface.

BSCs, whatever type I or II, should be vented to the outside through a thimble or with hard ducting. (See table 1).

The installation of both BSC class I and II has to be performed under the supervision of an engineer from the manufacturer's company.

3.2 Samples

Specimens for isolation and culture of tubercle bacilli

Cultures of tubercle bacilli and other mycobacterial species

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 09.01

Author(s): Lab-Microbiologist & lab staff

Class I or class II BSC models should have a visible front display for current face velocity. BSCs should be vented to the outside of the buildings according to the following table.

Differences between Class I and II biological safety cabinets (BSCs)

Table 1 according to *World Health Organization. Laboratory Biosafety Manual. 3rd ed. Geneva: WHO; 2004.*

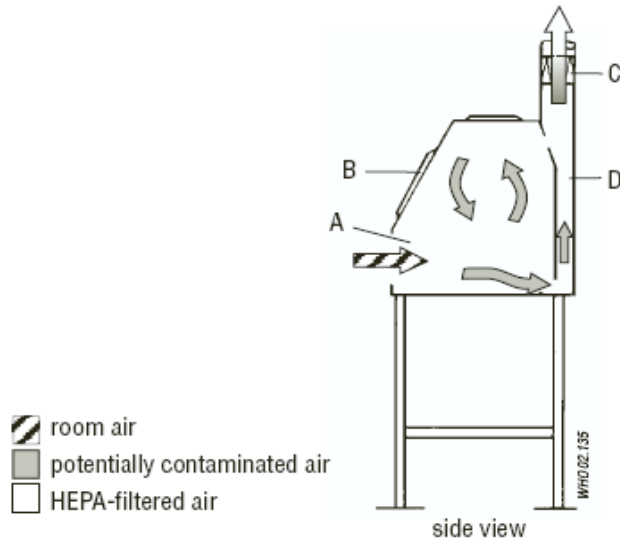
BSC class	Face velocity (m/s)	Air flow %		Exhaust system
		Re-circulated in the cabinet	Exhausted	
I	0.36	0	100	Hard duct
IIA1	0.38 - 0.51	70	30	Thimble connection*
IIA2	0.51	70	30	Thimble connection*
IIB1	0.51	30	70	Hard duct
IIB2	0.51	0	100	Hard duct

* For safety reasons dealing with risk group 3 pathogens (TB bacilli), the exhaust to the room should be avoided

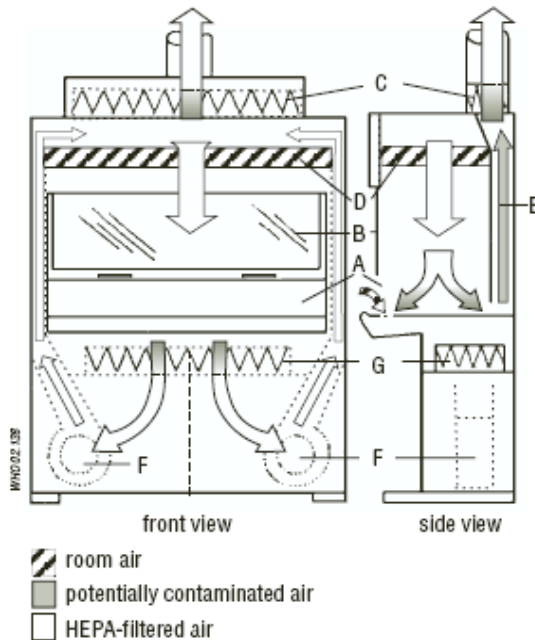
Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>
 Date: 1/04/2009

Procedure No. 09.01
 Author(s): Lab-Microbiologist & lab staff



Schematic diagram of a Class I biological safety cabinet.
 A, front opening; B, sash; C, exhaust HEPA filter; D, exhaust plenum.



Schematic diagram of a Class IIB1 biological safety cabinet.
 A, front opening; B, sash; C, exhaust HEPA filter; D, supply HEPA filter; E, negative-pressure exhaust plenum; F, blower; G, HEPA filter for supply air. Connection of the cabinet exhaust air system to the building exhaust air system is required.

World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: WHO; 2004.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 09.01

Author(s): Lab-Microbiologist & lab staff

All these BSCs are vented to the outside, including the IIA1 - IIA2 class.

BSC should be located away from air disturbances such as doors, windows, air-conditioning, and ventilation supply or return outlets, exhaust fans, etc. Personnel traffic should be minimized in front of the BSC in order not to disrupt the air flow.

BSCs must be connected to a suitable (capacity for at least 15 minutes of extra running time) UPS system or generator in any location where electricity supply may be irregular.

3.4 Reagents and solutions

70% ethanol as disinfectant solution

3.5 Detailed instructions for use

3.5.1 Certification

Certification must be done by a qualified service professional, BEFORE the BSC is placed into service and thereafter at periodic intervals. The BSC must be re-certified after change of HEPA filters or whenever it has been relocated, serviced or repaired. It should consist of HEPA filter leak tests (filter integrity and its sealing) and of airflow velocity tests (inward flow and laminar down flow) which is carried out with an anemometer.

3.5.2. Daily use

1. Switch on the BSC air flow 5-15 minutes prior to use (check manufacturer's instructions).
2. Check the air flow conditions on the display (models that indicate positive, negative or differential pressure, accurate within 2%, can be available).
If the BSC is equipped with a magnehelic gauge, record the reading. A change of more than 50% from the previous reading indicates a problem requiring discontinuation of use and the need for immediate service.
3. Complete the BSC logbook providing details of the BSC's operation.
4. Organize all items needed for work in the BSC to avoid frequent displacements. The work has to be organized to minimize arm movements.
Arm movements in and out of the BSC should be horizontal so as to minimize turbulence and prevent air inside the BSC from flowing out of it. Conduct all manipulations within the BSC as far as possible towards the back of the unit.
5. Do not overcrowd the working area as this disturbs the air flow. In BSCII types work from the grille, never allow the grille to be covered by anything.
6. Provide inside the BSC a container with disinfectant for liquid waste (and for sharps if needed). Do not accumulate waste in the BSC but remove it when activities are stopped.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.01

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

7. After use, wipe down the inside of the BSC and work surface with 70% alcohol.

8. Switch on the UV light and leave for a minimum of 30 minutes

Switch off the BSC fan

3.5.2. Weekly, wearing gloves clean the UV lamp with a gauze pad moistened with 70% alcohol. Care must be taken to never touch the lamp with bare hands.

3.6 Recording and reporting

Each use should be reported in the logbook. These records are used for maintenance/service schedules, specifically for filter checking and UV lamp replacement, reporting of incidents, accidents and/or mechanical problems. They should be kept on file for a minimum of one year, after which they may be archived.

After re-certification the service professional must issue a certificate indicating the performance characteristics of the BSC and safety compliance. The most current inspection certificate must be posted in close proximity to the BSC. Certificates have to be archived as long as the BSC is in use in the laboratory.

3.7 Quality control

If the daily check of the air flow shows values below the minimal velocity level indicated in Table 1 (according to the BSC type) and cannot be manually improved, alert the technical service in charge of the maintenance and do not use the BSC till the BSC is re-certified.

In the case of any problem or malfunction an 'Out of Service' notice must be placed on the BSC indicating that it is not to be used until the problem has been diagnosed and corrected.

BSCs have to be re-certified at least every year and after every replacement of filters by a qualified service professional.

3.8 Safety precautions

3.8.1 Waste management

At the end of each day, collect contaminated material, in an autoclavable bag and autoclave as soon as possible. Keep the bag in a safe, closed bin or large bucket till then.

3.8.2 Personnel protection

Refer to the specifics of protective clothing and practises in TB culture/DST laboratories

3.8.3 Others

Never work in the BSC with UV light on

Replace the UV lamps at the limit of their duration of use (according to the manufacture's recommendations and according to the cumulative duration of the BSC use reported in the logbook). Use gloves for their removal. Wipe with a disinfectant and discard following national guidelines for special waste.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 09.01

Author(s): Lab-Microbiologist & lab staff

Fumigation of the BSC is required PRIOR to:

- . replacement of HEPA filters.
- . release of the BSC for use after a major bio-hazardous spill.
- . repair work requiring access to the sealed plenum.
- . service or replacement of the circulation fan or components.
- . maintenance work in contaminated areas.
- . performance tests requiring entry into contaminated areas.
- . movement of the BSC cabinet to another laboratory.
- . changing work programmes, e.g. to non-TB work.
- . release of the BSC cabinet for resale or salvage.

Users are NOT to make any repair. Repair and service of the BSC must be done by a qualified service professional.

Any problem or malfunction detected must be reported to the microbiologist, who will contact the maintenance contractor to correct the problem/malfunction as soon as possible. HEPA filters MUST NEVER BE TOUCHED by the personnel and no attempt should be made to clean or disinfect filters using chemical or mechanical methods.

4. Related documents

Manufacturer's Manual, specific to each BSC

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.02

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

TITLE: Maintenance of the autoclave

1. Objectives and scope.

The autoclave using saturated steam under pressure is the most efficient means of sterilization of instruments, glassware or media solutions in the general diagnostic TB laboratory, and of decontamination of biological material consisting of infectious waste (= mycobacterial cultures and related infectious material).

The current SOP describes the procedure of use of a pressure cooker autoclave with fully manual operation. The SOP has to be adapted for other autoclave types with automatic air and condenser discharge, as per manufacturers' instructions.

Note: Never operate Autoclave or plug it into electric socket and Switch ON when you are not aware of its usage details. It may damage the instrument or physically harmful to you. Ask the supplier/manufacturer to provide a demonstration, learn and use.

2. Definitions and abbreviations

Disinfection – A physical or chemical means of killing microorganisms, but not necessarily spores.

Decontamination – Any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.

Sterilization – A process that kills and/or removes all classes of microorganisms and spores.

Inactivation - A process rendering an organism inert by application of heat or other means.

BI : biological indicator

°C Degrees Celsius.

1 kPa (kilo Pascal) = 0.01 bar

1 kg/cm² = 98 kPa

1 lb/in² (pounds per square inch, psi) = 6.8947 kPa

At sea level, atmospheric pressure is 1 bar or 760 mm Hg or 14.7 psi or 101 kPa

At sea level, 121°C is obtained at 1.06 kg/cm² or 15 lb/in² or 115 kPa or 1 atm.

Conversions Table

	Pa	bar	atm	mmHg	Kg/cm ²	psi
Pa	1	10 ⁻⁵	9.87x10 ⁻⁶	0.0075	1.02x10 ⁻⁵	1.45x10 ⁻⁴
Bar	10 ⁵	1	0.987	750	1.0197	14.5
Atm	1.01x10 ⁵	1.013	1	760	1.03	14.7
mmHg	133.3	1.33x10 ⁻³	1.32x10 ⁻³	1	1.36x10 ⁻³	1.93x10 ⁻²
Kg/cm ²	9.8x10 ⁴	0.98	0.97	735	1	14.22
Psi	6895	0.069	0.068	51.7	0.07	1

Pa=N/m²; Torr = mmHg ; psi= lb/inch²

Geobacillus stearothermophilus was previously *Bacillus stearothermophilus*

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.02

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

3. Procedure

3.1. Principle

The autoclave is a sealing chamber which can contain steam at more than 1.06 kg/cm^2 (15 lb/in^2). At this pressure, steam is most saturated and temperature is 121°C at sea level. The reason autoclaving is so efficient is the latent heat of steam, consisting of not only heat energy as hot air would but also the additional heat energy required to change water to steam as the steam condenses to water on objects in the autoclave.

Two factors are essential for the optimum function of an autoclave: (1) all of the air in the chamber should be replaced by steam (2) the temperature must be 121°C .

The 1.06 kg/cm^2 or 15 lb/in^2 pressure in the autoclave is shown as a comparison to atmospheric pressure by most pressure gauges and control devices. At an altitude of 1,500 m (5,000 ft) above sea level, the pressure would be reduced by 0.21 kg/cm^2 (3 lb/in^2) and the temperature would be 117.6°C although the gauge showed a 1.06 kg/cm^2 pressure. In sterilizing at high altitudes, it is best to maintain a temperature of 121°C without exceeding the pressure limits of the autoclave.

NOTE : any air remaining in the chamber will reduce the efficiency. Pans of material to be sterilized or decontaminated must have some water added to turn to steam as the pans are heated. This prevents air pockets from forming in the pans.

3.2 Samples

- instruments, glassware, media or solutions in the general diagnostic TB laboratory for sterile use
- mycobacterial cultures for waste disposal
- all infectious materials and waste from laboratories where culture is performed

3.3 Equipment and materials

Installation is according to Manufacturer's Manual instructions by the authorized engineer. Autoclaves should be installed away from the main working area as they are noisy, hot, release steam and may be a source of major hazards

Separate autoclaves should be used for sterilization of solutions or glassware (clean materials) and for decontamination of infectious materials.

- "sharp" containers consisting of specific commercially available autoclavable plastic boxes or metallic boxes

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.02

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

- Metallic containers

- Cloth (linen or cotton) or steam-permeable paper or parchment for items that must be wrapped
- Incubator for spore test: calibrated at 55-60°C, a dry block incubator is suitable for this purpose
- Chart or paper if autoclave is equipped with a recorder
- Autoclave indicator tape
- Thermoresistant gloves
- Eye protection such as safety glasses

3.4 Reagents and solutions

Heat-resistant *Geobacillus stearothermophilus* spores. See below

3.5 Detailed instructions for use

3.5.1 Check before use

The manufacturer's instructions must be followed at all times for operation and cleaning of the autoclave.

WARNING: Improper use of the autoclave can be very dangerous. It can explode if the pressure is too great, if a part is defective, or if the door or cover is opened while under pressure. The caps of containers of liquid must be left loose during autoclaving because the container may explode when the autoclave is opened if caps are tight.

3.5.2. Procedure of use of a manual autoclave

- Fill the bottom of the autoclave with distilled water, up to the autoclave basket support or to the level marked by the manufacturer. If the water reaches the basket, drain off excess water by opening the drainage tap.
- Place the autoclavable containers containing the material to be sterilized/decontaminated together with autoclave tape.
- **Do not load material to be sterilized with material to be decontaminated.**
- Do not over pack.
- Close the lid and make sure that the rubber gasket is in its groove. Screw down the clamps firmly.
- Open the air outlet valve.
- Turn on the heating.
- When a jet of steam is released from the outlet valve, wait 3 or 4 minutes until the jet of steam is uniform and continuous, indicating that all the air has been driven out of the autoclave, and close the outlet valve.
- Check and re-tighten the lid clamps.
- When the temperature falls below 80°C, open the air outlet valve **slowly** to equalize pressures inside and outside the autoclave.
- When the hissing sound stops, unscrew the lid clamps and open the lid.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.02

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

- Leave the material to cool before removing it from the autoclave.
- Check the autoclave tape has turned colour before release of the material.

3.6 Recording and reporting

Record details of the loading and of the autoclave's operation in the autoclave logbook. Incidents, accidents and/or mechanical problems must be recorded and reported to a supervisor immediately.

Most types of non-manual autoclaves have a device for monitoring temperature and air pressure during the different steps of cycles. These records have to be stored or printed with the appropriate log sheet.

3.7 Quality control

3.7.1. Chemical indicator

A visible chemical indicator, such as autoclave tape, must be used with each load to be sterilized.

Note: Temperature sensitive autoclave tape is not sufficient on its own to indicate that sterilization temperature (121 °C) reached the heart of the load

3.7.2 Biological indicator (BI)

Even if the autoclave has a paper printed monitoring system for autoclave effectiveness (temperature and air pressure), a biological indicator must be used periodically, after every 40 hours of use. The results must be documented and kept for at least one year.

- Heat-resistant *Geobacillus stearothermophilus* spores are used for autoclave efficiency testing. A spore vial is placed in a waste bag located in the center of the maximally allowable size load and exposed to 121°C for at least 15 minutes. A string can be tied around the vial to permit its easy retrieval.
- The autoclaved spore vial is incubated along with a non-autoclaved control vial at 56-60°C. The control BI should yield positive results for bacterial growth.
- BI negative growth in the autoclaved vial demonstrates proper autoclave efficiency.
- If the autoclaved vial shows positive growth the procedure is repeated with another tester vial.
- Consistent positive growth indicates a problem that must be corrected before the autoclave can continue to be used.

3.8 Safety precautions

3.8.1 Personnel protection

USE THERMORESISTANT GLOVES to protect from heat

Wear eye protection such as safety glasses or visor when removing materials from a hot autoclave (see 3.3, Equipment and Materials).

3.8.2 Maintenance

Preventive maintenance procedures for the autoclave must be carried out according to Sections 3.8.2.1 to 3.8.2.4.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.02

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Any problem or malfunction detected must be reported to the Laboratory Manager, who will contact the maintenance contractor to correct the problem/malfunction as soon as possible.

Users are NOT allowed to make any repairs. Repair and service of the autoclave must be done by a qualified service professional.

In the case of any problem or malfunction an 'Out of Service' notice must be placed on the autoclave indicating that it is not to be used until the problem has been diagnosed and corrected.

All potentially infectious materials from laboratories performing cultures must be autoclaved before disposal.

Refer to the Manufacture's manual for safe handling of the autoclave.

3.8. 2.1 Daily preventive maintenance

Check that the lid/door gasket is clean and not protruding.

Check for leaks while the autoclave is running, e.g. bubbles.

Clean the autoclave and work area after every episode of use.

Disinfect the work area as needed according to the SOP for Disinfection.

3.8.2.2 Weekly preventive maintenance

Remove and clean the drain strainer if necessary.

Register in the logbook

Check the operation of the pressure release safety valve to verify proper function. The operator must keep away from the release valve exhaust during this check to prevent a burn injury.

3.8.2.3 Monthly preventive maintenance

Inspect autoclave gaskets, lid/doors, and internal walls for residue built-up or wear.

Register in the logbook

Notify the Laboratory Manager if any deterioration is observed.

3.8.2.4 Annually preventive maintenance

All autoclaves must be inspected and certified annually by a qualified service technician. At the minimum, pressure gauges and thermometers should be tested.

The service technician must issue an inspection certificate indicating compliance with safety and proper operation. The most current inspection certificate must be posted in close proximity to the autoclave.

3.8.2.5 three-year preventive maintenance

Pressure testing and inspection must be done at three-year intervals on all pressure vessels. Test certificates must be kept on record.

4. Related documents; Manufacturer's Manual, specific to each autoclave

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.02

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

The Operation scheme illustration for the Horizontal autoclave provided at IRL by RNTCP- read the manufacturers Operation and maintenance manual for instructions before operation for safety and efficient use-High pressure steam sterilizer-Horizontal

Vacuum Dry

↓
Water inlet up to – Green Mark.

Off / Slow Exhaust

- ↓
1. Switch on M.C.B. (Main power supply).
 2. Switch on the Autoclave.
 3. Green light will glow till pressure of Boiler reaches – 17 P.S.I. & It will be off Automatically.
 4. Green light will be on when pressure decreases to 15 P.S.I.
 5. It goes on Automatically – maintaining The pressure of Boiler – 15 P.S.I. - 17 P.S.I. .
 6. Load articles and close door.

Ster

- ↓
1. Pressure and temp will increase in the chamber slowly.
 2. Sterilization starts when temp. reaches to 121°C .
 3. Maintain it for - 30 Minutes .

Vacuum dry

↓
(1) for 5 minutes.

Off / Slow Exhaust

- ↓
1. Chamber pressure will come down to – 0 - P.S.I.
- ↓
Open the Door.
(1). Remove articles.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.02

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Operation instructions (obtained from the Horizontal autoclave operation manual) of High pressure steam sterilizer-Horizontal, provided at IRLs by RNTCP:

1. Ensure proper electricity supply voltage, phase and frequency.
2. Properly earth the autoclave & control panel as per Indian standard.
3. Pre-sterilization Procedure:
 - a. Ensure that chamber try is provided inside the chamber, if not note that one side of load may become wet which leads to bacterial contamination
 - b. Always load the dressing drum circulation position. Don't keep one above the other
 - c. Keep open the sliding band of the dressing drum open for penetration of steam
 - d. Double thickness muslin cloth or brown paper wraps packaging should be made use of as a layer for the avoiding wetting of material
 - e. Don't Mix wet load with dry load
 - f. Ensure that sufficient space is provided between the packs for proper steam circulation space
 - g. Ensure that pre-sterilization procedure affixed near the autoclave
4. Daily maintenance before heating sterilizer:
 - a. Remove Plug screen from bottom of chamber and clean lint and sediment from pores
5. To fill water in Boiler:
 - a. Put operating valve on 'Vac dry' position
 - b. Close water drain valve and open water inlet valve when approximately three quarter of the boiler is filled, close water inlet valve
 - c. If too much water was been filled, then open water drain valve till water reaches the correct level
 - d. Important Note: Refer information regarding specification of water for boiler.
6. To heat boiler:
 - i. Put operating valve on 'Vac Dry' position
 - ii. Now switch ON the mains
 - iii. Switch ON the heater ON/OFF switch and know both read & green indicator will glow
 - iv. Pressure in boiler & jacket will gradually rise and will be indicated on pressure gauge
 - v. When pressure gauge indicates 17 p.s.i (1.2kg/cm²) approx. the pressure switch will cut off the heater circuit and the green indicator will be OFF. When pressure drops to 15 p.s.i (1.0kg/ cm²) approx, the heater circuit will be ON. This will go on through out the cycle.
 - vi. NOTE: Don't temper factory adjustment of the pressure switch
7. To Sterilize:
 - a. Put multiport valve in Ster Position. Air and condensate from the chamber will gradually flow out through the steam trap and simultaneously the dial thermometer will steadily indicate increased temperature

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.02

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

- b. When dial thermometer read 121°C sterilization begins and exposure period begins
 - c. The pressure lock mechanism keeps the door locked during sterilization and no attempt to turn the insulated handle or to push the pressure lock rod should be made. As the door gets locked positively, it can be tightened if desired in locked position
 - d. Note: In no case sterilization should be carried out unless temperature rises to 121°C.
 - e. Approximate Exposure period at 121°C depend upon the material being sterilized. 121°C. for 20 min is the standard.
8. Exhaust of Chamber;
- a. For solutions in flasks & bottles select multiport valve to slow exhaust/off position till compound gauge reads 0. Now select to Vac dry for only one minute and back to off position before opening door
 - b. For all other types of load, put multi port valve in fast exh. Position till compound gauge indicates zero pressure
 - c. If drying of the load is not required, put operating valve in vac dry position for about a minute only before opening pressure lock door. Remove load at once for use or storage
 - d. If drying of the load is required, put operating valve in vac dry position. Now the self-sterilized vacuum drier will permit filtered air to circulate in chamber. Put operation valve in OFF position before for use or storage. Drying time varies from 15 to 30 min or more depending on materials.
 - e. Note: safety valve are factory adjusted to open at about 18-20 psi.
9. Post sterilization procedure;
- a. After sterilization process is over, when chamber temperature falls below 70°C, take out the load & keep the drums in sterile room. Sliding band of dressing drum open for few hours, and subsequently closed.
10. Consult the manufacturers operation & trouble shooting guideline manual for further details.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.03

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

TITLE: Maintenance of the centrifuge

1. Objectives and scope

Description of optimal operation of a centrifuge through regular servicing and preventive maintenance.

2. Definitions and abbreviations:

The relative centrifugal force (RCF) is determined according to the formula

$$\text{RCF} = 1.12 \times 10^{-6} \times \mathbf{R} \times (\text{rpm})^2$$

Where RCF is in g

\mathbf{R} = radius in millimetres from the centre of the rotating head to the bottom of the spinning centrifuge tube

rpm = number of revolutions per minute

Sedimentation efficiency has to be determined in rcf (g, absolute value) and not in rpm, a measure of speed for a particular centrifuge head.

To generate a RCF of 3,000 g, the required rpm is calculated from the formula :

$$\text{rpm} = 1000 \sqrt{\frac{\text{RCF}}{1.12 \times \mathbf{R}}}$$

3. Procedure

3.1 Principle of procedure

A centrifuge is intended to separate particles in a liquid by sedimentation. Dense particles sediment first, followed by lighter particles. In a TB laboratory, centrifuges are used for the sedimentation and concentration of tubercle bacilli within liquefied sputa or body fluids (usually urine).

To obtain a high sedimentation efficiency of 95%, i.e. 95% of bacilli recovered in the sediment and only 5% of bacilli still in suspension to be discarded with the supernatant, it is necessary to maintain 3,000 g for 15-20 minutes.

The high speeds and time used to achieve effective sedimentation efficiency leads to heat build up in the centrifuge and the specimen. To prevent cell death due to over heat, the use of a refrigerated centrifuge model, operated at 18-20°C, is recommended.

Be aware that if specimens contain cetyl pyridinium chloride (CPC, see SOP 3.2), CPC crystallizes at low temperatures.

3.2 Samples:

Decontaminated and liquefied specimens, high volume effusions, watery specimens.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.03

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

3.3 Equipment and materials

Aerosol-free swing buckets. For use in a TB lab, the centrifuge must have safety aerosol-free (O-ring sealed) swing buckets that can be removed from the centrifuge and placed inside a BSC for the removal of individual centrifuge tubes. The sealed buckets protect the personnel from infectious particles in case of tube damage during centrifugation. It is advisable to use bucket covers that are transparent so that leakage can be detected before opening.

The centrifuge has to generate a 3,000 g force and should be preferably refrigerated.

Centrifuge tubes must tolerate g-forces of at least 3,000 g and be used with appropriate rubber or plastic cushions matched to the tube and bucket holder. If adaptors have to be used, use only those recommended by the manufacturer.

The centrifuge lid must have a locking mechanism for protection to prevent opening while centrifugation is still rotating.

Ensure operation of the centrifuge on a rigid, flat, level surface. Any change in the surface may influence the centrifugation process. Due to the vibration they produce, centrifuges must be kept apart from balances.

Allow sufficient free space around the centrifuge for adequate ventilation to prevent overheating.

Cloth or paper towels for cleaning and disinfection.

Balance with 0.1 g accuracy for balancing centrifuge tubes.

Tachometer for periodic calibration of centrifuge speed

3.4 Reagents and solutions

Ethanol 70% as disinfectant solution or another mild disinfectant (see SOP 2.8 and 3.0).

3.5 Detailed stepwise instructions

1. Before use, check inside of the centrifuge and the rotors to ensure that everything is dry. If there is any sign of corrosion, discontinue use until the corroded part has been repaired by a qualified service technician.

2. Check that shock absorbing pads are in the bottom of the centrifuge buckets.

3. Balance the opposing buckets by weighing them with their tubes on an open two-pan balance. Add water to an empty tube placed in the buckets to achieve final balance. **NEVER add water to a specimen to balance tubes.** Never fill centrifuge tubes to more than three-quarters

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.03

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

4. Symmetrically distribute balanced tubes in opposing buckets. Always operate the centrifuge with all buckets in place, even if two opposing buckets are empty.
5. Switch on and follow the manufacturer's instructions to set the conditions of centrifugation: 3.000 g, 15-20 minutes, 8-10°C
6. Close and lock the lid.
7. Start the centrifuge cycle.
8. While the centrifuge is reaching full speed, stand with your hand on the unit to detect excessive vibration (usually due to improper balance). If excessive vibration occurs, or if a crack is heard or tube breakage is suspected, switch off the unit
9. Open the centrifuge **only** after the signal of end of centrifugation is seen
10. Remove the sealed buckets (not tubes) slowly and carefully to prevent re-suspension of the sediments. Place the buckets inside the BSC. In the BSC, carefully open the buckets and remove the tubes. Check for tube damage before their removal from buckets.
11. Switch off the centrifuge

3.6 Reading and recording

Record conditions of centrifugation in the centrifuge logbook (see below)

3.7 Quality control and maintenance

Sediments and supernatants should be visible after centrifugation.

3.8 Initial installation

Initial calibration should be performed only by a qualified service technician.

3.8.1 Daily maintenance

Wipe the inside bowl with disinfectant solution and rinse thoroughly.

For a refrigerated centrifuge that is turned off at night, open the top to allow the bowl to dry. During the day when the unit is under refrigeration, leave the top closed to avoid condensation and ice buildup.

The centrifuge must not be used if the interior is hot, if unusual vibrations or noises occur, or if deterioration (corrosion of parts) is detected. A qualified service technician should be contacted.

Most vibrations are due to improper balancing and can be corrected by re-balancing the buckets and tubes.

3.8.2 Monthly maintenance

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.03

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Clean the centrifuge housing, rotor chamber, rotors and rotor accessories with a neutral cleaning agent.

Clean plastic and non-metal parts with a fresh solution of 5% sodium hypochlorite (bleach) mixed 1:10 with water (one part bleach plus nine parts water).

3.8.3 Annual maintenance

Service is to be performed by a qualified technician. The service technician must ensure that the unit operates safely and properly. This would include cleaning condenser coils, fans, screens, filters and checking the centrifuge brushes, bearings, timer, temperature, speed and for electrical integrity.

3.9. Waste management

Tubes broken during centrifugation must be discarded immediately. Put in a metallic container and autoclave. Label "broken glass" if necessary.

Clean metal bowls and parts with 70% ethanol and 5% bleach for plastic ones. Do not use bleach for metal parts as it causes corrosion.

4. Related documents

Manufacturer's Manual, specific to each centrifuge

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.03

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Specific Instructions for use of L 600 A Table-top low speed large capacity centrifuge provided by RNTCP to IRLs:

Model and name: L 600 A Table-top low speed large capacity centrifuge:

Rotor type 3 with adapter for 12X50ml tubes should would give maximum RCF of 3640, when fully loaded.

Operation Procedure:

1. Consult manufacturer's operational manual for full instructions and details of maintenance.
2. Ensure to place the centrifuge on a balanced vibration /shake-free platform.
3. Plug in power and press the power switch. The switch is located in the back of centrifuge.
4. Press "STOP" key, open the door (If no power supply, then draw the string in front of centrifuge for opening the door).
5. Put the MC bottles/Falcon tubes (in even numbers and balanced) into the rotor, and set them in balance.
6. Ensure the cap of the tubes is tightly screwed.
7. Rotate the Centrifuge rotor with hand, to check if it works smoothly
8. Close of door of centrifuge and conduct manual check if it is locked properly.
9. Set the speed and time accordingly to requirements.
10. AT stop position, you can "SET" rotor type, time and speed. The Stop indicator should be ON, and when centrifuge is in running stage the stop indicator should be flashing.
 - a. Set Rotor: Press the "SET" key when centrifuge is in status of stop and then press "up arrow" or "down arrow" indicator to set rotor. Rotor provided is No.3
 - b. Speed set: Press the "SET" Key and then press the "up arrow" or "down arrow" indicator to set the desired speed for operation.
 - c. Acc/Dec set: Press the "SET" key and then press the "up arrow" or "down arrow" to set acceleration or declaration of rotor speed. Recommended: Acc= A5-A6; Dec= d6-d7.
 - d. Time set: Press the "SET" key and the press the the "up arrow" or "down arrow" to set time (max time set is 99 min.)
11. After completing above four steps, press "ENTER" to confirm the set of speed and time, and then "START" to operate the centrifuge. Note: If it does not stop, check the firm closing of door lid.
12. Press "RCF" key to check the RCF value for corresponding of rpm.
13. When timer times off, to "0", centrifuge will stop working automatically; when the speed is 0 rpm, the buzzer will tweet 15 times, Press "STOP" in running status, centrifuge will stop working and buzzer will not tweet.
14. When rotor is not spinning, the door will open automatically. Take off the tubes into a strand and transfer to a BSC. If the door cannot be opened, please draw the sting under the centrifuge, to open manually.
15. Keep the centrifuge door open when not in Use.
16. Turn of the power and unplug the cable.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.03

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Important Notes:

- a. Go through operation manual of the instrument before operation.
- b. Use appropriate power and keep grounded/earthed outlet.
- c. Don't open the centrifuge lid during the operation. Don't place any material on the top of centrifuge obstructing the opening.
- d. Use balanced tubes in the rotor to even. Always firmly screw the aerosol protective lids.
- e. Always open the centrifuge buckets inside the BSC.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.03

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Centrifuge Log-sheet

Operator's Name	Date	Time	RCF (G)	RPM (rpm)	Time	Temp	Remarks, if any	Sign

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.03

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

MAINTENANCE FORM:

ITEM IDENTIFICATION			
Equipment	CENTRIFUGE	Brand name	
Purchase date		Model/type	
Location within the lab		Serial No	
Warranty expiration date			

Manufacturer			
Address			
Tel		Contact person	
Technical service representative		Tel	

PERIODICITY :		
Date	Maintenance operation	Operator

FAILURE EVENTS			
Date	Event	Corrective action taken	Operator

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.04

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

TITLE: Maintenance of the incubator

1. Objective and Scope

The incubator is intended for culture of *Mycobacterium tuberculosis* by ensuring optimum growth conditions. Incubators are available in various sizes, from small (on the bench), to large incubator rooms, with circulating fans to obtain a homogeneous temperature.

2. Definitions and abbreviations

°C Degrees Celsius.

UPS Uninterrupted power supply.

3. Procedure

3.1 Principle

Optimal operation of the incubator is achieved through proper installation, regular monitoring and maintenance.

Since *M. tuberculosis* growth is inhibited above 37°C, the incubator should be set at 36°C± 1°C.

3.2 Samples

Mycobacterial cultures.

3.3 Equipment and materials

Incubator with proper electrical system should be installed:

- according to the instructions in the Manufacturer's Manual, specific to each incubator,
- in a well-ventilated dry space, on a level surface, away from heat sources.

Follow the Manufacturer's Manual, specific to each incubator, for installing and operating the incubator.

Locate incubator in a well-ventilated dry space, on a level surface, away from heat sources.

3.4. Reagents and solution

70% ethanol.

3.5. Detailed procedures for use and disinfection

3.5.1. General

Keep door(s) closed to prevent heat loss to the environment.

Ensure that rack positions are clearly marked.

The incubator should be switched off when not in use.

Do not overload.

3.5.2. Procedure of disinfection

Every 6 months or in case of spillage of infectious material within the incubator:

Disconnect the plug from the main socket.

Identify adequate volume of available safe space for temporary storage of bio-hazardous material.

Ensure that materials kept in the incubator are clearly marked.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.04

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Clean metallic surfaces (racks, floor, walls and doors) with 70% ethanol.
Clean materials located in the incubator which may have been contaminated by spillage.
Connect the incubator to the main power supply and switch on.

Relocate incubator contents once the temperature has reached 36°C.
Notify owners of contents of relocation.

3.6 Reading and reporting

Use log sheet for daily record of the temperature.
Register regular maintenance on the maintenance card.

3.7 Quality control / Maintenance

Record temperature at least once daily and document in the Incubator logbook.

Perform monthly temperature spot checks by placing (max/min) thermometers at various positions in the incubator to verify that the internal incubator temperature is constant at $36 \pm 1^\circ\text{C}$. Repairs should be performed by a qualified service technician.

Note: There are inexpensive electronic mini/max thermometers available, which give a better overview of the temperature variation

Calibration

The temperature should be calibrated:

- Prior to use.
- After temperature changes have been detected and corrected.
- Following a power failure.
- After cleaning of spillages.

Calibration process

Ensure that the door is closed and that the incubator is switched on.
Set the required temperature using the temperature control and leave the incubator to run for 1 hour.
Place a thermometer into the centre of the incubator with probe away from the heating element.
Take the temperature reading after 30 min. If the temperature is not $36^\circ\text{C} \pm 1^\circ\text{C}$, adjust the control.
Repeat process every 30 minutes until the required temperature is reached.
Continue to take readings until two consecutive readings (30 minutes apart) are $36^\circ\text{C} \pm 1^\circ\text{C}$.
Record readings in the Incubator Log book. The incubator is ready for use at the calibrated temperature only.

3.8 Waste management

Cultures are autoclaved for disposal.

4. Related documents

Manufacturer's Manual, specific to each incubator.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.04

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

ITEM IDENTIFICATION

Equipment	INCUBATOR	Brand name	
Purchase date		Model/type	
Location within the lab		Serial No	
Warranty expiration date			

Manufacturer	
Address	
Tel	Contact person
Technical service representative	Tel

TEMPERATURE REQUIRED: 36°C +/- 2°C (variation acceptable)

FAILURE EVENTS

Date	Event	Corrective action taken	Operator

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.04

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

MAINTENANCE FORM:

Disinfection date	
Disinfection reason : specify (regular, spillage)	
Operator's name	
Calibration date	
Calibration reason (temperature correction, power failure, disinfection)	
Operator's name	

Disinfection date	
Disinfection reason : specify (regular, spillage)	
Operator's name	
Calibration date	
Calibration reason (temperature correction, power failure, disinfection)	
Operator's name	

Disinfection date	
Disinfection reason : specify (regular, spillage)	
Operator's name	
Calibration date	
Calibration reason (temperature correction, power failure, disinfection)	
Operator's name	

Disinfection date	
Disinfection reason : specify (regular, spillage)	
Operator's name	
Calibration date	
Calibration reason (temperature correction, power failure, disinfection)	
Operator's name	

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.04

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

TEMPERATURE REQUIRED : 36°C +/- 1°C (variation acceptable)

Incubator Log Sheet

Trimester :			Year:					
<i>Month</i>	Temp.	Visa	<i>Month</i>	Temp.	Visa	<i>Month</i>	Temp.	Visa
	°C	(Initials)		°C	(Initials)		°C	(Initials)
1			1			1		
2			2			2		
3			3			3		
4			4			4		
5			5			5		
6			6			6		
7			7			7		
8			8			8		
9			9			9		
10			10			10		
11			11			11		
12			12			12		
13			13			13		
14			14			14		
15			15			15		
16			16			16		
17			17			17		
18			18			18		
19			19			19		
20			20			20		
21			21			21		
22			22			22		
23			23			23		
24			24			24		
25			25			25		
26			26			26		
27			27			27		
28			28			28		
29			29			29		
30			30			30		
31			31			31		

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 09.05

Author(s): Lab-Microbiologist & lab staff

TITLE: Maintenance of the refrigerator

1. Objective and Scope

The fridge is intended for ensuring an environment for material that requires preservation at 2-8°C.

This equipment can be placed in a clean or an infectious area of the laboratory.

2. Definitions and abbreviations

°C Degrees Celsius

NA Not applicable

3. Procedure

3.1 Principle

Optimal operation of the fridge is achieved through proper installation and maintenance (defrosting and cleaning)

3.2 Samples

3.2.1 Fridge placed in a clean area of the laboratory

Culture media prepared for use

Antibiotics which require cold storage at +2-8°C, preferentially in a desiccator.

Reagents/solutions to be stored at +2-8°C.

Never store flammable solutions in a fridge that is not approved and certified for this purpose.

3.2.2 Fridge placed in an infectious area of the laboratory

Separate fridges should always be used to store mycobacterial cultures away from other type of potentially infectious samples such as the specimens to be examined.

3.3 Equipment and materials

3.3.1. Fridge with proper electrical isolation, installed according to the following:

Leave the fridge for some hours in the upright position after transportation according to producer's recommendations before connecting.

Follow the Manufacturer's Manual, specific to each fridge, for installing and operating the fridge.

Do not install fridge close to heat sources.

Locate fridge in a well-ventilated dry space.

Ensure that fridge is placed on a level surface.

3.3.2. Materials. N/A

3.4. Reagents and solution N/A

3.5 Procedure of cleaning and defrosting

3.5.1. Cleaning

Spillages should be wiped up immediately and cleaning should be proceeded.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.05

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

- Identify temperature sensitive reagents/solutions which need to be relocated during cleaning (e.g. antibiotic solutions). Other reagent solutions which have to be stored at +2-8°C for extended life term may stand for a while at room temperature. Similarly, mycobacterial cultures stored in the fridge may stand at room temperature with no viability reduction.
- Consider safety issues for the temporary space used for biological materials while defrosting.
- Identify adequate volume of available space in another fridge.
- Notify personnel staff of contents of relocation.
- Note location of relocated contents in fridge logbook.

Disconnect the plug from the main socket.

Note: Turning the thermostat to "0" does not switch the power off.

- Never use abrasives.
- Clean the interior of the fridge with a damp sponge soaked in cleaning solution (e.g. soap and water).
- Dry with paper towels or a soft cloth.
- Clean the outside of the fridge with a sponge dampened in cleaning solution.
- Dry with a soft cloth.
- Connect the fridge to the main power supply and switch on.
- Relocate fridge contents once the temperature has reached 0-4°C.
- Notify personnel staff of contents of relocation.
- Note location of relocated contents in fridge logbook.

3.5.2. Defrosting

- Ensure that materials kept in the fridge are clearly marked.
- Identify temperature sensitive reagents/solutions which need to be relocated during defrosting (see sample listing). Other items such as solutions/mycobacterial cultures/specimens may stand at room temperature during the time of defrosting process.
- Consider safety issues for the temporary space used for biological materials (mycobacterial cultures, specimens.) while defrosting
- Identify adequate volume of available space in another fridge

Disconnect the plug from the main socket.

Note: Turning the thermostat to "0" does not switch the power off.

- Open fridge door and leave open.
- Never use a sharp object to chip the ice during the defrosting process.
- Remove the drain cap from the low temperature compartment, if any.
- Place another container under the drain hole to catch the defrosted water.
- Sponge up any defrosted water.
- Once all the ice has been melted, wash with a sponge and cleaning solution.
- Dry the internal compartment using paper towels/soft cloth.
- Never use abrasives.
- Dry with a soft cloth.
- Replace the drain cap, if necessary.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.05

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Connect the fridge to the main power supply and switch on.
Relocate fridge contents once the temperature has reached +2-8°C.
Do not over pack.
The whole process should be performed in few hours.

3.6 Reading and reporting

Use log sheet for daily record of the temperature.
Register regular maintenance on the maintenance card.

3.7. Maintenance

3.7.1 Daily

Check the compressor for unusual sound or overheating.

3.7.2 Monthly

Clean filters and screens at the ventilator system with a brush or vacuum cleaner

3.7.3 Every six months

Clean fridge according to Section 3.5.1. Perform this maintenance more frequently if required, specifically in the event of leakage of biological materials onto the internal surface of the fridge.

Defrost fridge according to Section 3.5.2 when the ice build-up on the internal wall reaches 3 mm in thickness.

Clean the condenser coils and fan with a brush or vacuum cleaner.

Check the compressor for unusual sound or overheating. Alert the technical service for maintenance, even though the equipment is still performing.

Repairs should be performed by a qualified service technician.

3.8 Waste management

Fridge contents may ONLY be disposed off with prior consent by the Laboratory Manager.
Disposal details must be noted in the fridge logbook.

4. Related documents

Manufacturer's Manual, specific to each fridge.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.05

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

TEMPERATURE REQUIRED : +2-8°C (variation acceptable)

ITEM IDENTIFICATION

Equipment	FRIDGE	Brand name	
Purchase date		Model/type	
Location within the lab		Serial No	
Warranty expiration date			

Manufacturer	
Address	
Tel	Contact person
Technical service representative	Tel

Maintenance card

PERIODICITY : Every six months or when needed

Date	Maintenance operation	Operator
	Cleaning: Defrosting: Relocation: What/Where: What/Where: What/Where: Refilling:	

Remark:

Date	Maintenance operation	Operator
	Cleaning: Defrosting: Relocation: What/Where: What/Where: What/Where: Refilling:	

Remark:

Date	Maintenance operation	Operator
	Cleaning: Defrosting: Relocation: What/Where: What/Where: What/Where: Refilling:	

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.05

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Remark:

Date	Maintenance operation	Operator
	Cleaning: Defrosting: Relocation: What/Where: What/Where: What/Where: Refilling:	
Remark:		

FAILURE EVENTS

Date	Event	Corrective action taken	Operator

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.05

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Temperature record log sheet

Equipment : FRIDGE	Reference :
Location :	Installation date :

TEMPERATURE REQUIRED: + 6°C ± 2°C (variation acceptable)

Trimester:			Year:					
<i>Month</i>	Temp.	Visa	<i>Month</i>	Temp.	Visa	<i>Month</i>	Temp.	Visa
	°C	(Initials)		°C	(Initials)		°C	(Initials)
1			1			1		
2			2			2		
3			3			3		
4			4			4		
5			5			5		
6			6			6		
7			7			7		
8			8			8		
9			9			9		
10			10			10		
11			11			11		
12			12			12		
13			13			13		
14			14			14		
15			15			15		
16			16			16		
17			17			17		
18			18			18		
19			19			19		
20			20			20		
21			21			21		
22			22			22		
23			23			23		
24			24			24		
25			25			25		
26			26			26		
27			27			27		
28			28			28		
29			29			29		
30			30			30		
31			31			31		

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 09.06

Author(s): Lab-Microbiologist & lab staff

TITLE: Maintenance of the Balance

1. Objectives and scope

A laboratory balance is used to measure a mass to a high degree of precision. There are many types of laboratory balances however; this procedure will focus on electromagnetic balances.

2. Definitions and abbreviations:

Sensitivity: corresponds to the smallest mass that the balance measures accurately

NA: Not applicable

3. Procedure

3.1 Principle of procedure

Electromagnetic balances are fragile and precision instruments intended for accurate weighing of chemicals in the routine TB diagnostic laboratory. These are analytical balances, their maximum weighing capacity and sensitivity can be of 61 g and 0.01 mg. For other purposes, such as balancing loads for the centrifuge use an open two-pan balance (sensitivity of 0.5 g, and capacity of up to several kilograms). Use a balance with an appropriate sensitivity to the desired weight.

Optimal operation of the balance is achieved through regular calibration, verification and proper maintenance. Usually, initial calibration and verification services are provided by the manufacturer (and a calibration certificate update).

3.2 Samples: NA

3.3 Equipment and materials

- Balance should be installed on a solid, vibration-free surface, away from direct sunlight.
- Balance must be placed in a precisely horizontal position, checked with a spirit level.
- The levelling-foot of the balance is turned to correct the air bubble position in the centre of the spirit level
- Protect the balance from drafts of air. Air moving across the pans will cause an inaccurate reading.
- Always keep the balance and weights clean and dry to protect them from corrosion.
- Appropriate milligram/gram weights for regular control.

3.4 Reagents and solutions

Silica blue desiccant

3.5 Detailed stepwise instructions

1. Check whether the sensitivity of the balance corresponds to the amount of material to be weighed
2. The balance must be zeroed before use

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.06

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

3. Put material to be weighed in a container or on weighing paper, **never** directly on the pan
4. Determine the mass of the weighing container prior to filling
5. Place the sample to be weighed in the container, or weighing paper in the middle of the pan to avoid corner-load error
6. Do not return unused substance to the stock bottle to prevent contamination of stock materials.
7. Clean the balance with a soft, clean brush after use. Refer to manufacturer's manual for other instructions on cleaning. Balance pans and working area can be disinfected with 70% ethanol.
8. Always keep the work area clean.
9. Keep the balance under an airtight plastic cover when not in use for protection against dust, together with a dish filled with blue silica to dry the air under the cover for prevention of humidity. (When turned to red, the silica has to be regenerated by heating)

3.6 Reading and recording

Determination of the weight is directly read on the screen of the balance.

3.7 Quality control

3.7.1 Daily

Check with an appropriate milligram/gram weight that the weight reported by the balance agrees with counterbalance weight on each day the balance is used and register this in the logbook.

3.7.2 Annually

Service and calibration of the balance should be done annually by a qualified service technician and registered in the log book.

Repair of the balance should be done by a qualified service technician.

3.8 Waste management: N/A

4. Related documents

Manufacturer's Manual, specific to each balance

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.06

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

ITEM IDENTIFICATION

Equipment	BALANCE	Brand name	
Purchase date		Model/type	
Location within the lab		Serial No	
Warranty expiration date			

Manufacturer	
Address .	
Tel	Contact person
Technical service representative	Tel

MAINTENANCE FORM:

PERIODICITY :

Date	Maintenance operation	Operator

FAILURE EVENTS

Date	Event	Corrective action taken	Operator

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.07

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

TITLE: Maintenance of the freezer

1. Objective and Scope

The freezer is intended for ensuring an environment for material that requires preservation at <-18°C.

This SOP relates to equipment in an infectious area of the laboratory.

2. Definitions and abbreviations

°C Degrees Celsius

NA Not applicable

UPS Uninterrupted power supply.

3. Procedure

3.1 Principle

Optimal operation of the freezer (or deep freezer) is achieved through proper installation and maintenance (regular defrosting and cleaning)

3.2 Samples

1. Mycobacterial cultures and specimens stored for specific purposes.
2. Specific reagents and solutions in case of drug-susceptibility testing.
3. Never store flammable solutions in a freezer that is not approved and certified for this purpose. Use separate freezers for clean and infectious materials.

3.3 Equipment and materials

3.3.1. Freezer with proper electrical isolation, installed according to the following:

- Leave the freezer for some hours in the upright position after transportation according to producers' recommendations before connecting.
- Follow the Manufacturer's Manual, specific to each freezer, for installing and operating the freezer.
- Do not install freezer close to heat sources.
- Locate freezer in a well-ventilated dry space.
- Ensure that freezer is placed on a level surface.
- To prevent loss of valuable cultures, chemicals etc. in case of a power failure, the freezer should preferably be connected to a suitable UPS system.

Frost-free freezers (ones with an automatic defrost cycle) are not suitable for the storage of cultures or antibiotics.

3.3.2. Materials N/A

3.4. Reagents and solution N/A

3.5. Procedure of defrosting and cleaning

3.5.1 Defrosting

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.07

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Identify adequate volume of available space in another freezer

Ensure that materials kept in the freezer are clearly marked.

Disconnect the plug from the main socket.

- Open freezer door and leave open.
- Place a container at the bottom to catch the defrosted water.
- Never use (sharp) tools to break of the ice.
- Sponge up any defrosted water.

3.5.2. Cleaning

- Clean the interior of the freezer with a damp sponge soaked in cleaning solution (e.g. water and soap).
- Dry with paper towels or a soft cloth.
- Clean the outside of the freezer with cleaning solution.
- Dry with a soft cloth.

Connect the freezer to the main power supply and switch on.

- Relocate freezer contents once the temperature has reached -18°C.
- Do not over pack.
- Notify personnel staff of contents of relocation.
- Note location of relocated contents in freezer logbook.

3.6 Reading and reporting

- Use log sheet for daily record of the temperature.
- Register regular maintenance on a maintenance card.

3.7 Maintenance

3.7.1 Daily

Check the compressor for unusual sound or overheating.

3.7.2 Monthly

Clean filters and screens at the ventilator system regularly with a brush or vacuum cleaner

3.7.3 Every six months

Defrost and clean freezer according to section 3.5 Perform this maintenance more frequently if required, specifically in the event of leakage of biological materials onto the internal surface of the freezer or when the ice build-up on the internal wall reaches 5-6 mm in thickness.

- Clean the condenser coils and fan with a brush or vacuum cleaner.
- Check the periods for running and resting of the compressor.
- Alert the technical service for maintenance, even though the equipment is still performing.

Repairs should be performed by a qualified service technician.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 09.07

Author(s): Lab-Microbiologist & lab staff

3.8. Waste management

Freezer contents may **ONLY** be disposed off with prior consent by the Laboratory Manager. Disposal details must be noted in the freezer logbook.

Infectious cultures or contaminated materials must be autoclaved before discarding.

4. Related documents

Manufacturer's Manual, specific to each freezer.

<i>ITEM IDENTIFICATION</i>			
Equipment	FREEZER	Brand name	
Purchase date		Model/type	
Location within the lab		Serial No	
Warranty expiration date			

Manufacturer	
Address	
Tel	Contact person
Technical service representative	Tel

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.07

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Maintenance card

PERIODICITY : Every six months or when needed

Date	Maintenance operation	Operator
	Defrosting: Relocation: What/Where: What/Where: What/Where: Refilling:	

Remark:

Date	Maintenance operation	Operator
	Defrosting: Relocation: What/Where: What/Where: What/Where: Refilling:	

Remark:

Date	Maintenance operation	Operator
	Defrosting: Relocation: What/Where: What/Where: What/Where: Refilling:	

Remark:

FAILURE EVENTS

Date	Event	Corrective action taken	Operator

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.07

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Temperature record log sheet

Equipment : FREEZER	Reference :
Location :	Installation date :

TEMPERATURE REQUIRED :	-18°C ± 2°C (variation acceptable)
-------------------------------	------------------------------------

Trimester :			Year :					
<i>Month</i>	Temp.	Visa	<i>Month</i>	Temp.	Visa	<i>Month</i>	Temp.	Visa
	°C	(Initials)		°C	(Initials)		°C	(Initials)
1			1			1		
2			2			2		
3			3			3		
4			4			4		
5			5			5		
6			6			6		
7			7			7		
8			8			8		
9			9			9		
10			10			10		
11			11			11		
12			12			12		
13			13			13		
14			14			14		
15			15			15		
16			16			16		
17			17			17		
18			18			18		
19			19			19		
20			20			20		
21			21			21		
22			22			22		
23			23			23		
24			24			24		
25			25			25		
26			26			26		
27			27			27		
28			28			28		
29			29			29		
30			30			30		
31			31			31		

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 09.08

Author(s): Lab-Microbiologist & lab staff

TITLE: Maintenance of the pH meter

1. Objectives and scope

The pH meter is intended for accurate pH measurements of buffer solutions and/or culture media solution used in the general diagnostic TB laboratory.

2. Definitions and abbreviations:

NA: Not applicable

pH: negative \log_{10} of the H^+ concentration

3. Procedure

3.1 Principle of procedure

Optimal operation of the pH meter is achieved through regular, two-point calibration (standards at pH 4 and pH 7) and proper probe storage.

3.2 Samples: N/A

3.3 Equipment and materials

pH meter with electrodes well maintained within shelf-life, with an automatic temperature compensation probe if room temperature cannot be maintained around 20°C.

3.4 Reagents and solutions

Standard buffers may be commercially available, within shelf-life, or prepared on site.

- Reference buffer at pH 4

For preparation at 20°C

Dissolve 0.6 g of acetic acid (CH_3COOH , MW = 60.05) in 900 ml of pure water.

Add 8.88 g NaCl.

Titrate to pH 4 at the lab temperature of 20°C with monovalent strong base (NaOH) or acid (HCl) as needed.

Make up volume to 1000 ml with pure water

Aliquot in tubes convenient for pH testing

Expiration date is one month from date of preparation

- Reference buffer at pH 7

For preparation at 20°C and use at 37°C

Dissolve 1.2 g of NaH_2PO_4 (sodium dihydrogen phosphate, MW = 120) in 900 ml of pure water.

Add 7.68 g NaCl.

Titrate to pH 7 at the lab temperature of 20°C with monovalent strong base (NaOH) or acid (HCl) as needed.

Make up volume to 1000 ml with pure water

Aliquot in tubes convenient for pH testing

Expiration date is one month from date of preparation

- Reference buffer at pH 8

- For preparation at 20°C and use at 37°C

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.08

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

- Dissolve 1.2 g of NaH_2PO_4 (sodium dihydrogen phosphate, MW = 120) in 900 ml of pure water.
- Add 7.31 g NaCl.
- Titrate to pH 8 at the lab temperature of 20°C with monovalent strong base (NaOH) or acid (HCl) as needed.
- Make up volume to 1000 ml with pure water
- Aliquot in tubes convenient for pH testing
- Expiration date is one month from date of preparation

For preparation of reference buffers always use chemicals of highest grade, pure autoclaved water and sterile glassware.

Buffers are kept away from sunlight, at ambient temperature.

3.5 Detailed stepwise instructions

3.5.1 Calibration

Calibration must be done according to the manufacturer's instructions.

Calibration has to be performed once daily or, in case of infrequent use, at least on the day of pH testing. It must be performed *prior* to the first measurement of the day.

Temperature variation effects pH measurement. Therefore, calibration must be done at the same temperature $\pm 2^\circ\text{C}$ as the sample to be tested.

Select two buffer solutions for calibration within ± 3 pH units of the solution to be tested. Discard contaminated or cloudy standard buffers.

Calibration results are acceptable if the pH of the buffer solution is within 0.1 pH units of the expected pH.

3.5.2. pH measurement

Prior to use, rinse the probe with deionised water and blot dry with a soft clean paper towel. Transfer to the test solution.

Compensate for the temperature if necessary

Read the pH when the reading is stable (5-20 seconds after insertion of the electrodes within the solution)

Rinse the electrode with deionised water and store according to the manufacturer's instructions.

3.5.3 Cleaning and maintenance

- Clean the pH meter with a soft, damp clean paper towel after use. No solvents should be used.

- Replace the electrode filling solution on a regular basis, according to the manufacturer's instructions.

- Repair and service of the pH meter should be done by a qualified service technician.

3.6 Reading and recording

Calibration results should be recorded on the pH calibration sheet. The records should be kept on file for a minimum of one year, after which it may be archived.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 09.08

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

Record the pH reading of the pH solution on the same sheet with the calibration results of the day.

3.7 Quality control /Maintenance

Calibration consists of QC.

Discard contaminated or cloudy standard buffers.

Calibration results are acceptable if the pH of the reference buffer solutions are within 0.1 pH units of the expected pH.

3.8 Waste management N/A

4. Related documents

Manufacturer's Manual, specific to each pH meter.

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>
 Date: 1/04/2009

Procedure No. 09.08
 Author(s): Lab-Microbiologist & lab staff

ITEM IDENTIFICATION

Equipment	pH meter	Brand name	
Purchase date		Model/type	
Location within the lab		Serial No	
Warranty expiration date			

Manufacturer		
Address		
Tel		Contact person
Technical service representative		Tel

Calibration and recording log sheet

Date	Temp °C	Electrode serial no	Calibration solution			Signature	Solution test		
			pH 4	pH 7	pH 8*		Solution/ buffer	pH read	Signature

*or pH 10 if bought (pH 10 can not be prepared easily onsite)

FAILURE EVENTS

Date	Event	Corrective action taken	Operator

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Date: 1/04/2009

Procedure No. 10.0

Author(s): Lab-Microbiologist & lab staff

10. Washing of Glass-ware

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 10.0

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

TITLE: Washing of Glassware

1. Objective and scope:

To describe Washing procedure for glassware. This document contains the processes that are required for preparation of glass wares and similar such material necessary for TB Laboratory.

2. Definitions and abbreviations:

McCartney bottles: 1 oz capacity screw capped glass bottles (28ml capacity) used for culturing of TB bacteria.

3. Procedure

3.1. Material required

- (a) Source of hot and cold water, sink and draining board
- (b) Detergent powder and Detergent solution.
- (c) Cleaning Powder.
- (d) Containers for distilled water and 5% hydrochloric acid.
- (e) Continuous supply of distilled water.
- (f) Washing brushes of various sizes for Test tubes, conical flasks, McCartney bottles and Measuring Cylinders.
- (g) Discard bucket with lid.
- (h) Large size wire baskets, for bottles and small size baskets for test tubes.
- (i) Acid-resistant gloves
- (j) Washing sink.
- (k) Adequate bench space.
- (l) 5% Hydrochloric acid (HCl) solution:

Concentrated hydrochloric acid	5 ml
Distilled water make up to	100 ml

Slowly and carefully add acid to the water contained in a heat resistant flask. The amount of 5% Hydrochloric acid required for soaking the glassware like McCartney bottles will have to be estimated. The container should be acid proof. This acid should be replaced once a week or depending upon the use.

3.2 Washing of Glassware:

1. Soak the glass-ware in hot 1% liquid detergent in a bucket for 2 hours, or overnight.
2. Clean the articles with detergent solution using a brush.
3. Clean the articles in running tap water using a brush and allowing the water to drain.
4. Immerse them in 5%HCl solution for at least 2 hours, or preferably overnight. This is done to remove any trace of alkali left in the bottle.
5. Remove of the glassware from the hydrochloric acid and then washing in tap water.
6. Rinse/soaking the articles in distilled water or demineralised water twice each time for at least 15 minutes.
7. Dry them inverted in the wire baskets

3.3 Washing of McCartney bottles (universal bottles) containing Media:

1. Autoclave them at 121°C for 30 minutes,

Standard Operating Procedures (SOPs) For Tuberculosis Lab

Lab: IRL <<name & place>>

Procedure No. 10.0

Date: 1/04/2009

Author(s): Lab-Microbiologist & lab staff

2. Remove the caps
3. Remove the media by using surgical bottle washing machine or manually by vigorously using the brush
4. Place the bottles in a wire basket and immerse them in boiling water containing 50gms/ of sodium carbonate powder per 20 lit and leave it for 45 minutes.
5. Use soap water and test tube brush to clean the bottles and wash them in running tap water,
6. Immerse them in 5 % HCl for at least 2 hours or overnight,
7. Wash again in running tap water and keep them immersed in distilled water for 5 to 10 minutes.
8. Dry glass-ware inverted in the wire baskets

3.4 Washing Metal caps of McCartney bottles (universal containers):

1. Remove the rubber liners
2. Remove the numbers written on the caps with acetone
3. Check the liners, wash in soap water and then in running tap water before replacing them in the caps if they are in good condition

3.5 Bijou bottles (5ml capacity) are treated in the same way as it is mentioned above.

4. Safety Conditions:

All safety guidelines are to be followed throughout, including bio-safety, chemical safety and disposal guidelines.

Note: All articles discarded in the laboratory excepting those from media preparation room should be considered as infectious and sterilized before proceeding for washing. The lids discarded culture slopes and sputum cups should be loosened slightly before putting them into the autoclave for sterilization. All containers into which infectious material are discarded should be sterilized before washing. It is advised that the sputum cups and culture slopes are sterilized separately and not mixed up with the containers.

Laboratory Performance Indicators for Mycobacterial Culture and Drug Susceptibility Testing

Introduction

Under RNTCP, a large number of culture and drug susceptibility testing (DST) laboratories will eventually be available, and maintenance of this laboratory network will be a growing challenge. National reference laboratories (NRLs) and intermediate reference laboratories (IRLs) are also expected to provide culture and DST quality assurance for accredited culture and DST laboratories in public sector, private sector, and in medical colleges. This quality assurance is an ongoing activity. Systematic assessment of laboratory quality should be built into the system, that can guide supervisors and facilitate monitoring by reference laboratories. Culture and DST lab staff should understand that their service quality will be subject to ongoing scrutiny, in the form of quarterly reports and an annual round of proficiency evaluation.

These Standard Laboratory Performance Indicators for Mycobacterial Culture and DST can help NRL's maintain organized and standardized measures to assess laboratory quality and changes in quality over time, and allow them to focus and guide their supervision activities.

How to use these indicators

- Each accredited laboratory is responsible for reporting on a **quarterly** basis.
- Laboratories should **report numbers** for numerators and denominators, and percents where indicated.
- Most indicators refer to **all specimens processed in a quarter**,
- Suggested **data sources are listed** for each indicator. Standard RNTCP registers and formats should be used for day to day work, and the monthly abstracts used to summarize activities. These can be found in the "Manual of Standard Operating Procedures for Intermediate Reference Laboratory for Tuberculosis". Otherwise, existing registers & forms may have to be modified to collect the information.
- **Final data collection for reporting should begin 2 months after the end of the quarter**, to allow for diagnostic cultures inoculated from the previous quarter to be finalized.
- If the laboratory cannot "diagnostic" and "follow-up" specimens from existing records then, then revised registers and forms are required to allow that distinction. Till those are implemented, specimens submitted for culture and DST can be considered as "diagnostic" and those submitted for culture only can be considered as "follow-up".
- Those laboratories performing culture only can ignore DST specific indicators.
- **Reporting format should be submitted to NRL & CTD by the end of the following quarter.**
 - Example: 1st quarter report should be started on 1st June, and submitted by 30st June.
 - Softcopies should be emailed to: labreport@rntcp.org
 - Hardcopies should be maintained for NRL/CTD visits.

Acknowledgements:

Adopted from McCarthy K, Metchok B, Kanphukiew A, et al. Monitoring the performance of mycobacterial laboratories: a proposal for standardized indicators. Int J Tuberc Lung Dis 2008; 12(9):1015-1020.

Summary of Standard Laboratory Performance Indicators for Mycobacterial Culture & DST

Indicator	Numerator	Denominator	Expected Data sources	Frequency	Relevant activity evaluated by indicator
(1) The percentage of specimens received within 7 days of sputum collection	Number of sputum specimens received within 7 days of collection	Total number of sputum specimens received	Specimen register	Quarterly	Specimen transportation
(2) The percentage of all cultures reported as <i>Mtb.</i> complex	Number of specimens (all) inoculated in one quarter reported as <i>Mtb</i> complex	Total number of specimens (all) inoculated in one quarter	Culture register	Quarterly	Workload
(3) The correlation between positive smears and positive cultures in smear-positive diagnostic specimens	No. of smear-positive <u>diagnostic specimens</u> inoculated in one quarter that were culture-positive for MTB or NTM	No. of smear positive <u>diagnostic specimens</u> submitted for culture inoculated in one quarter	Culture register	Quarterly	Specimen quality, processing, decontamination
(4) The percentage of all cultures contaminated (If liquid culture or other culture system used, reported separately for each culture system)	Number of <u>specimens (all)</u> inoculated in one quarter discarded due to contamination (both slants cont)	Total number of <u>specimens (all)</u> inoculated in one quarter:	Culture register	Quarterly	Specimens quality and decontamination
(5) The percentage of all cultures reported as non-tuberculous mycobacterium (NTM)	Number of <u>specimens (all)</u> inoculated in one quarter reported as NTM	Total number of <u>specimens (all)</u> inoculated in the reporting quarter	Culture register	Quarterly	Specimen preparation
(6) The percentage of drug susceptibility results available within 84 days.	Total number of DST processed in reporting quarter with results available within 84 days	Total number of DST processed in the reporting quarter	Specimen register	Quarterly	Timeliness, quality of service
(7) The percentage of final culture/DST results¹ reported within 3 days	Total number of culture/DST with final results reported to referring provider within 3 days of final result being available	Total number of cultures inoculated in the reporting quarter	DST register, DOTS-plus IRL register	Quarterly	Timeliness, quality of service
(8) Performance on Drug Susceptibility Testing (DST).	Total number of correct responses	Total number of specimens provided for DST re-testing	Proficiency testing report	Annual	DST quality
(9) Annual on-site evaluation conducted	Not applicable	Not applicable		Annual	On Site Evaluation

¹ A “final culture/DST result” refers to final DST result if available, or final culture result (culture+ M. Tb complex, culture-negative, contaminated, NTM) if DST was not performed for that specimen.

Reporting Format	Quarterly / Annual report on Laboratory Performance
Laboratory:	
Quarter / Year of reporting	
Microbiologist:	
Contact Phone Number:	

Workload and DST results

Culture workload (from culture register)			DST workload and results (from DST register)				
Month	Diagnostic Sputum Specimens inoculated	Follow-Up Specimens inoculated	DST Processed	Total H+R Sens	Total H+R Res	Total H only Res	Total R only Res

Performance indicators

	Numerator (No.)	Denominator (No.)	Percent
(1) The percentage of sputum specimens received within 7 days of sputum collection			
(2) The percentage of all cultures reported as <i>Mtb. Complex</i>			
(3) The correlation between positive smears and positive cultures in smear-positive diagnostic specimens			
(4) The percentage of all cultures contaminated (reported by culture system)			
(5) The percentage of all cultures reported as non-tuberculous mycobacterium			
(6) The percentage of drug susceptibility results available within 84 days			
(7) The percentage of final culture/DST results reported to provider within 3 days of result being available			
INDICATORS (7 – 8) EXPECTED ON ANNUAL BASIS ONLY			
(8) Performance on Proficiency Testing for DST (Report Date _____) (Reference lab _____)	Sensitivity (%)		Specificity (%)
	H:		
	R:		
	E (opt):		
	S (opt):		
(9) Annual On-Site Evaluation	Date: Ref. Laboratory: Evaluator:		

Indicator 1: The percentage of sputum specimens received within 7 days of sputum collection

Numerator / Denominator

$$\frac{\text{Number of sputum specimens received in one quarter within 7 days of sputum collection}}{\text{Number of sputum specimens received in one quarter}}$$

What it measures

- This indicator measures the time lag between sputum collection and receipt by the culture laboratory, which strongly affects specimen viability and risk of contamination.
- Although laboratories are not usually responsible for specimen handling themselves, laboratories are the only place that can detect and indicate problems in the pre-laboratory specimen handling chain.
- At a minimum, 80% of specimens should be received within 7 days of sputum collection. This may vary if special activities such as drug resistance surveillance are ongoing, and if special transport media is being used.
- This indicator will assist in the interpretation of laboratory contamination rates.
- This indicator is dependent upon the reporting of date of specimen collection.
- The indicator does not address the quality of the sputum collection, nor the loss of viability during transportation. The benchmark may be exceeded during special surveys with the use of transportation media.

How to measure it

The numerator can be determined by calculating the time difference between date of collection and receipt, usually available in the specimen receipt or culture register, and determining the proportion that met the 7-day benchmark during the quarter of interest. The denominator is the total number of specimens received during that quarter.

Data Sources

- Specimen receipt register
- Laboratory information systems

Indicator 2: The percentage of all cultures inoculated in one quarter reported as *Mtb* complex**Numerator / Denominator**

Number of cultures inoculated in one quarter reported as *Mtb* complex

Total number of all specimens inoculated for culture in one quarter (regardless of result, i.e. including *Mtb*, NTM, negative, and contaminated cultures)

What it measures

- This indicator is useful for establishing an acceptable recovery rate of *Mtb* complex within a given population/province
- This indicator represents the percentage of mycobacterial cultures inoculated in one quarter reported as *Mtb* complex. This indicator is directly related to the population from which specimens are received, and may vary between different populations.
- Approximately 12-14 months of data should be evaluated before determining an acceptable recovery rate for the population, and before responding to major fluctuations in the established trend
- A dramatic decrease (>20%) in this indicator may reflect a change in the population, but should also prompt a sputum collection, handling, and laboratory investigation. Sputum collection may have changed or become inadequate. Sputum specimens collected may have been inappropriately delayed, transport medium used may have been improperly prepared. Excessive decontamination during specimen processing, the use of expired reagents and/or reagents of suboptimal quality, and the use of equipment performing outside of the expected limits may have a negative impact on the recovery rate. A systematic review of processing procedures, reagents used, instrumentation, and laboratory records should be performed.
- A dramatic increase (>20%) in this indicator may represent a change in the population, or may suggest the reporting of false-positive cultures due to cross contamination. Molecular techniques may be used to detect cultures that are cross contaminated.

How to measure it

The numerator is the total number of specimens reported as *Mtb* complex within one quarter. The denominator is the total number of specimens inoculated in one quarter regardless of result, including NTM, *Mtb* complex, culture-negative, and contaminated.

Data Sources

- TB laboratory culture register
- Laboratory information systems

Indicator 3: The correlation between positive smears and positive cultures in one quarter in smear-positive diagnostic specimens

Numerator / Denominator

No. of smear-positive diagnostic specimens inoculated for both culture that were culture-positive for MTB or NTM in one quarter

No. of smear-positive diagnostic specimens inoculated for both culture in one quarter

What it measures

- This indicator is useful in evaluating technical proficiency in microscopy and specimen processing.
- This indicator represents the percentage of positive smears from diagnostic specimens that yield positive cultures reported in one quarter.
- Smear-positive diagnostic specimens are chosen as the measure of specimen processing because a high yield is generally expected. This would include specimens from patients on treatment but with suspected MDR TB.
- Diagnostic specimens for this purpose are defined as those specimens submitted for both culture and DST.
- If “diagnostic” and “follow up” specimens are not clearly indicated, than those specimens submitted for both culture and DST should be taken as diagnostic specimens.
- Approximately 90% of all positive smears performed and reported should result in positive cultures, though this may vary by laboratory depending on the characteristics of the population tested. Less than 90% for this indicator should prompt an investigation by the laboratory. This investigation should include a review of: patient history including previous positive cultures and/or smears, accuracy of classification of type of specimen received {diagnostic vs. follow up}, anti-TB treatment at time of specimen collection, specimen handling and transportation, quality of smear microscopy in the culture laboratory, the decontamination methods used for specimen processing, and the adherence to decontamination procedures.
- Loss of bacterial viability between specimen collection and receipt by the culture laboratory and the effect of decontaminating agents used in transporting sputum may result in a difference in smear results between collection and receipt. Hence to maintain focus on culture laboratory specimen processing, the smear-result from the culture laboratory of the actual specimen inoculated should be used when calculating this indicator.

How to measure it

The numerator is the total number of smear-positive specimens (submitted for both culture and DST) inoculated with a positive MTB or NTM culture result in one quarter. This can be obtained from the TB culture registers, culture worksheets or other data management system. The denominator is the total number of smear-positive diagnostic specimens inoculated for culture within one quarter.

Data Sources

- TB laboratory culture register (which should also record microscopy results of inoculated specimens for ease of preparation of this indicator)
- TB laboratory smear-microscopy register (if necessary)
- Laboratory information systems

Indicator 4: The percentage of all cultures contaminated in one quarter [Culture system specific]

Numerator / Denominator

Number of cultures inoculated in one quarter discarded due to contamination

Total number of all cultures inoculated in one quarter

What it measures

- This indicator is useful for monitoring specimen collection and transport procedures, and evaluating processing procedures.
- This indicator represents the percentage of cultures documented as discarded due to contamination in one quarter.
- This indicator may be related to the patient population sampled; however in most cases it is directly related to the collection, storage, and transport of specimens; and/or the media, reagents, and procedures used in the laboratory for specimen processing.
- Separate indicators should be calculated for each culture system, if more than one is used.
- Lowenstein Jensen (LJ)
 - An acceptable range for this indicator is 2–4%.
 - Contamination rates of 1–2% suggest the use of harsh decontamination reagents and/or excessive decontamination. The laboratory should review the specimen processing procedures and the stringency of reagents used, specifically the final concentration of NaOH if 1–2% contamination rates are observed. NaOH is sensitive to storage conditions, and the concentration can vary over time.
 - A contamination rate of >4% suggests incomplete digestion/decontamination, the use of suboptimal reagents or contaminated media for specimen processing and inoculation, or problems with specimen collection, storage and/or transport. LJ media is intrinsically quite resistant to contamination, and high contamination rates usually indicate a significant problem. If contamination rates exceed 4%, a laboratory investigation should be initiated.
- Liquid cultures
 - The acceptable range for contamination established by the manufacturers is 8–9%.
 - If the liquid culture contamination rate >10%, and the contamination rate of the solid media (LJ) exceeds 5%, an evaluation of all procedures relating to specimen collection and processing should be evaluated as above.
 - If liquid culture contamination rate >10%, but solid media contamination rates are <5%, then procedures to troubleshoot liquid culture contamination should be prioritized. Attention should be given to the preparation of liquid culture (reconstitution and addition of antimicrobials [e.g. PANTA]) prior to specimen inoculation, and the handling of tubes. However, given the relative sensitivity of liquid culture systems to contamination due to specimen processing compared to LJ, it is possible that specimen processing procedures are suboptimal. Hence the investigation should include review of these procedures.
- Contamination beyond thresholds for any culture system should also prompt consideration of the timing of contamination. Early contamination (within the first few days of inoculation) suggests gross contamination of the media, bottle, or specimen. Late contamination suggests inadequate decontamination processing of specimens.

How to measure it

The numerator is the total number of cultures in that system inoculated in one quarter that were documented discarded due to contamination, and can be obtained from the TB laboratory culture register or other information management system. The denominator is the total number of all specimens inoculated for culture in one quarter in that system, and can also be obtained from the same data source.

Data Sources

- TB laboratory culture register
- Laboratory information systems

Indicator 5: The percentage of all cultures reported as nontuberculous mycobacterium (NTM) in one quarter [Culture system specific].

Numerator / Denominator

Number of final isolates reported as NTM in one quarter

Total number of all cultures inoculated in one quarter

What it measures

- This indicator is useful for establishing an acceptable recovery rate NTM within a given population and/or province; and will help laboratories monitor and troubleshoot contamination due to NTM.
- This indicator represents the percentage of mycobacterial cultures reported in one quarter as NTM. This indicator is related to the frequency of infection caused by mycobacterium other than tuberculosis within a given population, and the distribution of environmental mycobacterium.
- Laboratories using a liquid based culture system have frequently reported a marked increase in the proportion of NTM isolates recovered when compared to solid media. The significance of that observation has not been established, and requires clinical correlation.
- There is no specific target that can be applied across laboratories, but the trend within a laboratory should be followed. Approximately 12-14 months of data should be collected and evaluated before determining an acceptable recovery rate and responding to major fluctuations in the established trend.
- A dramatic increase (20%) in the same culture system (solid or liquid) in this statistic may suggest environmental contamination.
- Contamination can be introduced during the pre-analytical phase of testing (the use of contaminated water and/or equipment during specimen collection), or may be introduced during testing (through contaminated buffers and/or water). If the laboratory observes the above noted increase, an investigation to determine the possibility and cause of contamination should be initiated. Speciation of the NTM recovered should be considered as relevant to the investigation, if laboratory capacity for speciation exists.

How to measure it

The numerator is the total number of cultures reported within one quarter as NTM, which can be obtained from the TB laboratory notebook or other data management system. The denominator is the total number of all specimens inoculated for culture in one quarter in the same culture system, and can also be obtained from the same data source.

Data Sources

- TB laboratory culture register
- Laboratory information systems

Indicator 6: The percentage of drug susceptibility results available within benchmark turn around time [culture system specific]

Numerator / Denominator

Total number of DST processed in reporting quarter with results available within target turn-around time

Total number of DST processed in the reporting quarter

What it measures

- This indicator is useful in identifying problems with the timeliness and quality of service, which usually indicates weaknesses in laboratory administrative and technical procedures.
- This indicator measures the number of days from laboratory receipt to the availability of drug susceptibility test results.
- The turn around time is specific for the culture and DST system used.
- The standard benchmark is that turn-around times should be reached for 90% of all DST processed.
- The following turn around times (TAT) from specimen receipt to DST results are recommended:
 - Solid media: within 84 days from receipt of specimen
 - Liquid media: within 42 days from receipt of specimen
 - Line-probe assay: within 5 days of receipt of specimen

How to measure it

- Turn around time should be routinely documented in the TB laboratory registers.
- Calculation of turn around time days:
 - Culture TAT: [date of culture result report – date of specimen receipt]
 - DST TAT: [date of DST result report – date of specimen receipt]
- A minimum of 90% of specimens should meet turn around times, though this may vary if a laboratory is heavily involved in reference or research functions. Turn-around times that exceed these standards for more than 10% of isolates tested should prompt an investigation of timeliness of laboratory procedures for specimen receipt, smear preparation and processing, inoculation, and recording in laboratory.
- Delays in reporting to providers due to 'certification' of results by senior laboratory officials have been observed, and should be minimized. One solution to this common administrative delay is to deputize a senior laboratory technician to certify results.

Data Sources

- The RNTCP culture and DST register has two columns that should be used to calculate the turn-around time: [Date of result] – [Date of receipt]
- One technician can be assigned to calculate the turn-around time achieved for each specimen

Indicator 7: The percentage of final culture/DST results reported to provider within 3 days

Numerator / Denominator

$$\frac{\text{Total number of final culture/DST results reported to provider within 3 days}}{\text{Total number of all cultures inoculated in one quarter}}$$

What it measures

- This indicator is useful in identifying problems with the timeliness and quality of service, which usually indicates weaknesses in laboratory administrative and technical procedures.
- This indicator measures the number of days from the availability of laboratory results to the time when those results are communicated back to the referring provider. Quick communication of results is required for proper clinical management of patients.
- This reporting turn-around time should be reached for **100% of all specimens received**.
- In an effort to reduce turn around time due to courier delivery of paper results, all positive results should also be reported by email, telephone, or fax no later than 2 days from the availability of results in the laboratory.

How to measure it

- "Final culture/DST results" are defined as the DST result if available. If DST was not performed for that specimen, then the final culture result (culture+ *M. Tb* complex, culture-negative, contaminated, or NTM) should be taken.
- Date of results and date of report to providers should both be recorded in routine laboratory registers.

Data Sources

- The RNTCP culture and DST register has two columns that should be used to calculate the turn-around time: [Date of result] – [Date of receipt]
- One technician can be assigned to calculate the turn-around time achieved for each specimen

Indicator 8: Performance on Proficiency Testing (PT) in Drug Susceptibility Testing (DST).**Numerator / Denominator**

Total number of correct responses

Total number of specimens provided for DST proficiency testing

What it measures

This indicator is a measure of the laboratory's proficiency to perform and interpret first and/or second-line (if applicable) drug susceptibility tests. This indicator is dependent upon a commitment to a proficiency testing programme.

- This indicator is useful in measuring laboratory proficiency and identifying training needs. It does not measure routine performance, but only whether or not a laboratory is capable of conducting drug susceptibility testing.
- The laboratory should enroll in a proficiency testing program (e.g. those provided by a WHO Supranational Reference Laboratory, the National Reference Laboratory, or other internationally recognized program providing panels for proficiency testing).
- Ideally, laboratories should have acceptable performance before reporting patient results.
- The same staff members who will routinely do the work should conduct the PT, and not supervisors or senior microbiologists from the laboratory.
- Though not a measure of routine performance, this indicator lends some integrity to the patient test results reported.
- A laboratory's performance on DST PT should be equal or greater than the following:
 - Isoniazid: 90% accuracy
 - Rifampicin: 90% accuracy
 - (optional) Streptomycin: 80% accuracy
 - (optional) Ethambutol: 80% accuracy

How to measure it

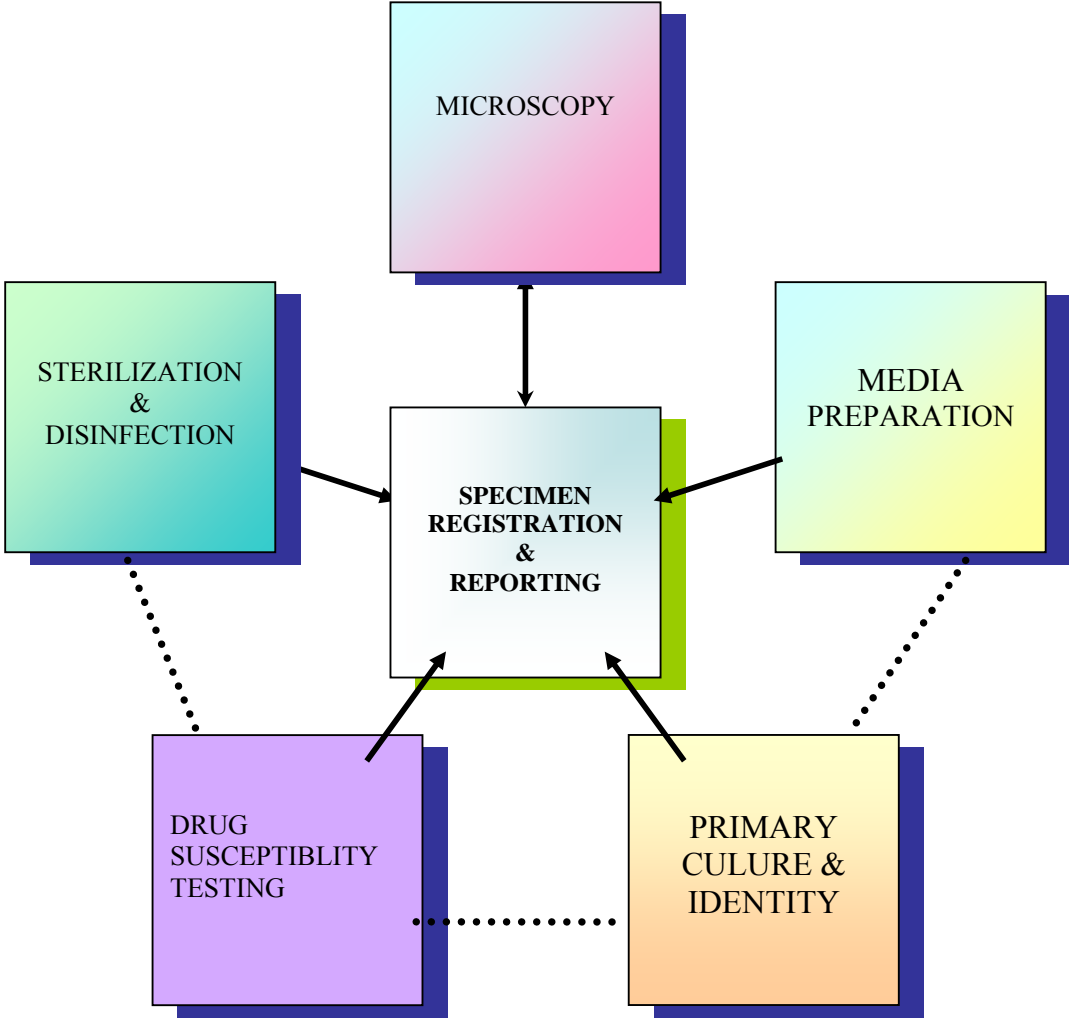
The laboratory should annually receive a panel of (20) cultures for drug susceptibility testing from a reference laboratory. The number of specimens is determined by National Guidelines. Performance can be measured by dividing the total number of points associated with correct responses by the total number of available points, or by comparing laboratory results with expected responses.

Data Sources

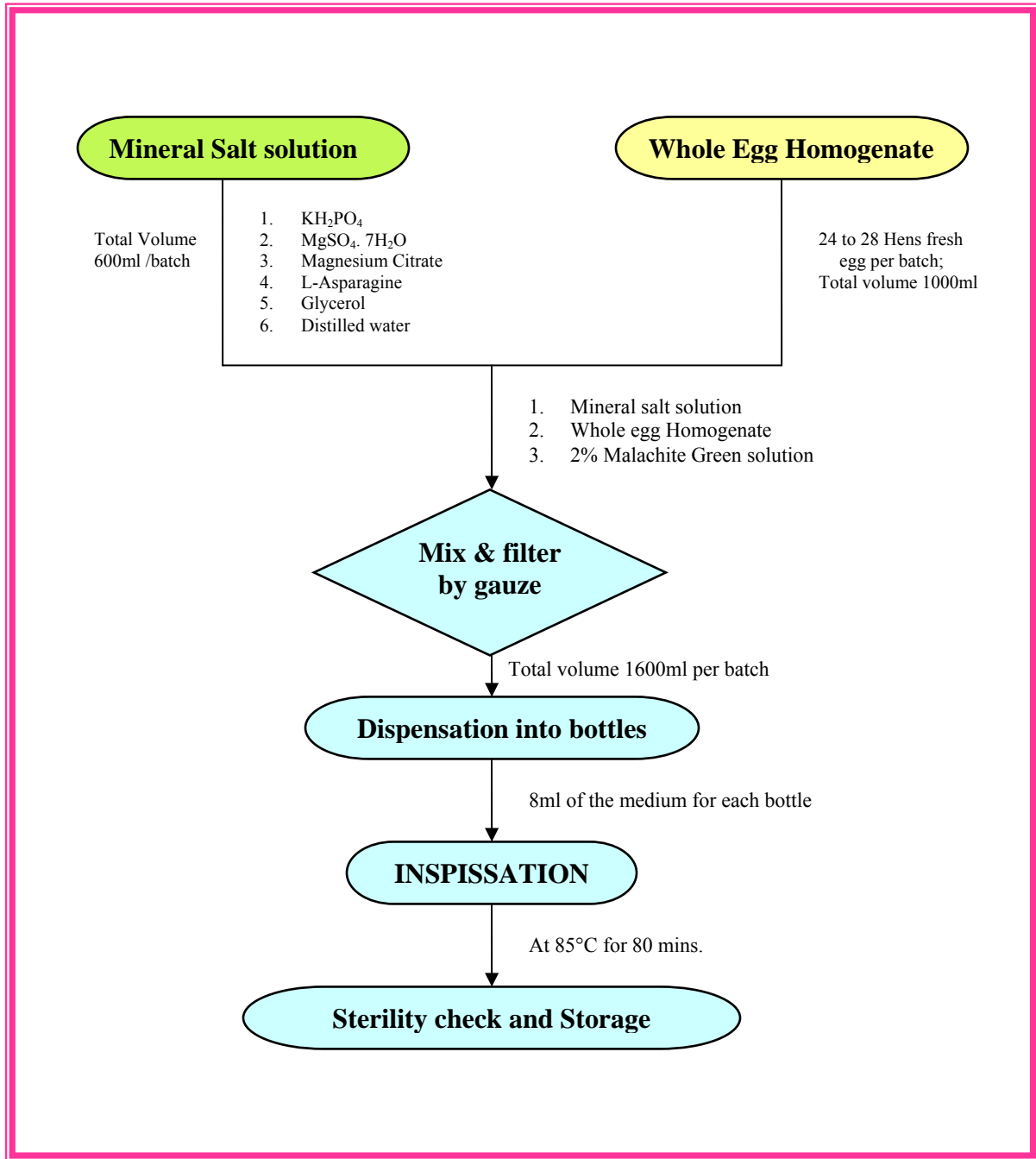
PT results certified by Reference Laboratory or external testing organization.

Flow diagrams

ORGANIZATION OF LABORATORY ACTIVITIES



PREPARATION OF LOWENSTEIN-JENSEN (LJ) MEDIUM



PRIMARY CULTURE OF *M. TUBERCULOSIS* & EXAMINATION

Homogenization & Decontamination of sputum [NaOH Method (Modified Petroff's)]

2 volumes of 4% NaOH
15 min at 37°C, water bath
3000xg for 15min (3500rpm)
D. water washing, 3000xg for 15min

In-Process, Sterility Test

1. At Beginning &
 2. At the end of process
for
- (a) Sterility of water
(b) Sterility of NaOH
Tested on
(a) Nutrient Agar (2 slopes)
(b) Nutrient broth (2 tubes)
(c) LJ medium (2 slopes)

Controls:

Positive- *M. tuberculosis* (H37Rv)

INOCULATION

One loop-full (5mm loop) of
sediment inoculated on 2 slopes
of LJ medium

INCUBATION

At 37°C for 2 week upto 8 weeks

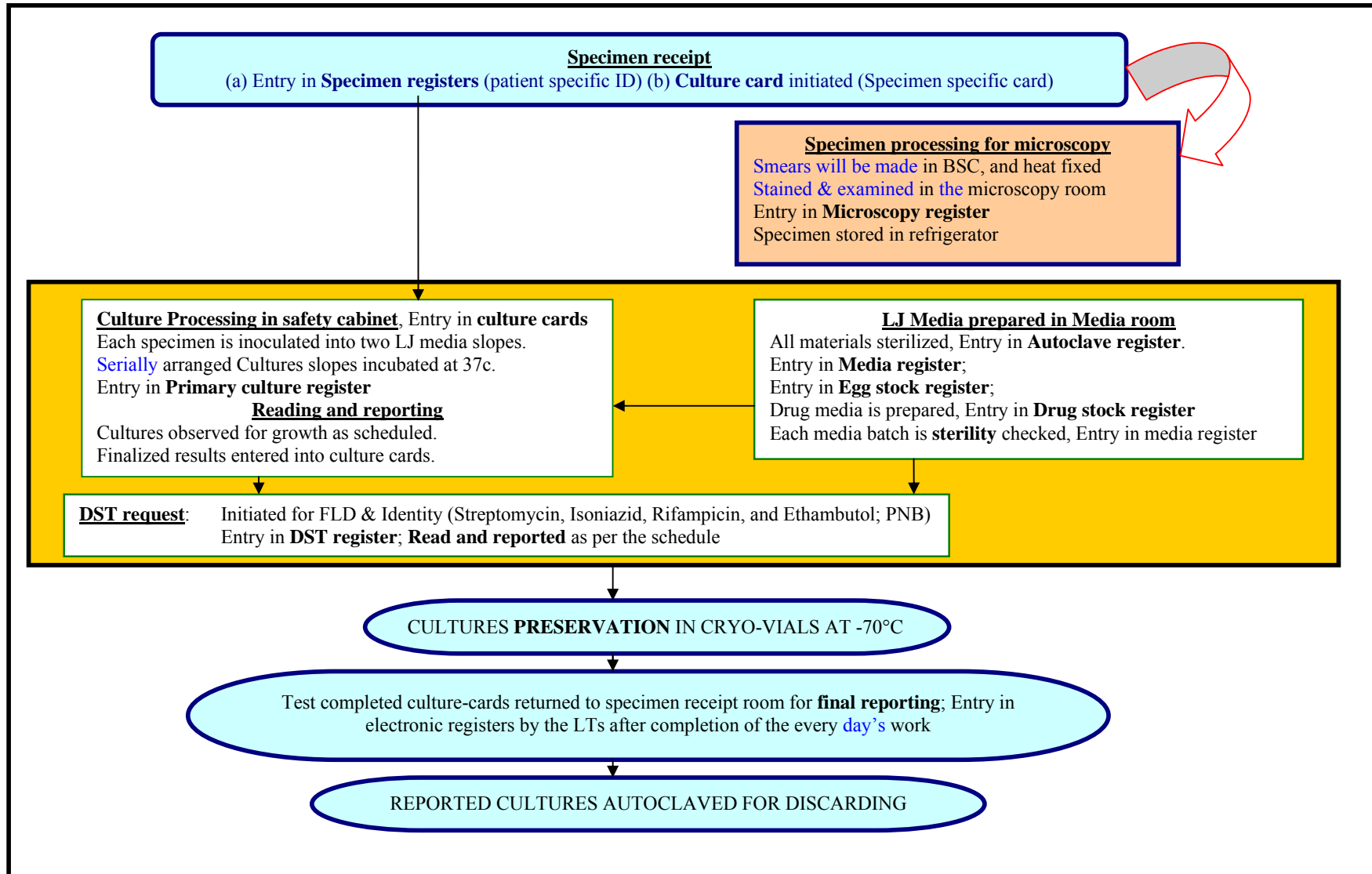
READING

At 1st week to 4th weeks
(5th, 6th, 7th, & 8th weeks)

Entries made in
(a) Culture cards &
(b) Primary culture registers

RECORDING & REPORTING

FLOW OF SPECIMENS FOR *MYCOBACTERIUM TUBERCULOSIS* CULTURE & DRUG SUSCEPTIBILITY TESTING



ANNEXURE- FORMAT OF REGISTERS

CULTURE CARD

1. Name/Group &		2. Age		3. Sex M _____ F _____		4. Date of collection		5. Laboratory No.	
6. Specimens Sputum : Spot/ ov _____ Others _____		7. Macro exam S M P B <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>		8. Smear Microscopy : Z.N. : _____ F.M. : _____		9. Culture Results			
10. Primary Culture Method : A/B Date :		11. Sub culture Date :		12. Batch No.		<i>M. tuberculosis</i> <input type="checkbox"/> Atypical Mycobacteria <input type="checkbox"/> Negative / No growth <input type="checkbox"/> Contaminated <input type="checkbox"/> Mixed Culture <input type="checkbox"/>			
Date of reading	1	2	Date of reading	37°C	RT	42°C	13. Sen. Test / Anti biogram		14. Iden. test
							Direct : _____		Niacin : _____
							Indirect : _____		Nitrate reduction _____
							Radio-matric : _____		PNB _____
							Sen. Res.		Pigment in
							SM _____		Dark _____
							INH _____		Light _____
							RIF _____		RG in days _____
							EMB _____		
							15. Remarks :		

Specimen Registration, Culture, and DST Results Register Month _____ Year _____ (part 1-Left half)

S. No.	Lab No.	Name (in full) & Address	Sex (M/F) & Age	Name of referring site (DMC/DOTS-plus site) & District	Reason for Testing (mark one)				Specimen	Date Specimen Collected from Patient	Date Specimen Received in culture lab	Specimen Condition (CPC or [MP, BLD, SAL, Contam]) †	Culture lab concentration & smear result ‡
					Diagnosis		Follow-up						
					TB No.	TB Type*	DOTS-Plus No.	Month of F/U					
									A				
									B				
									A				
									B				
									A				
									B				
									A				
									B				
									A				
									B				
									A				
									B				
									A				
									B				

* Using standard RNTCP definitions for TB type: **NSP, NSN, NEP, Relapse, TAD, Failure, or Other**
 † **CPC**=specimen contains CPC. If CPC present then no further description needed. For all other specimens with no CPC, describe condition: **MP**=mucopurulent specimen, **BLD**=gross blood in specimen, **SAL**=Salivary specimen, **Contam** if gross bacterial overgrowth is suggested by visual examination.
 ‡ Smear results for specimen deposit after concentration in culture laboratory, using standards definitions: **3+, 2+, 1+, Sc, Neg.**

Specimen Registration, Culture, and DST Results Register Month _____ Year _____ (part 2-right half)

Culture Results				Standard DST Results							Date Sending Report to DOTS-Plus Site & DTO		
Date Inoculated	Type (Solid / Liquid)	Culture Results §	Date Result Reported	Date Inoculated	Type (Solid / Liquid)	RIF (R/S)	INH (R/S)	SM (R/S)	EMB (R/S)	Date Result Reported	Culture	DST	Remarks

‡ R=Resistant, S=Sensitive, NA=no result.
 § Negative=no growth, Contam=contaminated, NTM=Non-Tuberculosis Mycobacteria/rapid grower, 3+=confluent growth, 2+= >100 colonies, 1+=10-100 colonies; Sc(number)=Scanty<10 colonies (indicate number of colonies).
 Positive culture results should only be reported after identity for *M. tuberculosis* is confirmed with PNB, Niacin, Catalase, Rapid Immunoassay, or other methods.

Primary Culture Register

Lab Number	Specimen					Patient		AFB smear Result		Quantified growth readings								Final result**		
	Date received	Date collected	Date processed	Type of specimen	Name of DMC	Diagnosis / follow-up & month	TB number (RNITCP 1st line drugs)	DMC	Culture Lab	Media inoculated*	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7		Week 8	
										LJ-1										
										LJ-2										
										LJ-1										
										LJ-2										
										LJ-1										
										LJ-2										
										LJ-1										
										LJ-2										
										LJ-1										
										LJ-2										
										LJ-1										
										LJ-2										
										LJ-1										
										LJ-2										

**No growth= Negative; Fewer than 10 colonies=Report exact number of colonies; 10-100 colonies= 1+ Positive; More than 100 colonies 2+ Positive; confluent growth= 3+ Positive. Contaminated=C; Non-Tuberculosis Mycobacteria/fast grower= NTM.

DRUG SUSCEPTIBILITY TESTING & IDENTITY RESISTER (left)

Lab number	Date of DST	Primary culture Positive date	Reading time*	Neat	-10 ⁻² dilution						
				Control (Drug free-media)	Control (Drug free-media)	Control (Drug free-media)	Strep	INH	RIF	EMB	
			4 weeks								
			6 weeks								
			4 weeks								
			6 weeks								
			4 weeks								
			6 weeks								
			4 weeks								
			6 weeks								
			4 weeks								
			6 weeks								
			4 weeks								
			6 weeks								
			4 weeks								
			6 weeks								

* Results are read for the first time on 28th day and second time on 42nd day. No growth= Negative; Fewer than 10 colonies=Report exact number of colonies; 10-100 colonies= 1+ Positive; More than 100 colonies 2+ Positive; confluent growth= 3+ Positive. Contaminated=C; Non-Tuberculosis *Mycobacteria*/fast grower= NTM. The colonies are counted only on the slopes seeded with the inoculum that has produced exact readable counts or actual counts (up to 100 colonies on the slope). Any strain with 1% (the critical proportion) of bacilli resistant to any of the four drugs – Rifampicin, Isoniazid, Ethambutol, and Streptomycin – is classified as resistant to that drug. Refer the manual/SOPs for details.

DRUG SUSCEPTIBILITY TESTING & IDENTITY RESISTER (right)							
-10⁻⁴ dilution						Identity	Remarks/ colony morphology
Control (Drug free-media)	Control (Drug free-media)	Strep	INH	RIF	EMB		

* Results are read for the first time on 28th day and second time on 42nd day. No growth= Negative; Fewer than 10 colonies=Report exact number of colonies; 10-100 colonies= 1+ Positive; More than 100 colonies 2+ Positive; confluent growth= 3+ Positive. Contaminated=C; Non-Tuberculosis *Mycobacteria*/fast grower= NTM. The colonies are counted only on the slopes seeded with the inoculum that has produced exact readable counts or actual counts (up to 100 colonies on the slope). Any strain with 1% (the critical proportion) of bacilli resistant to any of the four drugs – Rifampicin, Isoniazid, Ethambutol, and Streptomycin – is classified as resistant to that drug. Refer the manual/SOPs for details.

AJ-19/05/2009

MEDIA REGISTER (SOLID EGG based MEDIA)

Date	Batch No	Total media bottles	Inspissator		Sterility Test				Type of media prepared							Drug stock solution preparation date	Remarks/ prepared by		
			Time Started	Time Closed	Setup date	48 Hours*		14 days**		Plain LJ	PNB	Streptomycin	Isoniazid	Rifampicin	Ethambutol			Nutrient Agar	Nutrient Broth
						Read date	Result	Read date	Result										

* Bacterial sterility check for two days (37°C); ** Fungal sterility check for up to 14 days (25°C)

AUTOCLAVE REGISTER*

Sl.No	Date	Article(s) for sterilization	Pressure and duration for autoclaving	Autoclave Starting time	Time pressure reached	Time Switched off	Whether dry cycle ON & duration	Time of opening	Remarks/ Person sterilizing

* Follow the SOPs or manufacturers operation instructions.

EGG STOCK REGISTER**

Date	Opening Balance	No. of eggs received	Total	Eggs broken/ cracked	Eggs Used	Balance	No. of batches	Media Batch No.	Remarks

**Eggs should be fresh; should be stored at 4c, and should be used within 7 days.

DRUG STOCK REGISTER*

Sl. No.	Name of drug	Date of Preparation	Amount weighed (mgs)	Solvent name & Volume (ml)	Date of expiry*	Prepared by	Assisted by	Date discarded	Remarks

* Check the SOPs for solvents, never store the working stock.

Monthly Laboratory abstract of *M. tuberculosis* Primary Culture & DST activities

Month	Sputum smear status	Patient Type	Culture results								Total Primary culture	DST processed*				
			Positives		Negative		Contaminated		NTM/ fast growers			Total DSTs	All susceptible	MDR	Only H resistance	Only Rif resistant
			Nos.	%	Nos.	%	Nos.	%	Nos.	%						
	Smear +ve	Diagnostic														
		Follow-up														
	Smear -ve	Diagnostic														
		Follow-up														
		Total														
	Smear +ve	Diagnostic														
		Follow-up														
	Smear -ve	Diagnostic														
		Follow-up														
		Total														
	Smear +ve	Diagnostic														
		Follow-up														
	Smear -ve	Diagnostic														
		Follow-up														
		Total														

* DST is not advised for treatment follow-up cultures.

References

1. Canetti G, Fox W, Khomenko A, Mitchison D A, Rist N, Smelev N A. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. *Bull World Health Organ* 1969; 41: 21-43.
2. Canetti G, Froman S, Grosset J, Hauduroy P, Langerova M, Mahler H T, et al. Mycobacteria: laboratory methods for testing drug sensitivity and resistance. *Bull World Health Organ* 1963; 29: 565-578.
3. World Health Organization. Laboratory services in Tuberculosis control. Part III. Culture. WHO/TB/98.258. 1998.
4. Toman K. Toman's tuberculosis. Case detection, treatment, and monitoring. Questions and answers. Frieden T R, ed. Geneva, Switzerland: World Health Organization, 2004.
5. National tuberculosis Institute, Banglaore. Manual on Isolation, Identification and sensitivity testing of mycobacterium tuberculosis. 1998
6. Tuberculosis Research centre, Chennai. Bacteriological methods in diagnosis of tuberculosis. 2003.
7. Priorities for tuberculosis bacteriology services in low-income countries. Second edition. IUATLD. 2007.
8. Akiko Fujiki. TB bacteriology. Research Institute of Tuberculosis, Japan. 2003.
9. CDC & NIH. Biosafety in Microbiological and biomedical laboratories. Fifth edition. 2007.
10. World health organisation. Laboratory biosafety manual. Third edition. 2004.
11. World Health Organization. Maintenance and repair of laboratory, diagnostic imaging and hospital equipment. Geneva: WHO; 1994.
12. World Health Organization. Regional Office for the Eastern Mediterranean. Basics of quality assurance for intermediate and peripheral laboratories. 2nd ed. Cairo; 2002.