

Document type: procedure	GENERAL SAFETY PROCEDURES FOR FIND-UGANDA TUBERCULOSIS RESEARCH LABORATORY
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1. PURPOSE

This SOP describes the methods of good laboratory practice to be employed while working with potentially infectious material or samples known to contain *M.tuberculosis* and other mycobacteria at the FIND Research Laboratory, based at the National Tuberculosis Reference Laboratory in Kampala, Uganda.

This SOP covers access to the laboratory, personnel protection, working practices and safe handling of specimens and disposal of waste. The procedure is necessary to reduce the chance of laboratory acquired infection and cross-contamination of clinical specimens or experimental samples. Tuberculosis is transmitted via aerosol droplets. Any procedure that involves the production of aerosols may increase the potential of transmission of *M.tuberculosis* within the laboratory.

Most infections in the mycobacteriology laboratory can be attributed to the unrecognized production of potentially infectious aerosols containing acid-fast bacilli. All work performed with cultures or specimens suspected of containing *M.tuberculosis* must be performed in a Class II biological safety cabinet. Studies have shown that particular laboratory manipulations yield aerosols that are potentially infectious and special care must be taken while performing these procedures (e.g. pouring of liquid cultures or supernatant fluids, centrifugation or using automatic pipettes).

In addition to infection through inhalation, other routes of infection should also be considered. These include puncturing of skin with broken contaminated glassware, self-inoculation with a contaminated needle and infection through uncovered cuts and abrasions.

2. SCOPE

This SOP covers all procedures carried out in the FIND Research Laboratory, Kampala, Uganda.

3. RESPONSIBILITIES

The Head of Laboratory is responsible for the provision of the necessary safety equipment, reagents and training required to provide a safe working environment, as well as regular health monitoring of staff in accordance with provisions made in [*Health Monitoring of Laboratory Personnel_Uganda QP 07-01-03*](#).

All staff members working in the FIND Uganda Research Laboratory are responsible for the implementation of this SOP, including use of safety equipment and reagents as well as implementation of methods described herein.

All users of this procedure who do not understand it or are unable to carry it out as described are responsible for seeking advice from their supervisor.

4. CROSS-REFERENCES

Document Matrix_Uganda QP 01-03-03

Refer to SOPs listed under 07-01 (General Procedures), 07-02 (Specimen Handling) and 07-06 (Equipment Use and Maintenance)

See: ***Document Matrix_Uganda QP01-03-03.doc***

Location: ***Hard copy: FIND Uganda SOPs***

5. PROCEDURES***5.1 Access to the FIND Research Laboratory***

- The international biohazard warning symbol must be displayed on the door to the specimen processing laboratory where specimens containing viable mycobacteria are handled. A diagram of the Layout of the FIND laboratory can be found in ANNEX 1. The FIND research laboratory includes the “Processing laboratory”, “Molecular laboratory 1” (pre-amplification room), “Molecular laboratory 2” (post-amplification room) as well as office space.
- Only authorised personnel, stipulated by the Head of Laboratory Research in the Lab Access Authorisation Table, are allowed to enter the laboratory facilities.

See: ***Lab Access Authorisation Table_ddMMMyy.doc***

Location: ***Hard copy: FIND Uganda SOPs***

Access is by use of a code-lock, the combination of which is known only by authorised personnel and should be changed on a regular basis. The combination must not be given to other individuals. Visitors to the laboratories should always be accompanied by a member of the FIND laboratory staff.

- No children will be permitted to enter the laboratories under any circumstances.
- Laboratory doors should be kept closed at all times.
- Eating, drinking, applying cosmetics or storage of food or drinks is prohibited in the laboratories.

5.2 Personnel protection***5.2.1 Hand washing***

Personnel must wash their hands after handling infectious materials before leaving the working area. Hand wash basins are situated in the ante-room, processing laboratory and molecular laboratories.

All cuts and abrasions must be covered using an adhesive plaster when entering and working in the laboratory. These are available in the first aid kit which is stored in the FIND Research Laboratory Office.

5.2.2 Footwear

Open-toed footwear must not be worn inside the laboratory working areas. Laboratory shoes are provided for FIND staff members for use while working in each laboratory. Shoes should be disinfected on a weekly basis using appropriate disinfectant. Outdoor shoes should not be worn inside the laboratories, and laboratory shoes must not be worn outside the appropriate laboratory.

5.2.3 Safety glasses

Safety glasses should be worn when required to protect the worker from potential splashes or impacting objects, e.g. removal of tubes from the centrifuge.

5.2.4 Masks

A mask must be worn to enhance the personnel protection when performing experiments with potentially infectious materials and samples known to contain pathogenic mycobacteria. N95 Respirator masks will provide the wearer protection from aerosol particles between 0.1 - 10µm.

To don the mask, position it under the chin with the nosepiece up. Pull the top strap over the head so that it rests high on the back of the head. Pull the bottom strap over the head and position it around the neck below the ears. Pinch the nosepiece so that the mask fits snugly.

The mask must be replaced as soon as the user feels that breathing is becoming difficult.

When not in use, store the mask in the separate plastic bag provided for each laboratory worker. Used masks must be disposed of in the biohazard waste box.

5.2.5 Surgical gowns

Surgical gowns protect the skin and clothing from large droplets of infectious or hazardous material that may be splattered. They must be fully fastened and be worn when working in the biological safety cabinet.

Three gowns are issued to each staff member, and these must be changed, autoclaved and washed on a weekly basis. Soiled or dirty gowns must be changed, washed and autoclaved immediately. Gowns must be sealed in an autoclavable bag. After autoclaving gowns must be washed in soapy water, dried and ironed.

Gowns that have been used in the laboratory must not be stored in the same cupboards or lockers as street clothing. They must be stored on the designated hooks in the laboratory.

Surgical gowns must be worn during all work with infectious material. Gowns must not be worn outside the processing laboratory, e.g. in communal areas, toilets, offices etc.

5.2.6 White laboratory coats

White laboratory coats must be worn at all other times in the laboratory, and while working with non-infectious materials outside the biological safety cabinet.

Three white laboratory coats are issued to each staff member, and these should be changed on a weekly basis when in use or more frequently if soiled.

Laboratory coats that have been used in the laboratory must not be stored in the same cupboards or lockers as street clothing. They must be stored on the designated hooks in the laboratory.

Laboratory coats must not be worn outside the processing laboratory, e.g. in communal areas, toilets, offices etc.

5.2.7 Latex gloves

Latex gloves guard against infection through cuts and abrasions on the hands. Cuts and abrasions must be covered with a sticking plaster and/or suitable barrier dressing at all times while in the laboratory.

Gloves must be worn when working with potentially hazardous materials in the biological safety cabinet.

Gloves should be pulled over the cuff of the surgical gown to provide maximum protection.

Gloves must be changed at the end of each task or if soiled. Soiled gloves must be discarded in the biological safety cabinet in the bag provided for waste disposal.

Non-sterile latex gloves are routinely used. However, non-latex gloves can be made available to laboratory staff with known latex allergy.

5.2.8 Air handling system

Use and maintenance of the air handling system is described in *Use and Maintenance of Air Handling System_Uganda QP 07-06-13*.

See: *Use and Maintenance of Air Handling System_Uganda QP 07-06-13*.

Location: *Hard copy: FIND Uganda SOPs*

5.3 Working practices

The laboratory should be kept free of any materials that are not directly required for the work. Any materials that are required in the laboratory but are later to be removed from the laboratory (such as notebooks, documents) should be protected from contamination.

All work surfaces in the laboratory must be decontaminated after any spill and at least at the end of each working day. Specific work areas must be decontaminated after each task utilizing the area.

Mouth pipetting is extremely dangerous and is strictly prohibited.

5.3.1 Safe handling of specimens

- Specimens should be received and sorted in the biological safety cabinet. Under no circumstances should specimens (or experimental samples containing mycobacteria) be taken out of the processing laboratory and into molecular laboratories.
- Personal protective equipment should always be worn when handling specimens
- Broken or leaking specimen containers should be immediately discarded.
- Primary specimen containers should only be opened in a biological safety cabinet. Disinfectant should be used to wipe the outside surface of the container.
- Empty, contaminated specimen containers should be placed in the plastic discard bag inside the safety cabinet, and should be sealed while inside the cabinet before removal for decontamination and disposal.
- Following disinfection, specimens should be transferred to sealable containers. Outside of containers should be disinfected prior to storage in a designated refrigerator.

5.3.2 Working with needles and syringes

- The use of needles in the laboratory should be kept to a minimum due to the risk of needlestick injury. When the use of needles is unavoidable, special care should be taken that both hands are kept behind the needle when performing any manipulations. Care should be taken when the needle and syringe are removed from the packaging as this can cause the needle sheath to unclip.
- The needle and syringe must be discarded together into an appropriate sharps container. Under no circumstances should the needle and syringe be separated, or the needle be re-sheathed.
- A sharps container should be placed in each biological safety cabinet. The sharps container must be used for disposal of pipette tips, needles and syringes. The container should be filled to a maximum of two-thirds full, as indicated on the container label.
- Accidentally discarded items must never be removed from the container.
- The sharps container must be sealed with the press seals on the container lid before removal from the safety cabinet. The sharps container must be placed in a plastic bag and the bag sealed with tape prior to disposal.
- In the case of needlestick injury from a needle potentially infected with M.tuberculosis, the affected area should be washed immediately with copious amounts of cold water under a running tap, and the affected area squeezed to encourage bleeding. The area should be dried and covered, and medical advice sought immediately. The Head of Laboratory Research must be notified immediately and the accident reported in the Laboratory Accident Log.
 - Follow the procedures in *[Health Monitoring of Laboratory Personnel_Uganda QP 07-01-03](#)* if a needlestick injury or major spill occurs.

See: *Health Monitoring of Laboratory Personnel_Uganda QP 07-01-03.*

Location: *Hard copy: FIND Uganda SOPs*

5.4 Equipment

- All equipment in the FIND Research Laboratory must be monitored, calibrated and maintained according to standard operating procedures. Records of monitoring, calibration and repairs will be recorded in the appropriate Equipment Logbook, a bound copy of which will be stored next to each piece of equipment.
- Any equipment which has not been maintained according to this schedule must have a sign posted on it to prohibit its use until rectifying steps and re-calibration have been undertaken.

5.5 Disposal of waste

5.5.1 Liquid waste

- Liquid waste from supernatants and other suspensions of potentially infectious materials should be discarded in the biosafety cabinet in a suitable container (1 litre screw top containing mycobactericidal disinfectant. Sufficient undiluted mycobacterial disinfectant to cover the bottom of the container to the height of about 3 cm is needed to achieve mycobactericidal concentration when the 1 litre discard container is half full.
- Pouring of infectious materials if hazardous. Liquids should be gently poured down the side of the funnel to avoid splashing.
- The discard container should be replaced once a week or when half-full, whichever occurs first. The bottle and funnel should be placed inside a plastic discard bag and sealed before removing from the cabinet.
- Once the container has been removed it must be autoclaved. After autoclaving, the contents of the bin will be submitted for incineration by NTRL staff, and the container and funnel washed thoroughly by NTRL staff before re-use.

5.5.2 Non-infectious waste

Bottles containing non-infectious waste liquids will be discarded by NTRL staff and the containers washed and rinsed thoroughly before re-use. Non-hazardous liquids will be disposed of in the sink in the media room and rinsed thoroughly with running water.

5.5.3 Discard of surplus chemicals

Surplus chemicals must be disposed of in the biohazard waste box, unless stated otherwise in the Materials Safety Data Sheet (MSDS). MSDS for all chemicals will be stored in the filing cabinet in the lab office for reference.

5.5.4 *Potentially infectious solid waste*

- All potentially infectious solid waste must be placed in a plastic bag and sealed before disposal according to NTRL procedures. Solid potentially infectious waste materials are autoclaved, then incinerated by NTRL staff.

5.6 **Laboratory accidents**

- Details of accidents and spills in the laboratory must be recorded in the Accident log. All major accidents and needlestick injuries must be reported to the Head of Laboratory as soon as possible.
- An eye-wash station is situated near the door of the specimen processing laboratory. Any person having an accident involving the eyes should immediately wash their eye(s) in copious amounts of water or sterile saline in the eye-wash station.
- A spills kit must be kept in each biosafety cabinet, in the laboratory and in the ante-room. The spills kit in the safety cabinets must be used to disinfect spills within the cabinet. It should contain a pair of gloves, bottle of freshly prepared disinfectant (prepared on a weekly basis by a Scientific Officer during specimen processing room maintenance duties) and absorbent paper towel or cotton wool in a sealed plastic bag.
- The spills kit in the laboratory must be used to disinfect minor spills within the laboratory. It must contain a large plastic bag, a bottle of freshly prepared disinfectant, pair of gloves, mask and paper towel, cotton wool or absorbent cloth, spill sign "DO NOT ENTER".
- The spills kit in the ante-room must be used to disinfect major spills within the laboratory. It must contain large plastic bag, a bottle of freshly prepared disinfectant, pair of gloves, mask and paper towel, cotton wool or absorbent cloth, spill sign "DO NOT ENTER" and biohazard tape to seal the laboratory.
- The entire contents of the kit must be checked on a monthly basis and fresh disinfectant should be made weekly. Contents must be replaced immediately in case of a spill.

Record in the *Laboratory Cleaning and Maintenance Logbook*.

Use: *Laboratory Cleaning and Maintenance Logbook_form.doc*

Location: *Hard copy: FIND Uganda SOPs*

5.6.1 *Minor spill in a biological safety cabinet*

This includes breaking of an LJ slope, dropping a Petri dish or spilling contents of a sputum jar inside the BSC.

- Remove gloves if soiled and discard in the plastic bag provided in the cabinet. Put on clean gloves from the spill kit.
- Cover the spill with absorbent paper towel. Soak with disinfectant from spill kit and completely wet the area.
- Leave the room for at least 2 hours, leaving the BSC operating.
- Put on mask, gloves and gown to re-enter the laboratory.
- Place all broken tubes and containers and clean-up material in the disposal bag provided in the spill kit.
- Clean the inside of the BSC and mop all floor and worktops with disinfectant.
- Record details in the accident log.

5.6.2 Large volume aerosol spill in the biological safety cabinet

This includes breaking a tube of liquid culture.

- Evacuate the room immediately and do not enter for at least 4 hours. Post “DO NOT ENTER” sign on the outer door and seal the doors with biohazard tape. Leave the BSC operating.
- Put on protective equipment before re-entering the room.
- Soak the spill with disinfectant from spill kit and leave for at least 30 minutes.
- Place all broken tubes and containers and clean-up material in the disposal bag provided in the spill kit.
- Decontaminate the BSC using formaldehyde (see SOP-UG-E-001)
- Mop all floor and worktops with disinfectant.
- Record details in the accident log.

5.6.3 Limited volume spill outside biological safety cabinet

This includes breaking of an LJ slope, dropping a Petri dish or spilling contents of a sputum jar outside the BSC.

- Put on clean gloves, mask and gown. Discard soiled gloves in the discard bag in the spill kit.
- Remove gloves if soiled and discard in the plastic bag provided in the cabinet. Put on clean gloves from the spill kit.
- Cover the spill with absorbent paper towel. Soak with disinfectant from spill kit and completely wet the area.
- Leave the room for at least 2 hours. Put on mask, gloves and gown to re-enter.
- Place all broken tubes and containers and clean-up material in the disposal bag provided in the spill kit.
- Mop all floor and worktops with disinfectant.
- Record details in the accident log.

5.6.4 Large volume spill outside biological safety cabinet

- Evacuate the room immediately and do not re-enter for at least 4 hours. Post a “DO NOT ENTER” sign on the outer door and seal the doors with biohazard tape.
- Put on protective equipment before re-entering the room.
- Soak the spill with disinfectant from spill kit and leave for at least 30 minutes.
- Place all broken tubes and containers and clean-up material in the disposal bag provided in the spill kit.
- Mop all floor and worktops with disinfectant.
- Record details in the accident log.

A ***Laboratory Accident Flowchart*** summarizing actions in the event of laboratory accidents is posted on the wall in the specimen processing laboratory (See ANNEX 2).

5.7 After hours work

- After hours work may only be performed after permission has been granted by the Head of Laboratory Research. No work that involves the potential of a major spill may be performed after hours. If such work is unavoidable, at least two staff members must be present in the laboratory after hours.
- Staff must notify the Head of Laboratory and the senior technologist on duty at NTRL of their presence in the laboratory and the NTRL technologist on duty must be informed when the work is completed.

5.8 Training

- All personnel working in the FIND Research Laboratory must undergo biosafety training before starting work in the laboratory. Theoretical training by means of reading, presentations and feedback on safety issues will initially be undertaken. Following this, employees will undergo a period of fully supervised hands-on practical training alongside a trained member of staff. Only after competence has been demonstrated in general biosafety and specific techniques, will the employee be allowed to perform tasks independently.
- Biosafety training will be carried out on a regular basis (at least every year, or when new staff members start) or when new procedures are adopted.
- All new staff members, irrespective of their previous experience, will be expected to undergo biosafety training at FIND research laboratory. A needs assessment will be undertaken to establish the requirement for supervised hands-on training in particular techniques.
- For further details

See: ***Training of Laboratory Personnel_ Uganda QP 07-01-02.doc***

Location: *Hard copy: FIND Uganda SOPs*

5.9 Laboratory audit

A laboratory safety audit will be carried out at regular intervals (at least 3 times per annum) and after any major laboratory accidents according to *Laboratory Audit_Uganda QP 07-01-02*. A written report and recommendations for corrective actions will be disseminated to all laboratory staff members.

See: *Laboratory Audit_Uganda QP 07-01-02.doc*

Location: *Hard copy: FIND Uganda SOPs*

6. REFERENCES

Kent PT, Kubica GP. Public Health Mycobacteriology. A guide for the Level III Laboratory. US Department of Health and Human Service. Public Health Service, Centers for Disease Control, 1985.

World Health Organisation. Laboratory Services in Tuberculosis Control. Part 1. Organisation and Management. 1998. WHO/TB/98.258.

7. ANNEXES

ANNEX 1. Layout of FIND Research Laboratory

ANNEX 2. Laboratory Accident Flowchart

8. CHANGE HISTORY

New version # / date	Old version # / date	No. of changes	Description of changes	Source of change request

ANNEX 1. Layout of FIND Research Laboratory

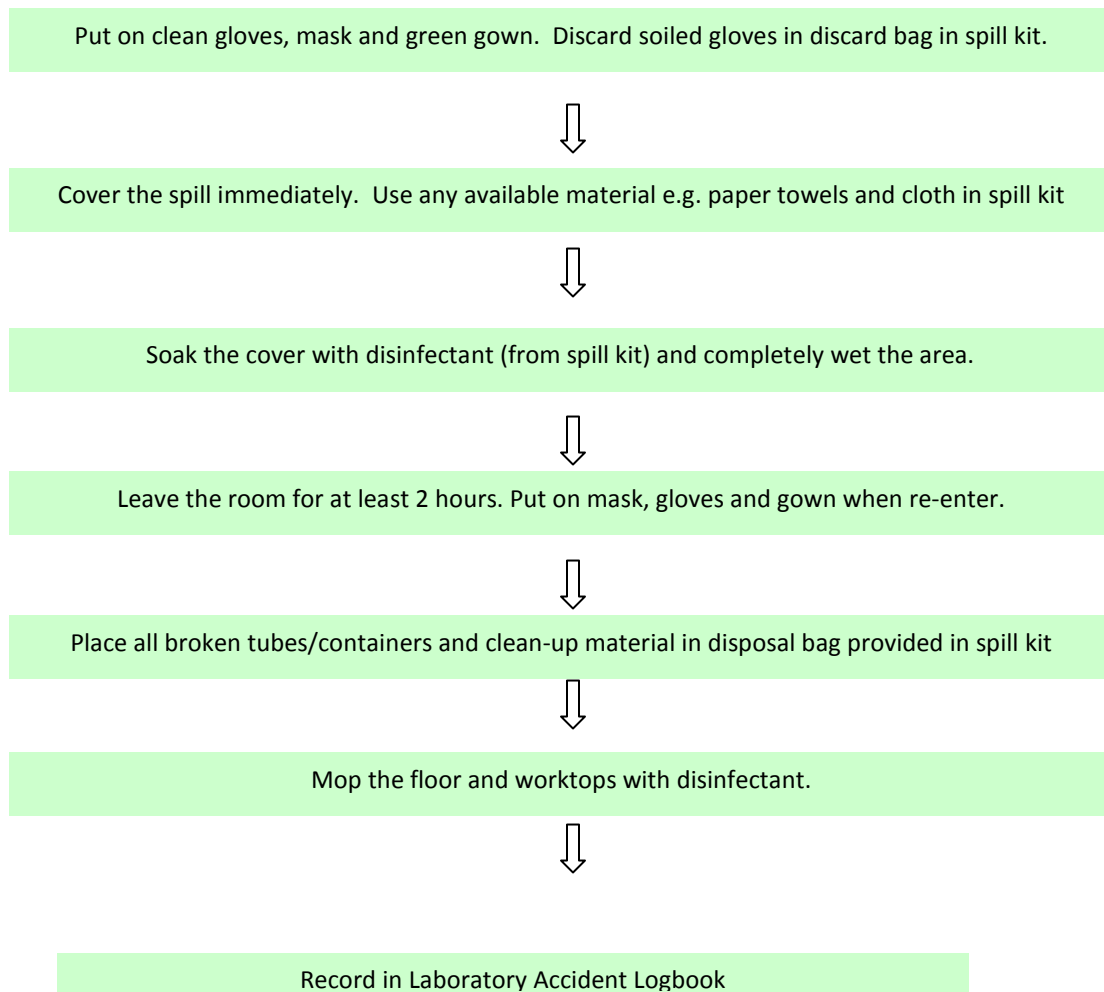
ANNEX 2. Laboratory Accident Flowchart

Laboratory Accident Flowchart.doc

Document type: flowchart	LABORATORY ACCIDENT FLOWCHART
Confidentiality: confidential	

Limited Aerosol Accident outside BSC

(e.g. breaking of LJ slope, dropping Petri dish or spilling contents of sputum jar)



Large Volume Aerosol Accident outside BSC

(e.g. breaking of tube of liquid culture, tube breaking in the centrifuge)

Evacuate the room immediately and do not re-enter for at least 4 hours. Post “DO NOT ENTER” sign on the outer door and seal the doors with biohazard tape



Put on protective equipment before re-entering room.



Soak the spill with disinfectant (from spill kit) and leave for at least 30 minutes.



Place all broken tubes/containers and clean-up material in disposal bag provided in spill kit



Mop the floor and worktops with disinfectant.



Record in Laboratory Accident Logbook

Limited Aerosol Accident in the Biological Safety Cabinet

(e.g. breaking of LJ slope, dropping Petri dish or spilling contents of sputum jar)

Put on clean gloves, mask and green gown. Discard soiled gloves in discard bag in spill kit.



Cover the spill immediately. Use any available material e.g. paper towels and cloth in spill kit



Soak the cover with disinfectant (from spill kit) and completely wet the area.



Leave the room for at least 2 hours, leaving the BSC operating.



Place all broken tubes/containers and clean-up material in disposal bag provided in spill kit



Clean the inside of the BSC and mop the floor and worktops with disinfectant.



Record in Laboratory Accident Logbook

Large Volume Aerosol Accident in the BSC

(e.g. breaking of tube of liquid culture)

Evacuate the room immediately and do not re-enter for at least 4 hours. Post “DO NOT ENTER” sign on the outer door and seal the doors with biohazard tape.



Put on protective equipment before re-entering room.



Soak the spill with disinfectant (from spill kit) and leave for at least 30 minutes.



Place all broken tubes/containers and clean-up material in disposal bag provided in spill kit



Mop the floor and worktops with disinfectant.



Decontaminate the BSC using formaldehyde (see [Uganda OP 07-06-01](#))



Record in Laboratory Accident Logbook