



## Module 2

### Safety Precautions for Tuberculosis Microscopy

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<b>Purpose</b>	To provide you with an understanding of safe handling techniques and precautions while performing AFB smear microscopy
<b>Prerequisite Modules</b>	None
<b>Learning Objectives</b>	<p>At the end of this module, you will be able to</p> <ul style="list-style-type: none"><li>▪ Explain airborne transmission of TB</li><li>▪ Describe the risks when collecting sputum</li><li>▪ Describe personal health and safety practices</li><li>▪ Describe why there should be three discrete areas in the TB laboratory</li><li>▪ Describe methods for the disposal of contaminated material</li><li>▪ Describe chemical safety precautions in the laboratory.</li></ul>
<b>Content Outline</b>	<ul style="list-style-type: none"><li>▪ Transmission of TB bacilli</li><li>▪ Proper collection of sputum</li><li>▪ Laboratory arrangement</li><li>▪ Safety practices in the TB microscopy laboratory</li><li>▪ Safe disposal of infectious waste</li><li>▪ Chemical safety</li></ul>
<b>Handout and Exercises</b>	None
<b>Appendix</b>	None

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## **Module 2: Safety Precautions for Tuberculosis Microscopy**

The most important factor in the prevention of laboratory-acquired infection is good technique on the part of the individual worker. Specialized equipment may aid good laboratory practice but does NOT replace it.

Aerosols may be produced in the TB laboratory when handling leaking specimens, opening sample containers, and preparing smears. When care and appropriate techniques are used, handling sputum presents a minimal risk of acquiring infection to a technician.

For laboratory staff, the greatest risk of infection involves sputum collection. People with suspected TB may cough and in doing so, spread TB bacilli in tiny droplets in the air which may infect others when they are inhaled. Precautions must be taken to minimize this exposure.

The laboratory technician is at considerably more risk when sputum is processed for culture and drug susceptibility testing. These procedures require shaking and centrifugation. Consequently, special equipment such as biological safety cabinets, which are costly to purchase and maintain, are required. However, this equipment is not justified in the AFB smear microscopy laboratory.

### **Transmission of TB bacilli**

The TB bacilli are almost always transmitted by patients with active pulmonary disease. The patient expels TB bacilli in small droplets of respiratory secretions. These secretions quickly evaporate leaving "droplet nuclei" less than 5  $\mu\text{m}$  in diameter. Droplet nuclei of this size containing 1–3 bacilli can remain suspended for long periods of time in the air and, following inhalation, are able to reach deep into the lungs to produce infection. Larger particles do not remain airborne for as long and do not transmit tuberculosis as efficiently.

The risk of infection depends on (1) the infectiousness of the source, (2) the environment (e.g., overcrowding and inadequate ventilation promote transmission of droplet nuclei), (3) the duration and intensity of exposure, and (4) the susceptibility of the recipient.

Smear-positive patients have  $10^6$ – $10^7$  bacilli per millilitre of sputum whereas smear-negative patients have about  $10^4$  or less per millilitre. This difference in bacterial load (as determined by smear status and radiologic extent of disease) is the most significant predictor of the infectiousness of a patient. Household contacts of smear-positive patients have tuberculin positivity rates of 30%–50% compared with contacts of smear-negative patients who have tuberculin positivity rates of only about 5%.

The infectiousness of the patient may also depend on how often that person coughs. Coughing is a good mechanism for producing droplet nuclei and a higher prevalence of tuberculin reactivity has been reported among contacts of frequent coughers (i.e., people who cough >48 times per night) than among contacts of infrequent coughers (i.e., people who cough <12 times per night). Interestingly, singing produces infectious droplet nuclei as effectively as coughing

and several outbreaks in choirs have confirmed that singing can spread infection. However, while coughing and singing may increase the contagiousness of a patient, the radiologic extent of disease and smear status remain the best indices of infectivity.

## **PROPER COLLECTION OF SPUTUM**

Collecting sputum represents the greatest hazard to a laboratory technician because infectious aerosols may be produced by coughing.

If a coughing patient comes into the laboratory, ask them to cover their mouth.

Wherever possible, collect specimens outside where air movement will rapidly dilute infectious droplets and UV rays from the sun will rapidly inactivate TB bacilli. NEVER collect sputum specimens in laboratories, toilets, waiting rooms, reception rooms, or any other enclosed space.

Always stand well clear and upwind when a patient is collecting a sputum sample.

## **LABORATORY ARRANGEMENT**

Ideally, the TB laboratory should be a well-ventilated area which is dedicated to microbiology with restricted access. Three separate areas are recommended for performing TB microscopy.

1. **Smear preparation and staining:** This area should be well lit and preferably near an open window to ensure adequate ventilation during smear preparation. A sink with running water is also required.
2. **Performing microscopy:** This area should have a flat bench or table for placement of the microscope. Subdued lighting is preferred. If no electricity is available, daylight must be used as the light source; in this case, place the microscope directly in front of a window.
3. **Record keeping and storage:** This third area is for entering data into the log book for Quality Control and for storing slides.

## **SAFETY PRACTICES IN THE TB MICROSCOPY LABORATORY**

Take the following precautions to protect yourself and all laboratory personnel.

- Assume ALL specimens are potentially infectious
- Never smoke, eat, or drink in the lab
- Wash hands frequently with soap and water at least before and after performing any procedures
- Establish airflow in working areas that will direct potentially infectious particles away from personnel. Air must be exhausted into a remote area. An extraction fan can be useful to vent air from a smear preparation area with poor ventilation that is closed off due to extreme climatic conditions.
- Do not rely on laboratory coats to protect you against infection with TB. They are useful protection against strong chemicals, staining reagents, and accidental spills but they will not prevent TB infection.
- Always follow safety procedures

### **Gloves**

Gloves do not provide any appreciable protection against airborne transmission of *M. tuberculosis*. Gloves are not required to prepare sputum smears and lack of their availability does NOT mean that sputum smears cannot be prepared. Indeed, wearing gloves can give technicians a false sense of safety and may result in contaminated gloves being used to handle or operate equipment that may otherwise not become contaminated (e.g., microscope or telephone).

If gloves are used, there should be a guaranteed supply. Reusing single use gloves is not advised. Never wear gloves outside the laboratory. Discard gloves at any interruption of smear preparation.

Hand washing and careful techniques are mandatory for safe laboratory practice in all countries.

### **Laboratory Coats**

Laboratory Coats are not required to undertake specimen collection or perform sputum microscopy. A lack of laboratory coats does NOT mean that sputum microscopy cannot be performed. If they are available, laboratory coats of various sizes should be provided (and cleaned) by the laboratory organisation. They should be tied at the back, not the front, and be made from water-resistant materials to avoid liquids soaking into the gown.

Laboratory coats must NOT be worn outside of the laboratory.

### **Masks**

One of the greatest false beliefs is that a standard surgical mask will protect the wearer from becoming infected with TB. These masks are made from porous material that will not trap TB bacilli, and have an extremely poor fit creating large gaps between the face and mask.

N95 "duck-bill" respirators (often incorrectly referred as "masks") and particulate respirators are expensive and are not necessary for laboratory technicians

carrying out sputum smear preparations only. Such equipment must be selected and fitted correctly to be functional.

### **Appropriate Disinfectants**

Phenolic agents (5% phenol in water or a phenolic disinfectant product diluted as per label) are excellent disinfectants for cleaning up sputum spills and for decontaminating equipment and single use items prior to disposal. Fresh household bleach (5% sodium hypochlorite) diluted 1:10 with water can also be used as a general disinfectant. Bleach solution works well for cleaning up blood spills, however, it is somewhat less effective than phenolic agents against TB. It is important that bleach dilutions be made fresh since it loses potency with time. Seventy percent alcohol is a good agent for cleaning bench tops.

**Surgical masks do NOT protect against TB infection as TB bacilli can pass through these masks. Therefore, surgical masks provide a false sense of protection.**

**Effective respiratory protection, such as an N95 respirator, is expensive and unnecessary if the technician uses appropriate technique.**

**Gloves are not required for use in smear preparation since TB infection is acquired by airborne inhalation.**

**Each country must evaluate the risks and decide on the level of protection that is appropriate with the resources that may be available.**

**Hand washing and careful techniques are mandatory for safe laboratory practice in all countries.**

### **Take the following safety precautions before and during laboratory procedures.**

- Reject broken or leaking containers. Request another specimen.
- Once collected, allow a sputum specimen to stand undisturbed for at least 20 minutes before opening to settle any aerosols.
- Cover sputum containers with their lids at all times except when removing specimen for smear preparation.
- Open sputum containers with care and away from the face. Gently open the sputum container, especially if the lid clicks or snaps on.
- Do not forcefully shake or stir the sputum in the container.
- Move slowly and carefully while sampling sputum particles and smearing onto slide.
- Avoid any rapid motion when making the smear as infectious aerosols may be produced.

## **Safety practices during procedures**

- Disinfect the working area before and after smear preparation. Immediately cover any sputum spills with disinfectant before cleaning up the area. A phenolic or hypochlorite disinfectant is appropriate.
- Where available, use disposable wooden sticks for smear preparation. Discard into a receptacle immediately after use.
- If wire loops are used, remove residual sputum on the wire loop before flaming. Do so by inserting the wire loop into a sand-alcohol flask and either moving it up and down or by rotating the loop. Never put a wire loop into a flame when sputum is still attached to it as sputum particles containing live AFB will produce infectious aerosols.
- Always keep a discard receptacle containing disinfectant in the immediate area.

After sputum is smeared onto the slide, let the slide air dry for 15–20 minutes. Wet slides can produce aerosols if disturbed. Do not flame slides to expedite drying. This can produce dangerous aerosols.

Fix smears by flaming only after they have dried completely.

## **SAFE DISPOSAL OF INFECTIOUS WASTE**

After smears have been processed, place all infected materials including closed sputum containers in a discard bag (polyethylene, if available).

Discard applicator sticks used for smearing immediately after use.

Since all sputum specimens are considered potentially infectious, treat all materials in the procedure as contaminated.

Discard specimens by one of the following methods:

- Burning
- Burying
- Autoclaving

To protect the surrounding population, the laboratory must dispose of waste safely. Burning waste in an incinerator is usually the most practical way for safe destruction of laboratory waste. If safe burning cannot be arranged, discard the waste in a deep pit of at least 1.5 meter depth. If an autoclave is available, place infected materials inside and follow procedures for safe and adequate sterilization.

## CHEMICAL SAFETY

AFB microscopy requires the use of several hazardous chemicals. These include concentrated acids, alcohols, and phenol. Take the following precautions when working with chemicals in the TB microscopy laboratory:

- Always wear laboratory coats, gloves, and safety glasses when handling strong acids.
- Take particular care in diluting concentrated acids. **ALWAYS ADD THE CONCENTRATED ACID TO WATER.** This avoids splashes of acid causing burns to the skin or eyes.
- Do not use alcohols near an open flame as they are flammable.
- Phenol is a toxic chemical. Avoid direct contact with the skin or mucus membranes. Reduce exposure to phenolic fumes by staining smears in a well-ventilated area and by limiting the number of slides in each staining batch to a maximum of 12.

### Key messages



- The greatest risk to a laboratory worker is a patient coughing and not the patient's sputum specimen.
- Never collect sputum in the laboratory.
- Never smoke, eat, or drink in the lab.
- Wash your hands frequently with soap and water at least before and after performing any procedures.
- Gloves, laboratory coats, and surgical masks do not provide any appreciable protection against airborne transmission.
- Protect the surrounding population by disposing of laboratory waste safely.
- Avoid hazards that may occur in a TB laboratory by paying careful attention to safety procedures.
- Always work carefully and in a safe manner.



## Module Review: Module 2

Find out how much you have learned by answering these questions.

**How is TB transmitted from person to person?**

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**What are the appropriate laboratory disinfectants?**

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**What precautions must you take when handling specimens?**

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**Why do surgical masks offer little protection against TB?**

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**What precautions should you take to prepare dilutions of strong acid?**

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