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From Basic Science  
to Patient Care



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## Chapter 11: Biosafety and Hospital Control

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### 11.1. Biosafety in the hospital

#### 11.1.1. Introduction

Tuberculosis (TB) drug treatment can be carried out mainly at the ambulatory level, but the diagnosis of the disease is not always accomplished in the ambulatory setting. In big cities of developing countries, the diagnosis of TB is often made in the hospital before these patients are assisted at the local or regional outpatient centers. The reference treatment centers for hospitalizing TB patients are frequently the only health centers with specialized ambulatory facilities for assisting these patients, including those with co-morbidities. The percentage of patients diagnosed in hospitals may be 30 % or even higher. This kind of situation favors the exposure to TB infection in the nosocomial environment.

Around 25 to 50 % of the persons exposed to an intimate contact with active pulmonary TB will become latently infected with *Mycobacterium tuberculosis*. Exposure to the index case for 12 or more hours implies a high risk of infection, especially in closed environments without biosafety precautions. Immunosuppressed persons have an increased risk of infection and active disease compared with immunocompetent persons. Initially, the evaluation of the risk of transmission of TB within a health institution can be classified as follows:

- Low, if the institution admits less than six patients with active pulmonary TB per year or if it has more than 100 healthcare professionals per hospitalized pulmonary TB patient per year;
- High, if there are less than 10 healthcare professionals per hospitalized pulmonary TB patient per year (Menzies 1995, Hopewell 1986, Harries 1997).

In the last decade, high rates of drug-resistant TB have been described in prisons and hospitals. Thus, it is essential that health facilities are adequate to assist patients with active pulmonary TB or those suspected of having TB in order to reduce the risk of *M. tuberculosis* transmission to healthcare personnel and other sick people, mainly immunosuppressed patients.

### 11.1.2. Healthcare Units

TB biosafety measures are often neglected. This increases the possibility of *M. tuberculosis* nosocomial transmission. During the '90s, transversal and longitudinal studies were accomplished on the risk of TB infection in general, as well as in reference and teaching hospitals in developed and developing countries. These studies identified a high rate of nosocomial transmission of TB to medicine, nursing, and physiotherapy students, as well as to healthcare personnel (Roth 2005, Alonso-Echanove 2001, Kruuner 2001, Harries 1997, Cuhadaroglu 2002, Do 1999, Tan 2002, Silva 2002, Resende 2004).

### 11.1.3. Tuberculosis infection control activities

Assuming the political commitment of the managers of public or private hospitals and the fulfillment of the international legislation suggested by the World Health Organization (WHO), TB transmission control measures in a health unit can be hierarchized into three levels: administrative, engineering, and individual protection (Jensen 2005, World Health Organization 1999, British Thoracic Society 2000).

Initially, the administrative measures can be deemed the most important. Besides being comprehensive, they are generally related to the permanent education and training of the healthcare personnel aimed at the implementation and appropriate fulfillment of the established norms.

The administrative measures include the evaluation of:

Number of pulmonary TB cases assisted annually in the health unit

- Number of annual pulmonary TB cases among the healthcare personnel
- Risk profile of the unit, by sector:
  - Low: < 6 pulmonary TB patients per year
  - Intermediate:  $\geq 6$  pulmonary TB patients per year and an annual average risk of TB infection (tuberculin skin test conversion) lower than 2 % among healthcare personnel
  - High:  $\geq 6$  patients with pulmonary TB and an annual risk of TB infection among healthcare personnel higher than 2 %

- Areas that potentially present a higher risk of transmission:
  - respiratory isolation rooms
  - ambulatory and phthisiology waiting rooms
  - thoracic radiology room
  - bronchoscopy and sputum induction rooms
  - pentamidine nebulization room
  - ventilatory assistance areas
  - day-hospital
  - emergency rooms
  - autopsy room
  - microbiology/mycobacteria laboratory

#### **11.1.4. General practices**

##### **11.1.4.1. Management of hospitalized pulmonary tuberculosis patients**

- The head nurse of the unit must have autonomy to isolate the patient if there is clinical suspicion of airborne TB.
- The patient in isolation must stay under the responsibility of the service that admitted him/her.
- The patients in isolation must be instructed to cover their mouth and nose when they cough or sneeze, even inside their room.
- The tests to be accomplished on the patients in isolation should be done as soon as possible, so that they spend a minimum time outside their room; the patient should not wait for the tests in the waiting rooms of the different services.
- When the patient needs to leave the room, a surgical mask must be used.
- Healthcare personnel must avoid unnecessary entry into the isolation rooms; in the same way, the number of visitors and attendants should be restricted to the smallest number possible. In this case, everyone should enter the isolation room using special masks (N95 or PFF2 respirators).

- In case of need, the priority in the isolation will be given to patients with acid fast bacilli (AFB) smear-positive microscopy results (bacilliferous) and shorter time of treatment.
- The patients with airborne TB (or suspicious cases) still in the infectious period should not be submitted to surgery unless in case of emergency.
- The hospital discharge of respiratory TB patients should be accomplished in the shortest possible time span.

### **Case searching**

Instruct the healthcare personnel in the screening area, emergency department, at the admission and discharge area to suspect TB in:

- respiratory symptomatic patients (cough with expectoration for more than three weeks)
- contacts of active pulmonary TB cases for more than 12 hours
- pulmonary TB radiological suspects
- persons with predisposition to TB (immunosuppression, diabetes)

### **Diagnosis**

- Ready request, accomplishment and release of the sputum AFB smear microscopy results in persons with presumptive TB diagnosis
- AFB smear microscopy result in 24 hours, at most
- Optimization of the diagnostic procedures, implementation of *M. tuberculosis* complex identification techniques and of anti-tuberculosis drug susceptibility testing

Every healthcare professional with signs or consistent symptoms of pulmonary TB should seek medical help and be submitted to laboratory tests (sputum AFB smear microscopy, when clinical specimen is available) and thoracic radiography. Until the pulmonary TB diagnosis is ruled out or the patient is considered non-infectious, healthcare workers with pulmonary disease should stay away from their activities. Healthcare personnel must be informed that patient care activities are not suitable for those harboring an immunosuppressive condition, such as human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), malign neoplastic disease or deficient cellular immunity. HIV testing and counseling should be offered to all healthcare professionals.

**Creation of a TB control committee responsible for:**

- evaluating annual trends of *M. tuberculosis* drug resistance in the institution
- identifying transmission risk areas
- performing operational studies for the surveillance of compliance with biosafety norms
- accomplishing biosecurity educational activities for the healthcare personnel
- accomplishing periodic tuberculin skin testing in the healthcare personnel
- preventing recently infected persons from becoming ill; indicating chemoprophylaxis to the healthcare personnel with latent TB infection.

**11.1.4.2. Guidelines for patient isolation**

- Suspected or confirmed airborne TB cases
- HIV-positive respiratory symptomatic patient with any change in thoracic X-ray
- HIV-negative patient with a radiological image suggestive of pulmonary TB (hypotransparency in the superior lobes of the lung or in segment six, or with a diffuse micronodular infiltration, suggestive of miliary disease)
- Patient with a request for sputum AFB microscopy examination and/or culture
- The head nurse of the unit must have autonomy to isolate the patient if there is clinical suspicion of respiratory TB

**Respiratory isolation area**

- Must be an individual room, with the door closed and windows that can be opened
- If absolutely necessary, two pulmonary TB patients can share an isolation room, provided they both have confirmed TB diagnosis and there is no epidemiological suspicion of drug-resistant TB (such as patients that have not received previous treatment and have not had contact with drug-resistant TB cases).

### **Time for discharge from respiratory isolation**

- In confirmed TB cases under a treatment scheme containing rifampicin: after two weeks of treatment and with three sputum AFB smear-negative results or one induced sputum or bronchoalveolar lavage AFB smear-negative result
- In confirmed TB cases under a treatment scheme not containing rifampicin: after four weeks of treatment and with three sputum AFB smear negative results or one induced sputum or bronchoalveolar lavage AFB smear-negative result
- If one of the tests is positive, repeat the series after one week
- In suspicious cases: with three negative sputum-smear microscopy or one induced sputum or bronchoalveolar lavage with negative AFB smear microscopy

#### **11.1.4.3. Ambulatory assistance under a standardized Reference Health System**

- Signal the TB risk areas of the unit
- Avoid movements of confirmed or suspected TB patients inside the health unit in order to minimize contact with people awaiting assistance for other ambulatory specialties in waiting or examination rooms
- In outpatient units where ambulatory patients with pulmonary TB and conditions other than TB are assisted in the same places and/or by the same healthcare personnel, consultation appointments should be separated in different hours or days, in such a way to avoid TB exposure of uninfected persons, mainly those who are immunosuppressed
- Avoid crowding in the waiting rooms, assigning consultations to specific days for TB and setting a time for consultation, giving priority to the assistance of infectious patients and suspicious cases, thus avoiding the gathering of potentially infectious patients
- Avoid assisting immunosuppressed patients and children less than 5 years old in rooms contiguous to the ones assisting TB patients
- Offer surgical masks or tissue paper to infectious or suspected TB patients, mainly when circulating through the unit (specialist consultation, X-ray exams, sputum delivery to the laboratory, search for exam results, etc.)

- Instruct patients to cover their mouth and nose when they cough or sneeze.

#### **11.1.4.4. Engineering measures**

Engineering measures for TB control are architectural and technical devices aimed at the adaptation of the unit, or of a certain area, to provide care to pulmonary TB patients. The implementation of such measures contributes to decreasing the risk of TB transmission and should be directed by qualified personnel with special knowledge on the characteristics of TB transmission.

#### **Objectives**

Basically, the objective is the removal or dilution of infectious particles taking into account the following factors:

- Ventilation exhaustion: captures and removes contaminants suspended in the air near the source (patient)
- General ventilation: ventilation rate or number of air changes per hour; for example, a complete air exchange per hour of a certain area reduces the concentration of infectious particles by 63 %, while six complete exchanges are needed to reduce it by 99 %
- Direction of the air flow within the facilities: contains the contaminated air in a certain area of the facility and prevents spread into non-contaminated areas
- Negative pressure in the room with directional flow: contains the contaminated air in a certain area of the room and prevents spread to non-contaminated areas
- Adjustment of air flow pattern inside the room: prevents air stagnation short circuit
- Air filters and/or ultraviolet (UV) light: disinfect air

#### **High efficiency particulate air (HEPA) filters**

HEPA filters or absolute filters are those able to remove 99.97 % of particles with a diameter larger than 0.3  $\mu\text{m}$  which pass through them. They can be placed in exhaustion ducts, in room ceilings or in movable filtration units.

The use of HEPA filters and/or UV light is strongly recommended for rooms where the following procedures take place: bronchoscopy, induced sputum, pentamidine nebulization, necropsy, and isolation. The combination of an adequate number of

air changes with negative pressure and a HEPA filter or UV light minimizes the risk of transmission in the environment in which the TB patient is assisted and in the area where the air is exhausted. The germicidal efficiency of the UV light is limited to its area of direct incidence and decreases with time.

HEPA filters are used:

- To purify the exhaustion of air of contaminated environments
- To recirculate the air inside the room or to other rooms facilitating the number of air changes per hour.

### **Basic engineering recommendations**

In areas with a high risk of infection, the main engineering measure is to facilitate ventilation so that the particles suspended in the air are removed at the highest speed possible. The speed of air removal is calculated in air changes per hour and should be:

- six air changes per hour for the isolation, the ambulatory, the X-ray, the waiting and the emergency rooms, and the ventilatory assistance areas
- twelve air changes per hour for the bronchoscopy, the sputum induction, the pentamidine nebulization and the autopsy rooms and the mycobacteria laboratory

### **The use of negative pressure**

Negative pressure prevents the dispersion of contaminated air into areas where people walk, mainly those in common use such as corridors. The exhaust air should never be directed towards these transit areas. If safe air exhaustion is not possible, the exhausted air should be filtered or sterilized.

### **Respiratory isolation room**

The isolation room must:

- Be private and with suitable ventilation characteristics
- Be under negative pressure
- Be submitted to six or more air changes per hour
- Have air exhaustion to the open-air
- Have HEPA filters if the air is recirculated or exhausted to circulation areas

- Have anterooms (they increase isolation effectiveness, minimizing the escape risk)
- Have UV light (optional)
- Be submitted to six air changes between a patient's discharge and the following patient's admission

### **Outpatient clinic**

In areas dedicated to ambulatory care, the minimum biosecurity conditions should include:

- Adequate (ventilated and sunny) site for sputum collection, preferentially outdoors
- Air flow adaptation of the waiting and consultation rooms, avoiding the use of ceiling fans; air conditioning is allowed only in combination with HEPA filters
- Suitable area for the waiting room, preferably outdoors, far away from any crowded area or other waiting rooms
- Within the assistance room, use of a standing fan either to direct the air flow towards the window (or door) or to produce an air "barrier" between the doctor and the patient
- The use of standing fans and exhaust fans in strategic points is a low-cost alternative to increase the number of air changes per hour
- Adaptation to the environment to which the air is being directed, avoiding other people being exposed to the risk of infection

#### **11.1.4.5. Individual Protection**

##### **Measures for healthcare personnel at risk**

- Masks: they can be of the N-95 type, with a National Institute for Occupational Safety and Health (NIOSH) certification of the United States (US) or of the PFF-2 type, with international standards certification; common surgical masks are not advisable: their effectiveness in preventing the inhalation of particles with diameters of 1 to 5  $\mu\text{m}$  is less than 50 % (they were specifically designed to prevent the exhalation of particles)

- The protection masks should be supplied by the health service where the TB patients are assisted, preferably in various sizes and models
- Even if administrative and environmental control measures are in force, healthcare workers should wear appropriate respiratory protection devices (N95 or PFF2) at all times while they are in patients' rooms, during bronchoscopy, induced sputum, pentamidine nebulization, surgery or autopsy performed on suspected or confirmed TB cases
- Instruct the personnel on the correct use of the special masks, reminding male employees that they should have their faces shaved as beards and/or mustaches can prevent perfect adjustment of the mask to the face
- Special masks (N95 or PFF2 respirators) can be used for indeterminate periods of time, as long as they are kept dry, clean and intact (without any torn, frayed or crumpled areas); their storage in plastic bags after use must be avoided because bags retain humidity

**For TB patients transiting through the institution**

- Indicate the use of common surgical masks for the respiratory symptomatic patients as soon as they enter the unit (triage, emergency, ambulatory, when being admitted or when passing through). The surgical masks work as a barrier, capturing the damp particles (usually larger than 5  $\mu\text{m}$ ) and, therefore, do not work as filters.
- In the day-hospital sector, HIV-negative patients who have been coughing for more than three weeks should wear a common mask all the time whilst there; HIV/AIDS patients with any respiratory symptom should use a common mask all the time. When the engineering measures are not working in the room, the asymptomatic patient in the same setting should be instructed to use a special mask (N95 or PFF2), particularly if immunosuppressed.

### 11.1.5. Tuberculin skin test survey

The evaluation of the risk of infection from *M. tuberculosis* (through tuberculin investigation) should be performed on healthcare personnel in the following situations:

- Recently admitted personnel
- Personnel that report frequent contact with pulmonary TB patient

It is important that every health unit knows the prevalence of TB infection and TB disease among the healthcare personnel. In this sense, the healthcare worker that reports a past history of active TB or household contact with a pulmonary TB case in the last two years must be submitted to medical examination, tuberculin testing and a chest X-ray.

A tuberculin skin test (TST) should be applied and read by one of a limited-number of trained nurses tested for intra- and inter-reader variability. Tuberculin purified protein derivative (PPD) will be injected subcutaneously and the amount of induration should be measured at 48–72 hours. For healthcare workers with an induration < 10 mm, the tuberculin skin test should be repeated 7–10 days later. Those with a two-step tuberculin skin test < 10 mm should be asked to undergo a repeated tuberculin skin test 6–12 months later. Those with a tuberculin skin test  $\geq$  10 mm and those who experienced a tuberculin skin test conversion should undergo a medical evaluation to rule out TB disease.

Since 1995, bacille Calmette-Guérin (BCG) revaccination has not been recommended by the WHO. Few countries still maintain the use of BCG revaccination. Recently, in an open, randomized clinical trial performed in Brazilian children, it was found that a second BCG vaccination at school age has low effectiveness. Because of these results and those described in the international literature, BCG vaccination is no longer recommended for healthcare personnel in some countries, including Brazil (Rodrigues 2005).

According to a study performed in the US, tuberculin investigation every 12 months in areas with a high risk of TB infection would be more cost-effective than other measures for preventing TB. Chemoprophylaxis should be indicated to recent PPD converters (induration increase of 10 mm in relation to the last test) (Nettleman 1997).

### 11.1.6. Recommendations

Flaws related to biosecurity measures (administrative, environmental or of individual protection) are factors known to be associated with higher nosocomial TB transmission.

The primary tuberculous infection may manifest itself as a light respiratory condition with hardly any clinical or radiological signs. Consequently, it usually remains undiagnosed. During this process, *M. tuberculosis* spreads both lymphatically and hematogenously and the bacilli implanted in extrapulmonary organs or tissues are a potential source of subsequent reactivation. Generally, the tuberculin skin test is the sole indication that *M. tuberculosis* infection has occurred.

It is estimated that 10 % of individuals infected with *M. tuberculosis* will develop active TB at some time during their lifetime. The risk of becoming sick with TB is highest in the first two years after the infection, when about 5 % of infected individuals undergo a progression from latent to active disease. The other 5 % can develop active TB at any time in their lifetime if they do not receive the treatment recommended for latent infection.

Even in places where TB is endemic and BCG vaccination is universal, the result of the tuberculin skin test reflects, with reasonable accuracy, exposure to *M. tuberculosis*. In countries with a high prevalence of TB, in which 25 to 50 % of the population is considered to be infected by *M. tuberculosis*, the tuberculin skin test is highly specific and a positive result has a high probability of indicating tuberculous infection. The adequate establishment and fulfillment of TB biosafety measures are the tools needed to reach the goal of reducing the annual risk of infection in healthcare personnel to levels similar to those of the general population.

## 11.2. Biosafety in the laboratory

### 11.2.1. Introduction

Microbiology laboratories are unique and special work environments, where the handling of infectious organisms may pose risks of infection to the laboratory personnel or the surrounding community.

Several cases of infections acquired in the laboratory have been reported throughout the history of microbiology. By the end of the 19<sup>th</sup> century and the beginning of the 20<sup>th</sup>, reports had already been published describing laboratory-associated cases of typhoid, cholera, brucellosis, and tetanus. By the middle of the 20<sup>th</sup> century, a few publications reported cases of laboratory-related infections in the United

States. Some of these cases were attributed to carelessness or inappropriate techniques in the handling of infectious material (Meyer 1941, Sulkin 1949, Sulkin 1951).

A laboratory survey was updated in 1976 (Pike 1976) totaling 3,921 cases. Brucellosis, typhoid, tularemia, TB, hepatitis and Venezuelan equine encephalitis accounted for most of the infections. Not more than 20 % of these cases were associated with a documented accident. Exposure to infectious aerosols was considered to be a likely but unconfirmed source of infection in more than 80 % of the reported cases, in which the infected person had “worked with the agent”. Pike, in 1979, concluded that “*the knowledge, the techniques and the equipment to prevent most laboratory infection were available*” (Pike 1979).

The actual risk of a laboratory-acquired infection is difficult to measure because there is no systematic reporting system. Besides, surveillance data on laboratory-associated infections are difficult to collect because the infections are often sub-clinical and have an atypical incubation period and route of infection. Another problem is that laboratory directors may not report incidents for fear of reprisal or embarrassment (Sewell 1995).

The risk of exposure to infectious agents tends to be lower for laboratory workers than other groups of healthcare workers. However, the risk of laboratory-associated infection in employees of clinical and research laboratories is greater than that of the general population, suggesting that unique risks are associated with the laboratory work environment (Kiley 1992).

The advent of the HIV/AIDS epidemic in the early '80s and the fact that the rate of new cases of TB began to rise in 1986 in developed countries (Tenover 1993), put laboratory safety and safety programs in the spotlight. The safety concerns led to the elaboration of guidelines and manuals (Centers for Disease Control 1987, Occupational Safety and Health Administration 1991). A decrease in the occupational risks associated with working in a clinic or laboratory was observed after these guidelines were adopted (Fahey 1991, Wong 1991).

The term “containment” is used when describing safe methods for managing infectious material in the laboratory environment where they are handled or stored. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other people, and the outside environment to potentially hazardous agents.

**Primary containment:** protection of laboratory workers and the immediate laboratory environment from exposure to infectious agents is provided by both good

microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increase in the level of personal protection.

**Secondary containment:** protection of the environment outside the laboratory from exposure to infectious materials is provided by a combination of facility design and operational practices.

Therefore, the three elements of containment include laboratory practice and technique, safety equipment, and facility design. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements (Blumberg 2000, Blumberg 2004, Centers for Disease Control and Prevention 1994, Centers for Disease Control and Prevention 2005).

The most important element of containment is the strict adherence to standard microbiological practices and techniques. People who work with infectious agents or potentially infected materials must be aware of potential hazards and must be trained and proficient in the practices required for the safe handling of these materials. The director of the laboratory is also responsible for providing or arranging the appropriate training of personnel.

Each laboratory should develop or adopt a biosafety manual or operations manual that identifies the hazards that are or may be found in the laboratory, and that specifies practices and specific procedures designed to minimize or eliminate the exposure to such hazards. Personnel should be informed about the special hazards and should follow the necessary practices and procedures.

A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures and hazards associated with the handling of infectious agents must be responsible for the conduct of work with any infectious agent or infected material.

*M. tuberculosis* is repeatedly ranked within the top-five most common laboratory-acquired infections (Collins 1998, Miller 1987, Sepkowitz 1994, Seidler 2005). Pike reported that laboratory and mortuary workers exposed to tubercle material have a TB incidence rate three times higher than that of the general population and indicated that only 18 % of infections could be traced back to a known event (Pike 1976). Despite the current knowledge and biosafety measures in place, a recent report in New York demonstrated rates from 2 to 6.6 % of TB conversion among healthcare workers (Garber 2003).

In addition, surveys suggest that the actual incidence of laboratory-acquired infections due to *M. tuberculosis* is greater than the number of reported cases. The documentation of a case of laboratory-acquired TB is difficult because the source of the infection is often unclear, as a result of the potential for exposure outside of

the workplace and the long incubation period before the development of symptomatic disease (Collins 1993, Pike 1979). The incidence of TB in laboratory personnel is estimated to be three to nine times that of individuals in other job environments (Harrington 1976, Reid 1957, Saint-Paul 1972).

Manipulation of specimens or cultures that generate aerosols is the most important risk factor for acquiring TB in the laboratory. Aerosolization occurs frequently during autopsies, preparation of frozen sections of infected tissues, and procedures involving liquid cultures (Centers for Disease Control and Prevention 1981, US Department of Health and Human Services 1993).

*M. tuberculosis* presents a low infective dose for humans of less than 10 bacilli (Riley 1957, Riley 1961), suggesting a high risk for laboratory-acquired infection.

Due to the nature of this organism, containment level 3 (CL3) laboratory operational and physical requirements have been recommended for manipulation of the live organism in North America (US Department of Health and Human Services 1995). Therefore, one would hypothesize that working in a CL3 with personal protective equipment, including a respirator, would be adequate to protect the worker. However, as tuberculin skin test conversion is still occurring (Blackwood, 2005), other practices and causes should be analyzed.

These recommended measures are implemented by healthcare facilities in high-income countries, but given their high cost, few facilities in low-income countries can afford to implement them (Pai 2006).

The WHO has proposed practical and low-cost interventions to reduce nosocomial transmissions in settings where resources are limited, and these are available on the internet at <http://www.who.int/docstore/gtb/publications/healthcare/index.htm> (World Health Organization 1999). Several simple interventions can ameliorate working conditions, such as training and supervising laboratory workers in good techniques and biosafety practices to provide the necessary organization (DeRiemer 2000, Joshi 2006).

### 11.2.2. Laboratory biosafety levels

Infectious microorganisms are classified by risk group. This type of classification is to be used for laboratory work purposes only.

- **Risk Group 1** (no or low individual and community risk)

A microorganism that is unlikely to cause human or animal disease.

- **Risk Group 2** (moderate individual risk, low community risk)

A pathogen that can cause human or animal disease, but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposure may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

- **Risk Group 3** (high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

- **Risk Group 4** (high individual and community risk)

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

**Biosafety Level designations:** are based on a combination of the design features, construction, containment facilities, equipment, practices and operational procedures required for working with agents belonging to the various risk groups (World Health Organization 2004).

Laboratory facilities are designated as:

- Biosafety Level 1 – basic laboratory
- Biosafety Level 2 – basic laboratory
- Biosafety Level 3 – laboratory with containment conditions
- Biosafety Level 4 – laboratory with maximum containment

A national classification of microorganisms, by risk group, may be determined taking into account regional characteristics:

- Organism: pathogenicity, mode of transmission
- Host: immunity, density vectors, environment
- Preventive measures
- Treatment

### 11.2.3. Risk assessment

Any laboratory work should be done under appropriate biosafety conditions based on risk assessment. Such an assessment will take into considerations the agent risk group as well as other factors to establish the biosafety level (World Health Organization 2004).

#### **Organism**

Factors that should be considered concerning the organism include:

- Pathogenicity of the agent and infectious dose
- Potential outcome of exposure
- Natural route of infection
- Other routes of infection, resulting from laboratory manipulations (parenteral, airborne, ingestion)
- Stability of the agent in the environment
- Concentration of the agent to be manipulated
- Presence of a suitable host (human or animal)
- Information available from animal studies and reports of laboratory-acquired infections or clinical reports
- Laboratory activity planned (sonication, aerosolization, centrifugation, etc.)
- Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment regimens
- Local availability of effective prophylaxis or therapeutic interventions

On the basis of the information ascertained during the risk assessment, a biosafety level can be assigned to the planned work:

- appropriate personal protective equipment
- standard operating procedures (SOPs)
- other safety interventions developed to ensure the safest possible conduct of the work

#### **11.2.4. General laboratory practices**

There are different types of laboratory hazards, such as biological, chemical, radiation and physical, as well as electrical hazards, slips, trips, and falls.

Attitude, behavior and common sense are the key to prevent such accidents.

The main causes of laboratory accidents are: lack of training, knowledge or experience; excessive self-confidence, negligence, fatigue, taking shortcuts, work load, working too fast, deciding not to follow safe practices, and skepticism about bio-hazard.

Laboratory workers can be classified as “unsafe” and “safe”. Unsafe workers are those who have a low opinion of safety programs, take excessive risks, work too fast and are less aware of the risk of agents. Safe workers are those who adhere to safety guidelines, practice defensive work habits and recognize potentially hazardous situations (Phillips 1986, Harding 1995).

The laboratory has to be organized with responsibility levels. The manager needs to have an attitude of support towards the safety program, should provide adequate resources and training, should supply a safe work environment, monitor work practices, and assess and assign risk level for hazardous materials (biologicals, chemicals, animals).

The employees, on the other hand, have to comply with occupational safety and health standards, rules, regulations and orders, use personal protective equipment and safety equipment when needed, and report all work-related accidents and illness to the supervisor.

The types of regulations that support working safely in laboratories are government regulations, institutional regulations, and laboratory regulations or guidelines.

#### **General safety guidelines**

- Eating, drinking or smoking are not permitted in laboratories or offices
- Wear personal protective equipment when needed
- Practice good personal hygiene
- Children are not allowed into laboratory areas
- Good housekeeping
  - clean up after each manipulation
  - do not store materials on work surfaces
  - keep aisles clear
  - keep chemicals in storage cabinets
  - purge work areas of unnecessary or unused equipment, supplies or chemicals
- Minimize all exposure
- Never underestimate the risks
- Become familiar with:
  - emergency exits
  - emergency procedures
  - first-aid
  - fire responses
- Report all accidents and injuries
- Ask questions when in doubt

**Personal safety guidelines**

Take pride in your workplace and practice accident prevention by banning negative attitudes and bad habits such as:

- Overconfidence
- Showing off
- Stubbornness
- Laziness
- Carelessness
- Impatience
- Ignorance

**Safety guidelines – slips, trips and falls**

- Clean up spills
- Watch out for loose carpet, polished floors, or objects on floor
- Keep all chair legs on the floor
- Use step stools and ladders when reaching for top shelves
- Never lay cords across walkways
- Use as much light as possible
- Do not carry loads which block your vision

**Safety guidelines – storage**

- Avoid overloading file cabinets
- Close file cabinet drawers when finished
- Store heavy items on lower shelves
- Keep pointed and sharp objects in a box in your desk drawer

**Safety guidelines – personal protective equipments**

Appropriate personal protective equipment must be worn whenever working with hazardous materials.

- Lab coat
  - must be worn in the laboratory
  - should be buttoned at all times
  - should cover the arms, torso, back and legs above the knees
  - should be selected based upon the chemicals used
  - should be removed and replaced if soiled or contaminated
  - should not be worn outside of the laboratory
- Eye and face protection
  - eye glasses, face shield, or mask are worn for work with small quantities (< 1 L)
  - goggles are worn for work with large volumes (1-5 L)
  - chemical fume hood or other shield should be used for volumes > 5 L
  - should not be worn outside laboratory

- Gloves
  - should be selected based on the chemicals being used
  - should be inspected for tears or holes before use
  - should be replaced or discarded when dirty or contaminated
  - should not be removed from the laboratory
- Respiratory protection
  - must be worn in the laboratory where there is an inhalation hazard
  - should not be worn if not trained in their use
  - should be cleaned and inspected after each use and allowed to dry completely
  - should be selected based upon the chemicals used or agents handled
  - should not be taken out of the laboratory

**Safety guidelines - controlling aerosols**

- Avoid splattering and spilling solutions
- Use plastic-backed absorbent paper on work surfaces
- Place caps or tops on bottles and tubes
- Place balances in ventilated enclosures
- Use safety cups when centrifuging
- Work under containment
  - biological safety cabinet
  - chemical fume hood
  - ventilated enclosures
  - directional airflow

**Risk assessment**

- Identify all hazardous materials and processes
- Consider possible routes of entry
- Consult information resources
- Evaluate biosafety level or toxicity of material
- Evaluate quantitative information on toxicity

- Select procedures to minimize exposure
- Prepare for contingencies

**Safety guidelines – Laboratory security**

- Prevent unauthorized entry into laboratory areas
- Prevent unauthorized removal of hazardous materials
- Recognize that laboratory safety and security are different
- Control access
- Know who is in the laboratory
- Know what materials are being brought into the laboratory
- Know what materials are being removed from the laboratory
- Have an emergency plan
- Have a protocol for reporting incidents

**11.2.5. Decontamination**

Mycobacteria are generally more resistant to chemical disinfection than other vegetative bacteria (Russel 1986).

The subject of disinfectants, which are really effective against mycobacteria, is very controversial and can generate confusion.

Table 11-1 shows disinfectants active against mycobacteria. The most common disinfectants used in the mycobacteria laboratory are: phenol 5 %, ethanol 70 % and sodium hypochlorite 2%.

There are interesting studies showing the efficacy of disinfectants against mycobacteria and *M. tuberculosis* (Best 1988, Best 1990).

The WHO manual of laboratory biosafety also gives good information on disinfectants in general ([http://www.who.int/csr/resources/publications/biosafety/WHO\\_CDS\\_CSR\\_LYO\\_2004\\_11/en/index.html](http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/index.html)).

Table 11-1: Disinfectants active against Mycobacteria

Disinfectant	Final concentration
Phenol	5 %
Sodium hypochlorite	10,000 ppm of Av Cl/mL
Sodium dichloroisocyanurate	6,000 ppm of Av Cl/mL
Ethanol	70 %
Glutaraldehyde-phenate	2 %

Av Cl = available chlorine

### 11.2.6. Handling of biological waste

It is strongly recommended that residues be segregated, packaged and properly labeled at the point of origin. They should then be immediately placed in distinctive containers according to their species and group, in order to reduce the amount of contaminated residues, as well as accidental risks, and to adopt the best conduction for the treatment of infectious or contaminant residues (Coelho 2000).

**Potentially infectious:** should be disposed of in plastic bags, made of polypropylene, resistant to autoclaving.

Bags should be filled up to 2/3 of their capacity and totally closed to prevent leaking of the content, even if turned upside down. Bags cannot be emptied or reused. In the laboratory, bags should be stored in garbage containers made of material that permits chemical or physical decontamination, identified with the label of hazardous biological waste having hinged-foot-activated mechanisms for opening and closing the lid, with rounded corners and edges.

Every biological residue generated in the laboratory should be previously treated before being disposed, even in the case of selective collection such as hospital or animal-house facility waste.

**Sharp and cutting residues:** should be disposed of in containers with rigid walls and lids resistant to sterilization.

The collecting containers for sharp and cutting material should be placed as near as possible to the area of use of such material. The containers should not be filled above 2/3 of their capacity.

After being filled, the collecting container should be closed and placed in plastic bags resistant to autoclaving. Such containers should be identified with self-adhesive labels, with the following information: "Do not reuse empty container".

**Decontamination:** autoclaving sterilization is the safest method, for its penetration power is higher than dry heat. Microorganisms are destroyed by thermocoagulation of cytoplasmic proteins.

Before disposal, bags should be sterilized by autoclaving; sterilization occurs at a pressure of 1 atm at temperatures of up to 121°C (250°F) for 62 minutes, with a 7-minute interval pre-vacuum, 25 minutes heating, 25 minutes sterilization for surface material or 30 minutes sterilization for thick materials and 15 minutes of cooling.

If final disposal occurs after a 24-hour period, anatomical pieces, human and animal organs and carcasses that have undergone treatment, should be refrigerated or kept in formalin.

**Leaking of biological residues:** in case of disruption or leaking of bags containing biological residues that have not undergone prior treatment, the procedures below should be followed:

- Cover the spill and spill site with paper towels
- Pour disinfectant solution (for example sodium hypochlorite: a minimum of 10,000 ppm available chloride) on the paper towels for 30 minutes contact time
- Pick up paper towels and discard them into a plastic bag
- Reapply disinfectant and wait for 10 minutes
- Carry out the cleaning
- Decontaminate all materials that had direct contact with the spill
- The professional responsible for the cleaning of the spill must wear the necessary Individual Protection Equipment

#### **Guidelines for internal collection and transport of residues**

- Never pour the contents of the garbage bin into another container. The garbage bag should be picked up, closed, and placed inside the internal waste collection trolley
- Check if there is any leakage in the plastic bag prior to picking it up from the garbage bin. In case of leakage, the bag should be placed into another bag with the same specification and the garbage bin must be washed and disinfected
- Residue transport from the laboratory to the disposal room can be done by hand or by the internal waste collection trolley

### 11.2.7. Transport of infectious materials

Guidance on regulations for the transport of infectious substances can be found at: [http://www.who.int/csr/resources/publications/biosafety/WHO\\_CDS\\_EPR\\_2007\\_2/en/index.html](http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2007_2/en/index.html)

### 11.2.8. Good Laboratory Practices (GLP)

Laboratories are complex and dynamic environments. Biomedical research and clinical laboratories must be able to adapt quickly to continuously increasing public health needs and pressures.

All biological research and clinical laboratories should be regularly certified to ensure that:

- Proper engineering controls are being used and are functioning adequately as designed
- Personal protective equipment is appropriate for the tasks being performed
- Decontamination of waste and materials has been adequately considered and proper waste management procedures are in place
- Proper procedures for general laboratory safety, including physical, electrical and chemical safety are in place

Laboratory certification is the systematic examination of all safety features and processes within the laboratory (engineering controls, personal protective equipment and administrative controls). Biosafety practices and procedures are also taken into account. Laboratory certification is an on-going quality and safety assurance activity that should take place on a regular basis.

Standardized practices can be found in the WHO Laboratory Biosafety Manual ([http://www.who.int/csr/resources/publications/biosafety/WHO\\_CDS\\_CSR\\_LYO\\_2004\\_11/en/index.html](http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/index.html)). Some important topics are summarized below:

#### **Safe handling of specimens in the laboratory**

Improper collection, transport and handling of specimens in the laboratory carry a risk of infection to the personnel involved.

### **Specimen containers**

Specimen containers may be of glass or preferably plastic. They should be robust and should not leak when the cap or stopper is correctly applied. No material should remain on the outside of the container. Containers should be correctly labeled to facilitate identification. Specimen request or specification forms should not be wrapped around the containers but placed in separate, preferably waterproof envelopes.

### **Transport of specimens within the facility**

To avoid accidental leakage or spillage, secondary containers, such as boxes, should be used, fitted with racks so that the specimen containers remain upright. The secondary containers may be of metal or plastic, should be autoclavable or resistant to the action of chemical disinfectants, and the seal should preferably have a gasket. They should be regularly decontaminated.

### **Receipt of specimens**

Laboratories that receive large numbers of specimens should designate a particular room or area for this purpose.

### **Opening packages**

Personnel who receive and unpack specimens should be aware of the potential health hazards involved, and should be trained to adopt standard precautions, particularly when dealing with broken or leaking containers. Primary specimen containers should be opened in a biological safety cabinet. Disinfectants should be available.

### **Use of pipettes and pipetting aids**

Mouth pipetting must be strictly forbidden. A pipetting aid must always be used for pipetting procedures. The most common hazards associated with pipetting procedures are the result of mouth suction. Oral aspiration and ingestion of hazardous materials have been responsible for many laboratory-associated infections.

Pathogens can also be transferred to the mouth if a contaminated finger is placed on the suction end of a pipette. A lesser known hazard of mouth pipetting is the inhalation of aerosols caused by suction. The use of pipetting aids prevents ingestion of pathogens.

Aerosols are generated when a liquid is dropped from a pipette on to a work surface, when cultures are mixed by alternate sucking and blowing, and when the last drop is blown out of a pipette. The inhalation of aerosols unavoidably generated

during pipetting operations can be prevented by working in a biological safety cabinet.

- All pipettes should have cotton plugs to reduce contamination of pipetting devices
- Air should never be blown through a liquid containing infectious agents
- Infectious materials should not be mixed by alternate suction and expulsion through a pipette
- Liquids should not be forcibly expelled from pipettes
- Mark-to-mark pipettes are preferable to other types, as they do not require expulsion of the last drop
- A discard container for pipettes should be placed within the biological safety cabinet, not outside it
- Syringes fitted with hypodermic needles must not be used for pipetting
- Devices should be used to open septum-capped bottles and to allow the introduction of pipettes, thus avoiding the use of hypodermic needles and syringes
- To avoid dispersion of infectious material dropped from a pipette, an absorbent material should be placed on the working surface; this should be disposed of as infectious waste after use

### **Avoiding the dispersal of infectious materials**

Disposable transfer loops do not need to be resterilized and can, therefore, be used in biological safety cabinets. These loops should be placed in disinfectant after use and discarded as contaminated waste.

Discarded specimens and cultures for autoclaving should be placed in leak-proof containers, e.g. laboratory discard bags. Tops should be secured (e.g. with autoclave tape) prior to disposal into waste containers.

Working areas must be decontaminated with a suitable disinfectant at the end of each work period.

Special care should be taken when drying sputum samples, to avoid creating aerosols. Smears should be dried at room temperature inside the biological safety cabinet, or outside the biological safety cabinet on a temperature plate.

### Use of biological safety cabinets

- The use and limitations of biological safety cabinets should be explained to all potential users, with reference to national standards and relevant literature. Written protocols or operation manuals should be provided to staff. In particular, it must be made clear that the cabinet will not protect the operator from spillage, breakage or poor technique
- The cabinet must not be used unless it is working properly
- The glass viewing panel must not be opened when the cabinet is in use
- Apparatus and materials in the cabinet must be kept to a minimum. Air circulation at the rear plenum must not be blocked
- Bunsen burners must not be used in the cabinet as the heat produced will distort the airflow and may damage the filters
- All work must be carried out in the middle or rear part of the work surface and be visible through the viewing panel
- Traffic behind the operator should be avoided
- The operator should not disturb the airflow by repeated removal and re-introduction of arms
- Air grills must not be blocked with notes, pipettes or other materials, as this will disrupt the airflow, causing potential contamination of the material and exposure of the operator
- The surface of the biological safety cabinet should be wiped using an appropriate disinfectant after work is completed and at the end of the day
- The UV-light should be switched on for 15 minutes before switching off the cabinet
- Paperwork should never be placed inside biological safety cabinets

The UV-light is very effective against mycobacteria when used for surface decontamination. For this reason we recommend its use in the cabinet to prevent cross-contamination of cultures (Ueki in press). In figure 11-1 the effect of UV light on a plate on which mycobacteria were inoculated is shown.

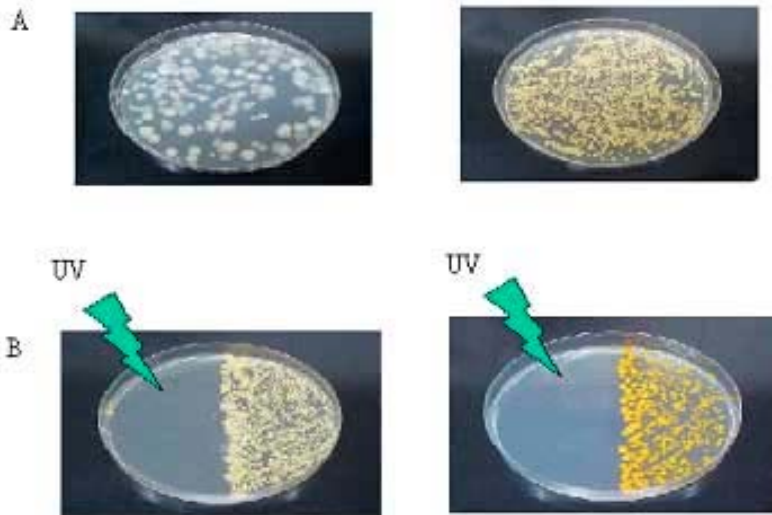


Figure 11-1: A) Two species of mycobacteria inoculated on Middlebrook 7H11 plates. The plates were then covered with aluminum foil and exposed to UV light inside the cabinet during 5 and 10 minutes. B) The same experiment, but only half of the plates were covered and the other half was exposed directly to the UV light.

### **Avoiding ingestion of infectious materials and contact with skin and eyes**

- Large particles and droplets ( $> 5 \mu\text{m}$  in diameter) released during microbiological manipulations settle rapidly on bench surfaces and on the hands of the operator. For this reason, laboratory workers should wear disposable gloves and avoid touching their mouth, eyes or facial skin
- No materials should be placed in the mouth – pens, pencils, chewing gum – when in the laboratory
- Cosmetics should not be applied in the laboratory
- The face should be shielded or otherwise protected during any operation that may result in the splashing of potentially infectious materials

### **Avoiding injection of infectious materials**

- Accidental inoculation resulting from injury with broken or chipped glassware can be avoided through careful practice and procedures; glassware should be replaced with plastic ware whenever possible, e.g. plastic Pasteur pipettes and tubes should replace those made of glass

- Needle-stick injuries can be reduced by: (a) minimizing the use of syringes and needles (e.g. simple devices are available for opening septum-stoppered bottles so that pipettes can be used instead of syringes and needles; or (b) using engineered sharp safety devices when syringes and needles are necessary
- Needles should never be recapped. Disposable articles should be discarded into puncture-proof containers fitted with covers

### **Use of centrifuges**

- Satisfactory mechanical performance is a prerequisite of microbiological safety in the use of laboratory centrifuges
- Centrifuges should be operated according to the manufacturer's instructions
- Centrifuges should be placed at such a level that workers can see into the bowl to place trunnions and buckets correctly
- Centrifuge tubes and specimen containers for use in the centrifuge should be made of thick-walled glass or preferably of plastic and should be inspected for defects before use
- Tubes and specimen containers should always be securely capped (screw-capped if possible) for centrifugation
- The buckets must be loaded, equilibrated, sealed and opened in a biological safety cabinet
- When using angle-head centrifuge rotors, care must be taken to ensure that the tube is not overloaded as it might leak
- Use of homogenizers, shakers, blenders and sonicators
- Homogenizers used for Risk Group 3 microorganisms should always be loaded and re-opened in biological safety cabinets
- Sonicators may release aerosols. They should be operated in biological safety cabinets or covered with shields during use. The shields and the outside of sonicators should be decontaminated after each use
- Domestic (kitchen) homogenizers should not be used in laboratories as they may leak or release aerosols: laboratory blenders and stomachers are safer
- Caps and cups or bottles should be in good condition and free from flaws or distortion; caps should be well-fitting and gaskets should be in good condition

- Pressure builds up in the vessel during the operation of homogenizers, shakers and sonicators, as aerosols containing infectious materials may escape from between the cap and the vessel; plastic, in particular, polytetrafluoroethylene (PTFE) vessels are recommended because glass may break, releasing infectious material and possibly wounding the operator
- When in use, homogenizers, shakers and sonicators should be covered by a strong transparent plastic casing that should be disinfected after use; where possible, these machines should be operated under their plastic covers, in a biological safety cabinet
- At the end of the operation, the containers should be opened in a biological safety cabinet
- Hearing protection should be provided for people using sonicators

#### **Use of tissue grinders**

- Glass grinders should be held in absorbent material in a gloved hand; plastic (PTFE) grinders are safer
- Tissue grinders should be operated and opened in a biological safety cabinet

#### **Care and use of refrigerators and freezers**

- Refrigerators, deep-freezers and solid carbon dioxide (dry-ice) chests should be defrosted and cleaned periodically, and any ampoules, tubes, etc., that have broken during storage should be removed. Face protection and heavy-duty rubber gloves should be worn during cleaning; after cleaning, the inner surfaces of the cabinet should be disinfected
- All containers stored in refrigerators, etc., should be clearly labeled with the scientific name of the contents, the date stored and the name of the individual who stored them; unlabelled and obsolete materials should be autoclaved and discarded
- An inventory must be maintained of the freezer's contents
- Flammable solutions must not be stored in a refrigerator unless it is explosion proof; notices to this effect should be placed on refrigerator doors

#### **Films and smears for microscopy**

Fixing and staining of blood, sputum and fecal samples for microscopy do not necessarily kill all organisms or viruses on the smears. These items should be

handled with forceps, stored appropriately, and autoclaved before disposal. References about sputum smear microscopy and safe handling of cultures can be found in the literature (Giacomelli 2005, Chedore 2002, Schwebach 2001).

### **Decontamination**

Hypochlorite and high-level disinfectants are recommended for decontamination. Freshly prepared hypochlorite solutions should contain available chlorine at 1 g/L for general use and 5 g/L for blood spillages. Glutaraldehyde may be used for decontaminating surfaces. More specific information on mycobactericidal agents can be found in Best 1988, Best 1990, and Rutala 1991.

### **DNA extraction**

DNA extraction is better accomplished by heating for 10 minutes at 100°C. This procedure inactivates the bacilli (Zwadyk Jr 1994, Bemer-Melchior 1999).

For genotyping purposes, DNA extraction should be less drastic to avoid damaging the DNA. Heating for 20 minutes at 80°C is recommended in this case. There is controversy among some authors that heating at 80°C for 20 minutes might not inactivate the bacilli completely (Blackwood 2005, Doig 2002, Van Embden 1993, Warren 2006). Therefore, a sample submitted for such a procedure should be handled as infectious material and should not be removed from containment.

### **Opening of ampoules containing lyophilized infectious materials**

Cultures of *M. tuberculosis* should not be lyophilized because of the high risk of aerosol production during ampoule preparation and opening.

### **Contingency plans and emergency procedures**

Every laboratory that works with infectious microorganisms should institute safety precautions appropriate to the hazard of the organisms and the animals being handled.

A written contingency plan for dealing with laboratory and animal facility accidents is a requirement in any facility that works with or stores Risk Group 3 or 4 microorganisms (containment laboratory – Biosafety Level 3 and maximum containment laboratory – Biosafety Level 4). National and/or local health authorities should be involved in the development of the emergency contingency plan.

### **Contingency plan**

The contingency plan should provide operational procedures for:

- Precautions against natural disasters, e.g. fire, flood, earthquake, and explosion
- Biohazard risk assessment
- Incident-exposure management and decontamination
- Emergency evacuation of people and animals from the premises
- Emergency medical treatment of exposed and injured persons
- Medical surveillance of exposed persons
- Clinical management of exposed persons
- Epidemiological investigation
- Post-incident continuation of operations

In the development of this plan, the following items should be considered for inclusion:

- Identification of high-risk organisms
- Location of high-risk areas, e.g. laboratories, storage areas, animal facilities
- Identification of personnel and populations at risk
- Identification of responsible personnel and their duties, e.g. biosafety officer, safety personnel, local health authority, clinicians, microbiologists, veterinarians, epidemiologists, and fire and police services
- Lists of treatment and isolation facilities that can receive exposed or infected persons
- Transport of exposed or infected persons
- Lists of sources of immune serum, vaccines, drugs, special equipment and supplies
- Provision of emergency equipment, e.g. protective clothing, disinfectants, chemical and biological spill kits, decontamination equipment and supplies

## **Emergency procedures for microbiological laboratories**

### **Puncture wounds, cuts and abrasions**

The affected individual should remove protective clothing, wash hands and any affected area(s), apply an appropriate skin disinfectant, and seek medical attention if necessary. The cause of the wound and the organisms involved should be reported, and appropriate and complete medical records kept.

### **Ingestion of potentially infectious material**

Protective clothing should be removed and medical attention sought. Identification of the material ingested and circumstances of the incident should be reported, and appropriate and complete medical records kept.

### **Potentially infectious aerosol release (outside a biological safety cabinet)**

All persons should immediately leave the affected area and any exposed persons should be referred to the appropriate center for medical advice. The laboratory supervisor and the biosafety officer should be informed at once. No one should enter the room for an appropriate period of time (e.g. 1 h), to allow aerosols to be carried away and heavier particles to settle. If the laboratory does not have a central air exhaust system, entrance should be delayed (e.g. for 24 h).

Signs should be posted indicating that entry is forbidden. After the appropriate time, decontamination should proceed, supervised by the biosafety officer. Appropriate protective clothing and respiratory protection should be worn.

### **Broken containers and spilled infectious substances**

Broken containers contaminated with infectious substances and spilled infectious substances should be treated in the same way as biological residue leaks. Infectious substances spilled onto working areas should be covered with a cloth or paper towels. Disinfectant should then be poured over these and left for the appropriate amount of time. The cloth or paper towel and the broken material can then be cleared away; glass fragments should be handled with forceps. The contaminated area should then be swabbed with disinfectant. If dustpans are used to clear away the broken material, they should be autoclaved or placed in an effective disinfectant. Cloths, paper towels and swabs used for cleaning up should be placed in a contaminated-waste container. Gloves should be worn for all these procedures. If laboratory forms or other printed or written matter are contaminated, the information should be copied onto another form and the original discarded into the contaminated-waste container.

**Breakage of tubes containing potentially infectious material in centrifuges without sealable buckets**

If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (e.g. for 30 min) to allow settling. If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed (e.g. for 30 min). In both instances, the biosafety officer should be informed. Strong (e.g. thick rubber) gloves, covered if necessary with suitable disposable gloves, should be worn for all subsequent operations. Forceps, or cotton held in the forceps, should be used to retrieve glass debris. All broken tubes, glass fragments, buckets, trunnions, and the rotor should be placed in a non-corrosive disinfectant known to be active against the organisms concerned. Unbroken, capped tubes may be placed in disinfectant in a separate container and recovered. The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried. All materials used in the clean-up should be treated as infectious waste.

**Breakage of tubes inside sealable buckets (safety cups)**

All sealed centrifuge buckets should be loaded and unloaded in a biological safety cabinet. If breakage is suspected within the safety cup, the safety cap should be loosened and the bucket autoclaved. Alternatively, the safety cup may be chemically disinfected.

**Fire and natural disasters**

Fire departments and other services should be involved in the development of emergency contingency plans. They should be told in advance which rooms contain potentially infectious materials. It is useful to arrange visits from these services to the laboratory to acquaint them with its layout and contents.

After a natural disaster, local or national emergency services should be warned of the potential hazards within and/or near laboratory buildings. They should enter only when accompanied by a trained laboratory worker. Infectious materials should be collected in leak-proof boxes or strong disposable bags. Salvage or final disposal should be determined by biosafety staff on the basis of local ordinances.

### Emergency services: whom to contact

The telephone numbers and addresses of the following should be prominently displayed in the facility:

- The institution or laboratory itself (the address and location may not be known in detail by the caller or the services called)
- Director of the institution or laboratory
- Laboratory supervisor
- Biosafety officer
- Fire services
- Hospitals/ambulance services/medical staff (names of individual clinics, departments, and/or medical staff, if possible)
- Police
- Medical officer
- Responsible technician
- Water, gas and electricity services

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