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***Mycobacterium tuberculosis*: Biorisk, Biosafety and Biocontainment**

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1. Introduction

1.1 Biorisk, biosafety and biocontainment: A life or death process

Safety at workplaces, occupational diseases, pandemics, international travel, public transportation, day care centers, nursing homes, and jails, among others, are situations we are frequently involved in and whose impact and risks we are not aware of but could change the course of our lives. When we travel abroad on business or vacation usually our expectations are high, but we have no information on the process undertaken to secure passengers health, as well as community and environmental safety. When we share a closed space such as an airplane, we should be aware of some diseases, especially air borne diseases, to be able to assess exposure risk and minimize it by adopting biosafety measures (individual, collective or both), and have the proper infrastructure to contain or isolate such risk.

Based on this multifactor approach, scientists and experts on *Mycobacterium tuberculosis* biology, on chemical agents, personal protective equipment (PPE), industrial air purifiers, building design, laboratory equipment and different transportation means join their efforts to harmonize the life of communities around the planet.

Every community should make an effort to acquire a culture aimed at preserving their physical integrity and environmental quality because this process is not restricted to expert laboratories. Anti-pandemic plans presently emphasize that communities are the main pillar to contain these devastating events and such a perspective must transcend the usual scenarios such as hospitals, laboratories, universities, public transportation, supermarkets, movie theaters, etc.

The debate today is heated, and there is an extensive and sometimes emphatic documentation. Many of the aspects involved are described separately, but we must remember that they should be integrated so as to avoid implementing isolated components that may have been successful elsewhere but are not reproducible in our own settings. The present chapter is no exception: we collected the minimum information required to develop the safety data sheet and start the risk assessment; we describe the basic, most general biosafety measures adopted internationally, and we offer a guide on essential biocontainment measures (some of which are described in the section dedicated to

biosafety). Such a structure responds solely to academic purposes, but we have to remember that in reality they are inclusive, inseparable processes aimed at the same results. They are the starting point to strengthen the process in any institution, and, therefore, risk assessment should be done together with experts from different disciplines and based on updated knowledge, as well as on the identification of protection elements that may be included in the process and adapted to specific infrastructures and resources. This is the only way to develop a practical and reliable process. Besides, contingency plans should be designed to respond to unexpected catastrophic events such as bioterrorist attacks (Wheelis 2002) and natural disasters.

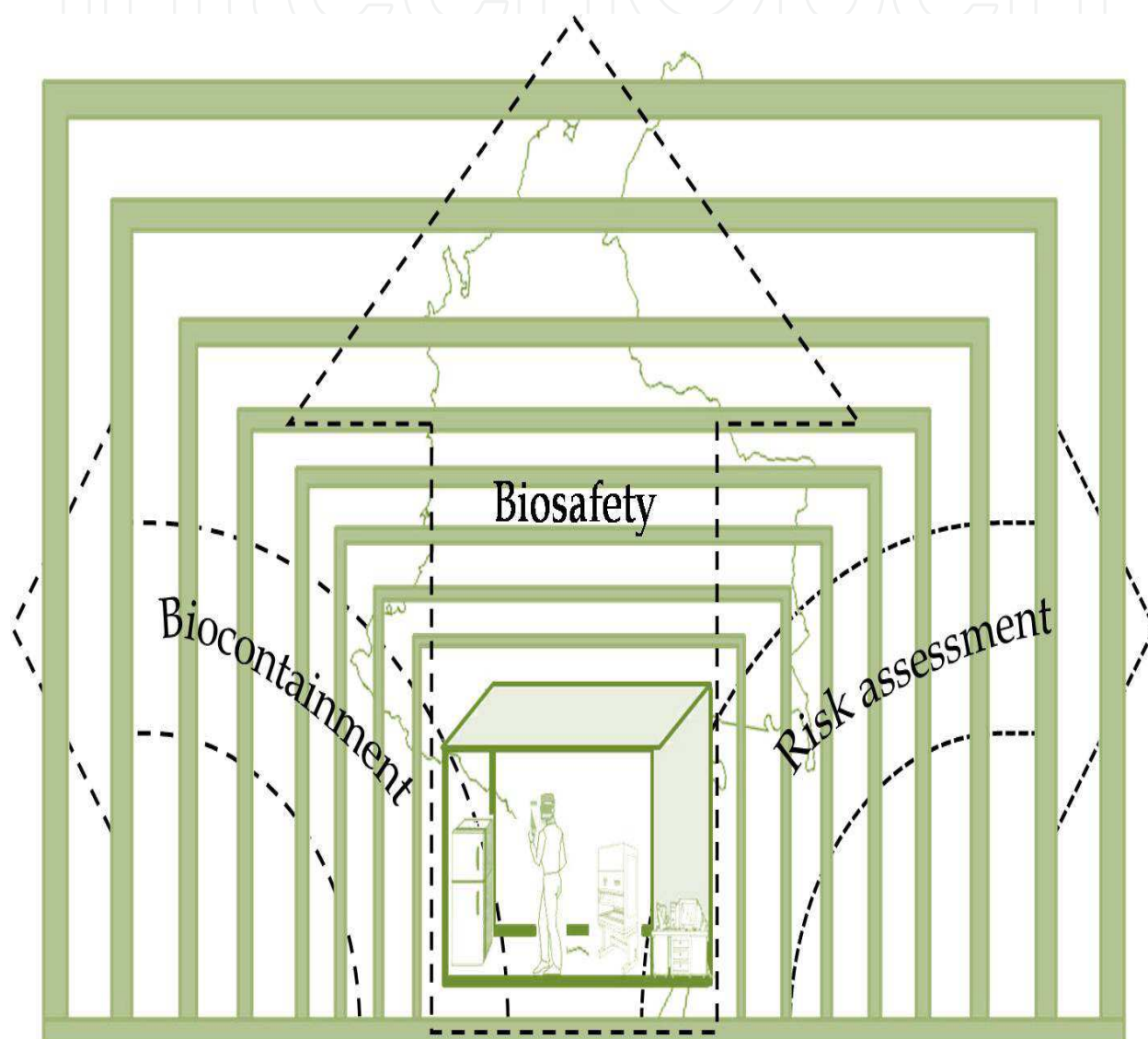


Fig. 1. Calculating risk levels and negative and positive impact according to the safety procedures.

Finally, as this is a complex and dynamic process, you should determine a specific, known and daily situation and then identify exposure risks, the PPE presently in use and consider and assess if you can contain the risk and prevent it from having greater magnitude. Figure 1 will help you in calculating risk levels and negative and positive impact according to your

present procedures. Remember human fragility and the fact that probably many of the elements mentioned are not at your disposal, but you can resort to those available in your day-to-day scenario. Human beings are not disposable or reusable, but they are biodegradable or incinerable depending on the circumstances, and that is why the process described in this chapter must be seen as conducive to a better quality of life or to death.

2. Biorisk

The following is a description of various characteristics of the microorganism which are useful in understanding biorisk, biosafety, and biocontainment. *M. tuberculosis* is a pathogen that has been extensively studied; the majority of information required for risk-evaluation of each procedure or work situation can be easily consulted in publications of such renowned international organizations as: The World Health Organization (WHO); The Centers for Disease Control and Prevention (CDC); the National Institutes of Health (NIH); Sandia National Laboratories; American Biological Safety Association (ABSA); Asociación Mexicana de Bioseguridad (AMEXBIO), and in the domestic regulations of each country. The updated versions of this information can be accessed through the systematic review of professional scientific publications. Each institutional biosafety committee is responsible for implementing, and ensuring compliance with, the relevant guidelines and regulations. The *M. tuberculosis* safety data sheet should be prepared in each laboratory, posted in a conspicuous location, and accessible to all personnel who work in the area. Staff should be diligent in the risk assessment of their tasks and procedures, request the required PPE, and comply with containment measures in order to guarantee their safety and that of their colleagues and the environment.

2.1 Safety data sheet

2.1.1 Identification of the microorganism, (Riley 1961, Kunz 1982, Wayne 1984 and Grange 1990)

Agent name: *Mycobacterium tuberculosis*

Taxonomy

Domain: Bacteria

Phylum: Actinobacteria

Class: Actinomycetes

Order: Actinomycetales

Family: Mycobacteriaceae

Genus: *Mycobacterium*

Species: *tuberculosis*

2.1.2 Biological characteristics

Condition: bacteria aerobic

Grow: slow-growing

Motility: non-motile

Spore: non-endospore forming* (aspect in discussion by Ghosh 2009 and Traag 2010)

Acid-Fast Bacillus (AFB)

Allergenic: no

Cancerous: no

Abortive: no

Toxins forming: no

Immunosuppressor: no

Capable of mutating in the host: yes

Recommended pictogram:



2.1.3 Mode of transmission

Usually airborne human to human (inhalation of infectious aerosols or infected droplets) or dermal inoculation and possible ingestion, it is not transmitted through sexual contact, and there has been no documentation of vertical transmission. (Wells 1955 and Verhagen 2011)

Disease:

Tuberculosis (TB) (second leading cause of death worldwide)

Latent TB infection

Multidrug resistant TB (MDR TB)

Extensively drug resistant TB (XDR TB)

Host:

Humans, nonhuman primates, and commonly used laboratory animals: pigs, cats, dogs, sheep, cattle, rodents and seals. Some domestic animals, in contact with people suffering from TB, are able to develop TB and become themselves a source of infection. (Grange 1990, National Research Council. 1997 and 2003, Hankenson 2003, Krauss 2003, and Cousins 2003)

Infectious dose for humans: is very low (ID₅₀ 1-10 bacilli by inhalation route), a sputum of an infected patient can contain several millions of bacilli per milliliter. (Riley, 1957 and 1961 and CDC 1999)

Communicability: high, human to human with symptoms.

Incubation period: long (years), may progress to pulmonary or disseminated disease.

Vectors: none

Zoonosis: by inhalation or direct contact with infected animal or tissues from infected animal.

Survival on inanimate surfaces at different relative humidities: *M. tuberculosis* can survive for several days on inanimate surfaces; 70 days on carpet, 45 days on clothing, 105 days on paper, 90 to 120 days on dust, 6 to 8 months in sputum in a cool and dark room, 45 days in manure, and 49 days in guinea pig tissue. (Kunz 1982, and Rubin 1991)

Geographical localization: worldwide

2.1.4 Detection

Latent TB infection has been traditionally identified by the tuberculin skin test (TST, Mantoux or PPD); currently the new generation of test entails interferon gamma (IFN- γ) release assays (IGRAs: QuantiFERON and T-SPOT.TB).

Diagnosis of TB: acid fast stain of sputum samples, culture, phenotypic and genotypic identification of *M. tuberculosis*, DNA fingerprinting (Rozo 2010) and drug susceptibility testing and tissue exams.

Possibility of viewing the bacillus in clinical specimens: yes, through examination of clinical sample for acid-fast bacilli (AFB), fluorescence microscopy and light-emitting diode (LED)

Growth in culture media: yes, frequently in Lowenstein-Jensen (LJ), Ogawa Kudoh (OK) or liquid culture as modified Middlebrook 7H9 broth, the *M. tuberculosis* is of slow growth, between 4 to 8 weeks, from clinical samples. (Welch 1993)

Rapid identification of *M. tuberculosis*: through the employment of methods such as nucleic acid hybridization methods, lateral flow assays, line probe assays, and DNA sequencing.

2.1.5 Epidemiology

Risk population: it is relatively more prevalent in immigrants, minorities, the elderly, persons with acquired immunodeficiency syndrome (AIDS) and among healthcare workers, and laboratory personnel who are occupationally exposed.

Mortality and morbidity: mortality for TB, depending on the country, MDR TB 50% to 70% of the patients not treated within a period of two years. Mortality is higher in patients with TB/AIDS. The morbidity in TB is high.

Perception of malicious use: low

2.1.6 Surveys of laboratory-acquired infection and prophylaxis

Laboratory personnel should undergo an annual PPD or IGRAs, and workers with a positive test should be evaluated for active TB; in accidental exposure the laboratory personnel should be tested 3 to 6 months after the event and should be offered prophylaxis

if is required in TB, but the referenced isoniazid (INH) prophylaxis is not applicable in MDR TB.

Vaccine: Bacille Calmette Guerin (BCG), attenuate live vaccine, with limited protection. (Lietman 1999)

Treatment: therapy with multiple drugs

First line: INH, rifampin (RIF), pyrazinamide (PZA), ethambutol (EMB), streptomycin (SM).

Second line: Amikacin, capreomycin, ciprofloxacin, ethionamide, kanamycin, levofloxacin. Ofloxacin and *para*-aminosalicylic acid for MDR TB and XDR TB.

2.1.7 Prevention and control

Exposure control-personal protection: PPE such as respiratory protection (fit-tested respirators with N-95 rating) (Occupational Safety and Health Administration –OSHA- 2003 and 2004), hand protection, eye protection, skin and body protection, and hygiene measures.

Containment:

Objective: to prevent aerosol exposure or dermal inoculation.

Biosafety level (BSL) 2 can be used for low-risk procedures, such as making smear and diagnosis activities including primary culture of clinical specimens potentially infected by bacilli of *M. tuberculosis* s with PPE. (Welch 1993)(American Thoracic Society 1993)

BSL-3 can be used for high-risk procedures, such as handling solid and liquid positive culture, secondary cultures for diagnostic or research activities, DNA or RNA extraction (only in the initial stages of the procedure)(Castro 2009, Warren 2006, and NIH 2002), biochemical test, centrifugations, pipetting, mechanical homogenizing, sonication, heating or boiling, work with bacteriological loops, preparation and manipulation of frozen sections, animal studies, infected clinical specimens and others with PPE. (CDC. 1999 and 2006, and Hankenson 2003)

2.1.8 Disinfection, inactivation and sterilization of *M. tuberculosis*

Efficient disinfectants are:

0.4-5 phenol during 10 minutes

5% formaldehyde during at least 10 minutes

1-2% Glutaraldehyde during 30 minutes

0.2-5% Sodium hypochlorite for one minute

70-96% Ethyl alcohol during 2 minutes

3-10% Hydrogen peroxide during 5 minutes

2-10%, 75ppm Iodophore

Mix of iodine + iodophores or ethyl alcohol

Mix of paraformaldehyde + glutaraldehyde or formalin

Susceptible to moist heat sterilization at 121°C for 15 minutes at 121 pounds of pressure in autoclave.

Note: quaternary ammoniums inhibit tubercle bacilli but do not kill them. Please check the efficacy of each disinfectant in your own laboratory conditions.

Ultraviolet dosage required: 10.000 μW-s/cm² at 254 nanometer for 99.9% destruction of bacillus.

2.1.9 Transport information

WHO classification of infective microorganisms by risk groups (RG):

RG 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 (WHO 2004).

Cultures positives for *M. tuberculosis* require packing measures, and labeled as “Infectious substance”. The triple packaging should be utilized according to the International Air Transport Association (IATA) and Dangerous Goods regulation and WHO recommendations. *M. tuberculosis* (cultures only) is included in Category A, UN 2814 (Infectious Substances, affecting humans) see table 1. (IATA 2006)

Infections substance	Class	Division	Category	Proper shipping name	UN Number	Packing instruction (PI)
<i>M. tuberculosis</i> cultures only	6	6.2	A	Infections substance	2814	602

Table 1. General information of *M. tuberculosis* for international shipping.

2.1.10 Biosafety functions officer

Mistakes and accidents, which result in over-exposure to infectious materials, should be immediately reported and corrective measures should be taken to avoid a repetition of the event.

Mandatory: personnel concerned with mycobacteria activity should be experienced and under the supervision of the head of the laboratory.

2.2 Epidemiology of hospital and laboratory acquired TB

The epidemiology is defined as the study of the distribution and determinants of diseases and injuries in human populations. Inherent in the definition of epidemiology is the necessity of measuring the amount of disease in a population by relating the number of cases to a population base. One of the unfortunate consequences of working with infectious materials is the potential for acquiring an infection. The laboratory acquired infections (LAI)

due to a wide variety of viruses, bacteria, parasites and fungi have been described. In the absence of precise data on LAIs, epidemiological methods provide the necessary tools to evaluate the extent and nature of personnel exposures. Although the precise risk of infection after an exposure remains poorly defined, surveys of LAIs suggested that *Brucella* species, *Shigella* species, *Salmonella* species, *M. tuberculosis* and *Neisseria meningitidis* are the most common causes. Early surveys of laboratory acquired TB found an incidence of TB among laboratory personnel 3-9 times greater than that in the general population (Harrington 1976 and Reid 1957). The *M. tuberculosis*, the causative organism of TB, has distinction of repeatedly being ranked within the top five most commonly LAI (Pike 1976, 1978, and 1979). The OSHA in 1996, 1997, 2003 and 2004, promulgated withdrawal of the 1997 proposed standard on occupational exposure to TB. Along with the withdrawal of the 1997 standard, the respirator-specific standard, 29 CFR 1910.139, was also withdrawn. The effect of withdrawing these standards is the application of the general industry respiratory protection standard, 29 CFR 1910.134, for all occupations, to those workplaces that provide respiratory protection from TB.

According to WHO, TB remains the second leading cause of death worldwide, killing 2 million people each year. In many developed countries, TB is considered a disease of the past. However, the impact of this disease can be devastating even today specially in poor countries. An estimated 9.4 million new cases of TB globally, with most cases occurring in resource limited or resource poor countries. In addition to that, much of deadliness of TB epidemic has to do with the virulent synergy between Human Immunodeficiency Virus (HIV) and TB. Recently, MDR-TB and XDR TB have had devastating effects on populations of HIV infected individuals in developing countries.

2.3 Risk assessment

Risk assessment was defined by Boa (Boa 2000), as “the use of factual information to define the health effects of exposure to individuals or populations to hazardous materials and situations”

The CDC and the NIH provided the basic definition for risk in their *Biosafety in Microbiological and Biomedical Laboratories*: -“Risk” implies the probability that harm, injury, or disease will occur. In the environment of the research, microbiological, teaching, and biomedical laboratories, the assessment of risk focuses first and foremost on the prevention of laboratory associated infections and the likelihood that the agent can be used as a weapon and the consequences of bioattack with the agent. The risk assessment helps to assign the BSL, PPE required, laboratory and facilities design, equipment that can be used, procedures and practices that can be implemented and that reduce, to a minimum, the personnel and the environmental risk of exposure to an agent. The risk evaluation should be made by the person with the best knowledge of the microorganism (*M. tuberculosis*) and the available containment measures. The risk assessment can be quantitative in the presence of known hazards or qualitative when the data will be incomplete or unknown.

The CDC and NIH recommend that the laboratory director or principal investigator, in close collaboration with the institutional biosafety committee, be responsible for assessing risk in order to set the BSL for the work.

The risk assessments should be conducted periodically and the analysis should include: new variables; updated information and procedures related to *M. tuberculosis*; management of the TB; MDR TB or XDR TB, and new international regulations applicable to the malicious use of the bacillus or bacillus-infected substances.

The CDC and NIH include the following important factors in a risk assessment:

1. The pathogenicity of the infectious or suspected infectious agent, in this case *M. tuberculosis*, including disease incidence and severity (morbidity and mortality). **Remark:** The more severe the potentially acquired disease, the higher the risk. With respect to *M. tuberculosis*, one has to consider the types of resistance (TB, MDR TB, and XDR TB).
2. The route of transmission (parenteral, airborne or ingestion). **Remark:** The greater the aerosol potential, the higher the risk (for *M. tuberculosis* the mode of transmission is usually airborne).
3. Agent stability: aerosol infectivity and the agent's ability to survive over time in the environment. Factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfectants must be considered.
4. The infectious dose of *M. tuberculosis*.
5. The concentration (number of infectious organisms per unit volume).
6. The origin of the potentially infectious materials.
7. The availability of data from animal studies, in the absence of human data.
8. The established availability of an effective prophylaxis or therapeutic interventions.
9. Medical surveillance.
10. Evaluation of the experience and skill level of at-risk personnel.

Sandia National Laboratories International Biological Threat Reduction department worked with biosafety, infectious disease, and risk experts to develop a systematic and standardized methodology for biological safety risk assessments. This standardized methodology will enhance biosafety risk assessments by allowing them to be both repeatable and quantifiable. This methodology is not intended as an all-hazards assessment, but is focused on the risks associated with biological materials being handled in a laboratory setting. Sandia National Laboratories defined criteria related to:

- Agent factors which impact the biosafety risk to humans,
- Agent factors which impact the biosafety risk to animals,
- Procedures used for the activity being assessed, procedures and processes involving animals used for the procedure being assessed.

A "scoring system" was developed for each criterion, with zero defined as the absence of the element defined by the criterion and four defined as the highest possible value for the element (for some elements the highest possible value is the worst case and for others the highest possible value is the best case). For example:

Is this agent known to cause infection via inhalation in humans (to cause infection via droplets or droplet nuclei that have entered the upper or lower respiratory tract) in a laboratory setting?

4=Preferred route

2= A possible route

1=Unknown

0=Not a route

Taking the via inhalation in humans scenario for an agent which cannot cause infection via inhalation the score will be zero; for an agent which via inhalation as *M. tuberculosis* is the preferred route of infection the score will be four.

The biosafety risk assessment model has been coded into a software package which runs on Microsoft's .Net Framework. The software, titled "BioRAMSoftware.exe" (Version 1.0 dated September 2010), was planned to be released open source and discussions have started to freely license the software to organizations. The BioRAMSoftware allows visitors to provide the scores for all the criteria in a simple tool by answering a set of questions. The BioRAMSoftware calculates the risk scores using the algorithms and weights defined in the model and methodology. The BioRAMSoftware also allows visitors to modify the wording of questions and the definitions of the scoring scales to better reflect a unique laboratory situation or language differences. The software then produces a numeric and graphical document with the relative risk rankings for the visitors and a chart identifying the impact each question had on the final results. This feature is useful in understanding and communicating the risks, as well as providing guidance on risk management or mitigation efforts. Also, visitors can view and, if needed, modify the weights. The methodology outlined is consistent with internationally accepted risk assessment schemes and also parallels international biosafety risk assessment guidance.

Biosafety RAM includes generalized definitions of how to conduct a biosafety risk assessment:

Evaluate the biological agents that exist at the facility.

Evaluate the facility processes and procedures.

Evaluate the existing biorisk mitigation measures.

You can establish the procedures and concrete situations based upon your institution's particular environment, geographical conditions, and risk assessment. The BioRAMSoftw is available on: <http://www.sandia.gov/>

The selection should include all situations and procedures that represent a risk for employees, the community, the natural environment, and animals. Biosafety and biocontainment measures should then be implemented based upon each institution's particular situation. A risk assessment should be initiated that defines the specific problem. The method of risk assessment should be simple; easy to apply and interpret; and should permit a quantitative classification of risk on a scale ranging from very low, low, moderate, high, and very high risk.

A risk assessment should include the characteristics of *M. tuberculosis* that are described in the safety data sheet. The intrinsic properties and the laboratory techniques that are likely to generate infectious aerosols should be evaluated based upon each particular situation. For example, in a research laboratory one should establish the difference between the characteristics of bacteria's under study; the H37Ra (ATCC 25177) used in some experiments is classified as a RG 2 pathogen, while the H37Rv (ATCC 2618) strain is designated as RG 3

pathogen. The information collected in the risk assessment may confirm changes in the pathogenicity of the specific microorganism and, therefore, the risk assessment may be altered enough to require an increase in the BSL and PPE for its containment. For example, bacteria which have developed resistance to multiple therapeutic drugs, such as *M. tuberculosis* MDR or XDR are considered to be a higher risk due to the lack of treatment alternatives and are to be handled with more stringent precautions. This bacteria is RG 3, but the extra precautions required for safe work with *M. tuberculosis* MDR would not be expected to take it to a higher containment level than BSL 3.

The laboratory diagnosis of TB should determine the percentage of positivity of pathogenic mycobacteria of clinical specimens submitted for the *M. tuberculosis* test; reported studies estimate that only 1% is positive; however, this data will obviously vary according to region and the number of samples that each laboratory processes. Additionally, the following factors are of crucial relevance in the risk assessment:

- *M. tuberculosis* can be isolated from virtually any type of human or animal specimens.
- The infectious dose in humans is very low and some samples processed in a diagnostic laboratory, such as the sputum of an infected patient, can contain several millions of bacilli per milliliter.
- The infection predominantly occurs by inhalation of airborne bacilli and the manipulation of liquid clinical specimens that likely involves generation of infectious aerosols, although percutaneous injury or infection by secondary transmission through the use of contaminated PPE or laboratory surfaces may also result in infection (Miller 1987, and Muller 1988).

Studies of air transmission of TB conducted during the first half of the last century by Wells (Wells 1955), led to the framing of the concept of the “droplet nucleus.” The great majority of laboratory technicians generate droplets of liquid or aerosols and each droplet may contain one or more bacillus. The aerosols that are produced can be classified according their size:

Droplet nuclei: with a size ranging from 1 to 10 μm in diameter and a velocity of propagation of 0.2 to 18 cm/minute or 0.1 to 1 μm in diameter and a velocity of propagation of 0.005 to 0.2 cm/minute.

Dust: with a size ranging from 10 to 100 μm in diameter and a velocity of propagation of 18 to 1800 cm/minute.

Droplet: with a size ranging from 100 to 400 μm in diameter and a velocity of propagation of 1800 to 15200 cm/minute. These particles containing *M. tuberculosis* can remain airborne from minutes to hours.

Larger droplets would not dry and could rapidly contaminate laboratory equipment and surfaces, and fingers or gloves, resulting in a secondary contamination of mouth and nasal cavities. The droplets settle very slowly and dry, and they are transformed into droplet nuclei. These droplet nuclei float in the air of a room and are spread by very small air currents; when inhaled they can settle in alveolar spaces and infect the employee.

Remark: among the laboratory techniques used for the identification and characterization of *M. tuberculosis*, the following ones are likely to increase the risk of contamination or to

generate infectious aerosols producing droplet nuclei, such as: centrifugations, pipetting, mechanical homogenizing, sonication, heating, boiling, work with bacteriological loops, preparation and manipulation of frozen sections, handling of containers with clinical specimens, acid-fast staining, manipulation of solid and liquid cultures, flow cytometry, and animal studies.

The risk assessment should also be conducted in hospitals, healthcare units, respiratory isolation areas, ambulatory assistance spaces, and for the TB or non- TB patients transiting through the institution.

3. Biosafety

Biosafety currently involves a large, interdisciplinary group of professionals gathered with the unique objective of guaranteeing that the risk of contracting infection for employees of the institution, and animals, is reduced to a minimum, and that the environment is protected. Currently, however, many of the decisions implemented to reduce biorisk, and contain infectious agents, are also employed by the community-at-large as part of anti-pandemic programs. These activities are dynamic and are strengthened by recent scientific and industrial advances. Changes in the biological characteristics of microorganisms, and the pace of modern life, have generated host-parasite relations that facilitate the transmission of illnesses that are devastating for humanity. Thanks to the groups engaged in interdisciplinary, scientific work, many of these unusual relationships have been disclosed. General and basic recommendations, of a compulsory nature, to ensure biosecurity in the manipulation and containment of *M. tuberculosis*, are discussed below.

WHO classifies microorganisms within four RGs according to infectious characteristics, availability of treatment, preventive measures, and the possibility of containing dissemination (WHO 2004):

RG 1: no or low individual and community risk

RG 2: moderate individual risk, low community risk

RG 3: high individual risk, low community risk

RG 4: high individual and community risk

Current classifications, similar to those created by WHO, have been developed by other institutions, such as: Standards Australia/New Zealand 2002, Canadian Laboratory Biosafety Guidelines (Laboratory Centre for Disease Control 1996), European Economic Community Directive (Comission of the European Communities 2000), NIH Recombinant DNA guidelines, and CDC/NIH guidelines (NIH 2002).

M. tuberculosis is located within these classifications as a microorganism RG3; therefore, its management requires the implementation of PPE consistent with its biological characteristics; a level of security in facilities and laboratory equipment that will minimize the risk of infection and maximize the capability of containment; and measures focused on preventing the intentional, malicious use of this microorganism by the institution's staff or outside parties.

The WHO's *Laboratory Biosafety Manual*, 3rd ed., 2004, which addresses the general principles of biosecurity, establishes the recommended BSLs for the management of microorganisms according to their RG, offers examples of laboratory practices that should be frequently conducted, and the requisite safety equipment. The BSL designations are based on a combination of the containment facilities; design features, equipment, construction, practices and operational procedures required for working with agents belonging to the various RGs. The laboratory facilities are designated as:

- BSL 1 - basic laboratory: for microorganisms in RG 1; an example of a facility would be a basic teaching laboratory; requirements include a good microbiological technique, open bench work, and an autoclave for sterilization of material. The use of PPE is recommended in all procedures; however, safety equipment such as a biological safety cabinet (BSC) is not required.
- BSL 2 - basic laboratory: for microorganisms in RG 2; examples of laboratories are primary health services, primary level hospitals, diagnostic, teaching and public health. Require an implementation of good microbiological technique plus PPE in all procedures; biohazard signs, open benches or ventilation (inward air flow or mechanical via building system) plus BSC for the potential aerosols and autoclave. Various procedures related to the identification of *M. tuberculosis* through clinical samples are conducted at this BSL; therefore, specific PPE measures are obligatory in order to minimize the risk of infection. The following are some accepted procedures for the identification of *M. tuberculosis* through clinical samples:
 - Making smear microscopy and diagnostic activities including primary culture of clinical specimens potentially infected by bacilli of *M. tuberculosis*. (Welch 1993)
 - Extraction of DNA, RNA, proteins, cell compounds, and molecular methodologies, following the inactivation, death, and lysis of the microorganism; and, having previously determined that the experimental protocol for the extraction and separation of the bacterial components is completely secure in the particular laboratory conditions in which it is conducted (Burgos 2004, Castro 2009 and Warren 2006).
- BSL 3 - laboratory with containment conditions for microorganisms in RG3; examples of laboratories are special diagnostic, production facilities, national tuberculosis reference laboratory and research laboratories; all of the conditions that apply for BSL 2 are included, plus specific PPE, controlled access with double-door entry, isolation laboratory, room sealable for decontamination, directional air flow, ventilation (inward air flow, mechanical via building system and filtered air exhaust), safety equipment as BSC class II or III and autoclave, preferably double -ended. Positive, viable samples of *M. tuberculosis*, *M. tuberculosis* MDR, and *M. tuberculosis* XDR should be handled at this BSL (Sessler 1983), including the following activities:
 - Handling solid and liquid positive culture
 - Secondary cultures for diagnostic or research activities
 - DNA or RNA extraction (initial step of each protocol)
 - Biochemical test for *M. tuberculosis* identification
 - Bacterial suspension preparation
 - Detection drugs resistance
 - Centrifugations
 - Pipetting
 - Mechanical homogenizing

- Sonication, heating or boiling
- Work with bacteriological loops
- Preparation and manipulation of frozen sections (biopsies)
- Animal studies
- Infected clinical specimens and others

BSL 4 – laboratory with maximum containment: for microorganisms in RG 4; this is the maximum containment-BSL and includes all of the requirements of BSL 3 plus airlock entry, airlock with shower, effluent treatment, BSC class III, shower exit, and special waste disposal.

Remark: although some of the precautions may appear to be unnecessary for some organisms, and no clinical or hospital laboratory has complete control over the specimens it receives, the staff may occasionally and unexpectedly be exposed to organisms in higher RGs; therefore, each employee is responsible for his/her own safety; this implies the obligatory and continuous use of PPE, and biosecurity and biocontainment measures, equipment and facilities while in areas of biological risk.

General considerations to guarantee biosecurity in each of the established levels are provided in the recommendations issued by WHO, CDC, NIH (American Thoracic Society 1983, Kent 1985, and CDC 1999 WHO 2004). Compliance with these measures should be part of a culture of biosecurity and professional responsibility. We consider it important to stress some of the following, specific measures for the handling of potentially contaminated material, or material infected with *M. tuberculosis*, in accordance with the requisite level of biosecurity:

BSL 2:

- The international biohazard sign should be displayed on the doors of rooms where the clinical specimens for the search of *M. tuberculosis* are being processed.
- The laboratory personnel have specific training in handling agents such as *M. tuberculosis* and are directed by a competent scientist.
- Access to the laboratory is limited when work is being conducted.
- Extreme precautions are taken with contaminated sharp items.
- The procedure in which infectious aerosols or splashes may be created are conducted in CBS (remember that the risk of aerosols possibly infected with *M. tuberculosis* in a clinical sample such as sputum, or a biopsy, vary according to the number of cases in a region and the number of samples processed by a laboratory; therefore, procedures including making smear microscopy should be conducted in a color booth with biological containment and specific filters for the retention of chemical vapors emitted during the coloration process) and additionally, films and smears for microscopy should be handled with forceps, stored appropriately, and sterilized before disposal.
- Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.

- The work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against *M. tuberculosis*.
- The wastes are decontaminated before disposal by an approved decontamination against *M. tuberculosis*.
- An insect and rodent control program is in effect.

Recommendations for special procedures:

- Access to the laboratory is restricted by the laboratory director when the work with *M. tuberculosis* includes possibly infectious substances. **Remark:** persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not permitted in the laboratory. Every person, upon initiating employment, should have a medical exam and laboratory tests in order to confirm that he/she is not at risk. Each institution should establish requirements to guarantee that all staff members are clinically suited for this type of work; this process should be conducted in coordination with occupational health professionals, health insurers, and experts in risk assessment and biosafety. In some countries, such as Colombia, vaccination with BCG is indicated; in those nations that have imposed this requirement, the employer should request the appropriate certificate to document the vaccination.
- A degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes and scalpels. These items should be used only when absolutely necessary and should be discarded in appropriate containers for subsequent decontamination, thereby preventing the formation of aerosols.
- Spills and accidents that result in over-exposure to substances possibly infected with *M. tuberculosis* are immediately reported to the chief. Medical evaluation and surveillance, and prophylaxis or treatment, should be provided based upon the severity of the accident and estimated risk of the procedure that was being conducted. Each institution should implement protocols for biorisk containment in order to maintain biosecurity; all personnel (housekeeping, professional, administrative, students, and others authorized to enter work areas) should be familiar with these policies. All laboratory areas should include a containment kit that facilitates the rapid implementation of corrective measures following an accident, including an appropriate disinfectant for laboratory surfaces and equipment, PPE, absorbent paper, tweezers for removal of glass particles, and signs to restrict access to the area where the accident occurred. The accident response should conclude with an analysis of causes and implementation of corrective measures.

BSL 3:

All general guidelines governing standard microbiological practices are applicable, in addition to the measures included in BSLs 1 and 2.

- The two person rule should apply, whereby no individual ever works alone in the laboratory.
- All procedures involving the *M. tuberculosis* manipulation are conducted within BSC.

- The laboratory has special engineering design features; however, in the case of those laboratories that do not possess all of these features, good ventilation, illumination, and disinfection of surfaces should be employed in order to guarantee good biosafety. Access to the laboratory is restricted, utilize standard microbiological practices. The decision to implement this modification of BSL 3 recommendations should be made only by the laboratory director.
- The laboratory doors are kept closed when experiments are in progress.
- The laboratory personnel receive the appropriate immunizations with BCG Vaccine or test for the infection surveillance with *M. tuberculosis*, TST or IGRAs.
- The biosafety manual specific to the laboratory is prepared or adopted by the laboratory director and biosafety precautions are incorporated into standard operating procedures.
- The laboratory and support personnel receive specific training about the potential hazards associated with *M. tuberculosis*.
- The laboratory director is responsible for ensuring that before working with *M. tuberculosis*, all workers demonstrate proficiency in standard microbiological practices and experience in handling *M. tuberculosis*.

The CDC and NIH, besides providing orientation about standard microbiological practices and special practices for each BSL, also describe the safety equipment or primary barriers and the laboratory facilities or secondary barriers (CDC 2000). In the following section we reproduce some of these recommendations and include information pertaining to the handling of *M. tuberculosis*:

Safety equipment or primary barriers for BSL 2

- All procedures should be conducted in BSC class II; the selection of BSC should be based upon the actual conditions of each laboratory as reflected in the risk assessment; all BSC's should include at least one HEPA (high efficiency particulated air) filter with at least 99.97% efficiency in retaining particles of 0.3 micrometers, protecting both the operator and the environment; various types may also provide greater protection for the product.

BSC class II type A1:

- Re-circulates 70% and removes 30% to the interior of the laboratory
- Minimum inflow 75 fpm
- Gas jets, volatile toxic chemicals, and radionucleotides cannot be used
- Includes a front aperture and guillotine-type window

BSC class II type A2:

- Re-circulates 70% and removes 30% to the interior of the laboratory
- Minimum inflow 100 fpm
- Gas jets, volatile toxic chemicals, and radionucleotides cannot be used
- Includes a front aperture and guillotine-type window
- Installation of a tube can allow 30% of the air to be ventilated to the exterior of the lab; traces of radionucleotides and small quantities of volatile, toxic liquids can be used

The two types of BSC class II can be used in BSLs 1, 2, and 3 (National Sanitation Foundation International -NSF- 2002).

- The centrifugation of specimens should be done in closed containers, i.e., centrifuge safety cups; these containers are opened only in BSC class II.
- Protective elements such as goggles, mask, and face shield, should be used in order to prevent splashes or sprays of material possibly contaminated with *M. tuberculosis* or other infectious substances. These measures should be used continuously while working, preferably when BSC protection is not available.
- Clothing appropriate for the laboratory should be used, such as impermeable uniforms, gowns that close at the back, caps, shoe protectors, and other items that the institution may consider to be necessary. Laboratory clothing should be used only in the laboratory and is not permitted in other areas such as cafeterias, bathrooms, libraries, on public transportation, offices, etc.

Remark: “all protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.”

- When working with contaminated material, on contaminated surfaces or equipment, it is recommended that two pairs of gloves be used. Every laboratory should establish a protocol for the disinfection of gloves and hand washing upon completing work.

The laboratory facilities or secondary barriers for BSL 2:

- Laboratory installations should be isolated from public areas, when possible
- Hand-washing and eyewash should be available
- Laboratory facilities, furniture and chairs, should be easy to clean and disinfect; avoid the use of carpets and rugs.
- The laboratory should not be accessible to unauthorized persons.

Safety equipment or primary barriers for BSL 3

- All PPE specific for the manipulation of *M. tuberculosis* should be used, including: caps, shoe protectors, impermeable clothing, and gowns that close in the back. These elements should be disinfected prior to leaving BSL 3 for the laundry or to be discarded.
- Frequent change of gloves and hand washing is recommended. Disposable gloves are not reused. Respiratory and face protection are used when handling or monitoring infected animals.
- In the case of *M. tuberculosis*, infected material is handled in BSC class II; the possibility of using BSC class II type B should be considered based upon the characteristics and risk assessment of each laboratory.

BSC class II type B1:

Re-circulates 40% and removes 60% to the exterior of the laboratory

Minimum inflow 100 fpm

Permits traces of radionucleotides and small quantities of volatile, toxic chemicals; the use of gas burners is not recommended.

BSC class II type B2:

Re-circulates 0% and removes 100% to the exterior of the laboratory

Minimum inflow 100 fpm

Recommended for the handling of radionucleotides and volatile, toxic chemicals; the use of gas burners is not recommended.

Remark: each laboratory should develop its own protocols for the use and disinfection of BSC, according to the instructions of the manufacturer, frequency of use, and risk assessment. The decision to use a BSC class III should be based upon the risk assessment of each laboratory.

Some PPE serve an important function, especially given the current, particular situation concerning *M. tuberculosis*. The short-term prospects of obtaining a vaccine or new, alternative methods of treatment are remote, and the evolving strain of *M. tuberculosis* that is resistant to various, contemporary therapies dictate effective methods of personal protection. Presently, individual respiratory protection is the most recommended measure, and not only for laboratory staff; these devices should also be used by hospital personnel (physicians, nurses, respiratory therapists, and administrative personnel who attend patients, among others); additionally, it is necessary to remark upon the differences between respirators and surgical masks (American National Standard Institute 1992, CDC 1994). The surgical masks provide protection against pathogens present in droplets emitted by coughing; protection is limited to the nose and mouth as the mask does not completely cover the face; therefore, masks do not provide protection against infection contained in droplet nuclei. These masks are now recommended for use by TB patients as they move throughout the hospital or when they are within confined spaces. Respirators, on the other hand, are designed to provide protection against pathogenic microorganisms contained in droplet nuclei; these can include respirators for the retention of particulates or the purification of air. Respirators that purify air function with batteries that power a ventilator providing filtered air to the user; this protective item can be disinfected, allows the change of HEPA filters, and guarantees a level of 100% purification of air. A particulate respirator can be reusable and employ filters that are easily replaced; the equipment can be disinfected and permits installation of a new filter. These respirators do not prevent transmission when used by infected persons. Disposable face respirators are made of filtered material that impedes the passage of large and small particles contained in the air; some include a valve for expelling air. The National Institute of Occupational Safety and Health –NIOSH– 2003 and 2004) has approved nine types of respirators for the retention of particulates. Differences include capacity to filter air, and resistance of the filter to oil (partially or strongly resistant). See table 2. Any of these can be used when handling *M. tuberculosis*.

General characteristics of respirators			
Class of respirators		Resistance to oils	% of retention
R	95	Resistant	95
	99	Resistant	99
	100	Resistant	99.97
P	95	Partial	95
	99	Partial	99
	100	Partial	99.97
N	95	No	95
	99	No	99
	100	No	99.97

Table 2. General characteristics of respirator.

Correct training in the use and care of these items is indispensable in order to guarantee their protective function; the respirators should be properly adjusted to the face; the perception of odors or the presence of air leaks is an indication that the respirator is not functioning properly. **Remark:** These elements are for individual use and should be discarded when alterations, stains, porosity, or humidity are present.

The laboratory facilities or secondary barriers for BSL 3:

- All doors and windows should remain closed.
- A double-door system should be in place that does not allow both doors to be open at the same time (an alarm should sound if this occurs).
- A special ventilation system with HEPA filters and negative pressure should be installed.
- All procedures should be conducted in BSC.
- Autoclaves, preferably double-ended, should be on-site, in the laboratory room.
- A constant supply of electricity, water, disposal, and gas should be guaranteed; filters and other necessary items should be available in order to ensure the containment of *M. tuberculosis* and other pathogens.
- All necessary equipment should be available in order to conduct all processes and avoid the entrance and exit of material that should be contained.
- All procedures that occur at this BLS should be documented and approved by the laboratory director and the institution's biosafety committee, who should then monitor compliance with the policies.
- Illumination should be adequate and should avoid reflections on cabinet windows and on other materials that would impair the vision of the operator.
- Installations and integrated systems at this BSL should be monitored and inspected periodically.
- Work areas should include decontamination systems and an adequate waste-disposal program (a company should be employed that specializes in this area).
- Equipment should be located in such a manner that facilitates disinfection below and between the items.
- Equipment and work surfaces should be resistant to the action of disinfectants.
- Professionals are now available who specialize in the planning, construction, and maintenance of laboratories at BSL 3; they should be consulted and evaluated by the biosafety committee of each institution.

3.1 Biosafety and hospital control

Hospital patient care areas, waiting rooms, healthcare units, respiratory isolation areas, and TB patients transiting through the institution, are just some examples of areas that require PPE for workers personnel and the requisite BSL in order to minimize the risk of exposure (CDC 1996). The implementation of biosafety and biocontainment measures in the hospital should begin with the creation of a TB control committee responsible for risk assessment (CDC 2003). The committee should be responsible for the following functions:

- Comprised of professionals who are expert in the area of biological changes of *M. tuberculosis* and its forms of resistance.

- The risk area should be identified
- Provide preventive measures and guidelines for patient isolation; identify, intervene and monitor the transmission risk areas
- Develop protocols for the management of patients, accidents in areas of risk, anti-pandemic plans, and for other situations that may arise
- supervise the compliance with protocols and guidelines

The areas that comprise the greatest risk of transmission in a hospital, and over which the TB control committee should focus its attention, are: ambulatory waiting rooms; radiology room; bronchoscopy and sputum induction rooms; respiratory isolation rooms; ventilator assistance areas; emergency room; autopsy room; and microbiology or micobacteria laboratories. A detailed analysis indicates that these areas comprise almost 70% of the services provided by a general hospital; therefore, the activities of the TB control committee should be continuous and rigorous.

One of the effective measures used to diminish the transmission of MDR TB and XDR TB in the community is the placement of the patient in a respiratory isolation room; the room should have HEPA filters for the recirculation of air with a minimum replacement of six air changes per hour. The room should have negative pressure, guarantee the privacy of the patient, permit effective disinfection measures, and preferably contain an anteroom in order to minimize escape risk.

The TB control committee should delegate authority to a designated professional to decide which ambulatory or hospitalized patients should be located within the isolation area. The patient should receive clear instructions regarding behavior while in the respiratory isolation area (Garner 1996). For example, the patient should cover his/her mouth and nose when coughing in the room; upon leaving the room, the patient should cover his/her mouth and nose with a surgical mask. Healthcare personnel should avoid, to the maximum extent possible, entering the isolation area (Chen 1994). A small number of professionals should care for the patient and, when doing so, utilize a special mask such as N95, or respirators.

Remark: the TB control committee should monitor areas where patients gather, and should prepare guidelines to ensure that patients with suspected respiratory illness, pediatric patients, infectious persons, and geriatric patients are not in the same waiting room.

The TB control committee should also develop guidelines for ambulatory care areas that provide for adequate ventilation, illumination, and periodic disinfection with effective chemical agents against the pathogens most frequently encountered in this area. Air conditioners and ventilators are permitted only when used in conjunction with HEPA air filtration systems (American Institute of Architects 2001). The assistance room should include a ventilator that maintains a barrier between the physician and patient. Air flow should be directed toward the air that enters through doors and windows. See figure 2.

3.2 Biosafety in the teaching laboratory

The teaching laboratories are usually found in academic institutions to provide a venue for instructing students on how sciences are conducted and for training in specific applications. This laboratory in all discipline is unique in at least one important aspect. As a general rule and particularly at introductory levels, teaching laboratories tend to be densely populated

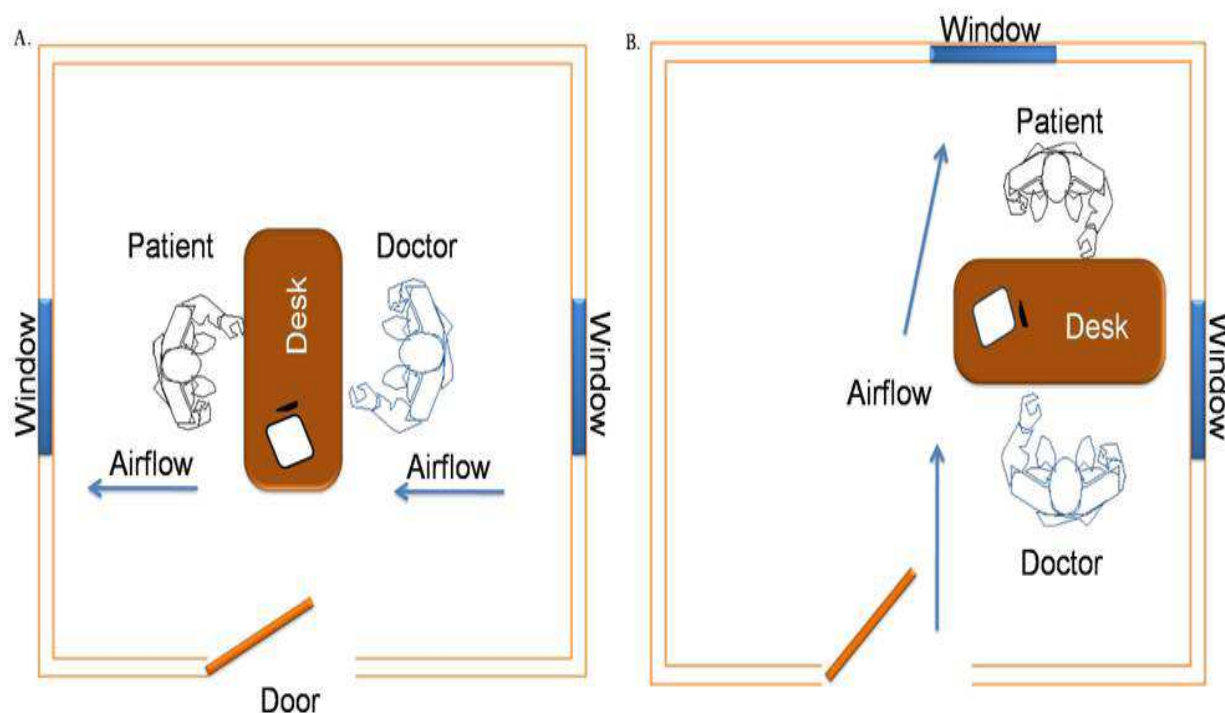


Fig. 2. Basic biosafety recommendations at the healthcare units.

On <http://www.who.int/docstore/gtb/publications/healthcare/index.htm> the WHO has proposed practical and low cost interventions to reduce nosocomial transmission.

with large numbers of individuals with limited experience in hazard of a science laboratory and certain number of them may be immunocompromised. Please answer the question ¿Are teaching laboratories less safe than others laboratories? The correct answer is no, because in this space the student must learn the specific topics for risk assessment, biosafety and biocontainment. It is a responsibility of educational institutions to teach about biosafety with the international and national guidelines and the use of PPE. (WHO 1992, CDC 1999, 2002, and 2005, Food and Drug Administration 2004).

3.3 Biosafety in the pharmaceutical industry

The microorganisms used in pharmaceutical companies are extremely diverse, encompassing bacteria, viruses, fungi, helminthes and protozoa. The pharmaceutical companies that use pathogenic microorganisms to produce or testing drugs, and vaccine must establish a broad range of biosafety practice to ensure the safety of their workers and their product. During the scale up, the biosafety practices employed should be in harmony with the international guidelines to ensure that the manufacturing process and product may be used and sold internationally. The biosafety in the pharmaceutical encompasses both laboratory scale practices and requires a well organized and implemented program of risk assessment, risk management, program evaluation and modification (Advisory Committee on Dangerous Pathogens 1998). More information: CDC 1997 and <http://pharmacos.eudra.org/F2/eudralex/vol-4/pdfs-en/anx02en200408.pdf>

4. Biocontainment

The biocontainment measures are very important, they arise from an adequate handling that an institution must do of their risk assessment, biological level, procedures, biosecurity measures, PPE, standards and protocols in order to prevent malicious use. The implementation and strict adherence of standard microbiological practices, it is currently considered the best measure of biocontainment for *M. tuberculosis* and other infectious substances is. The adoption of a biosafety culture together with a good laboratory practice and facilities design is a guarantee to preserve the environment and control risk.

The design of laboratories, as well as the supportive health and engineering staff faces great challenges like: to guarantee the maintenance of long-term infrastructure, to build efficiently at a reasonable cost and conscious planning of energy and water, localization of these laboratories (If the possibility is offered, this should be discussed with the regional development plans in each region). The biocontainment culture in an institution should anticipate the management of unexpected situations and for this; the institution must have contingency plans and emergency procedures.

4.1 The contingency plan must include

Operative procedures for the risk assessment, identification of high risk areas, to identify as much specific as possible to the population at risk and their characteristics, emergency transportation for the personnel exposed and prioritize this work when an incident occur, inventory of resources, suppliers commitment with availability of treatments, availability of PPE and properly trained personnel in the proper use and final disposal. Precise actions and simulations to verify the effectiveness of the evacuation plan and estimate the possibility of natural disaster like earthquakes powerful, storms and flooding, depending on the geographic region. (Lindell 1996 and, Young 2004)

Emergency procedures should include practical protocols, effective and achievable depending on the resources of the institution, these biocontainment protocols should include all possible events or accidents according with common activities like: ingestion or inhalation of potentially infectious material, broken containers and infectious spilled substances, breakage of tubes in centrifuges, puncture wounds, cuts and abrasions.

The police and fire departments should be involved in the development of emergency and contingency plans for fire or natural disasters, but they need to take a special training. Since, it is impossible to prevent all incidents of this nature, some precautions must be followed. These are some examples of what can be done to minimize the possibility of releasing pathogenic organism into the environment as the result of a natural disaster: post notices on all incubators, refrigerators, freezers and other storage facilities and contents listing persons to be notified in case of incident, secure store in culture collections, damage resistant cabinets or containers, cabinets or shelving provide for storing books, equipment, chemicals and others that close securely with doors, do not store heavy boxes and equipment above bench level.

5. Disinfection and sterilization

Understanding the importance of decontamination, cleaning, sterilization and disinfection is vital for implementing a laboratory biosafety plan. The descending order of resistance to

Disinfection					
Reagent	Concentration	Time exposure	Action	Funtion	Dificulties
Phenol	0.4 - 5%	10 minutes	Protein denaturation	Efficient disinfectants	Irritant, toxic, corrosive
Formal-dehyde	5%	10 minutes	Protein alkylation	Efficient disinfectants	Cutaneous irritant, respiratory irritant, eye irritant
Glutaral-dehyde	1 - 2%	20 - 30 minutes	Membrane disruption	Efficient disinfectants, or decontaminating surfaces	Toxic, cutaneous irritant, eye irritant
Sodium hypochlorite	0.2 - 5%, 5000 ppm, 1g/L	1 - 2 minutes	Enzymatic inhactivation	Efficient disinfectants	Toxic, corrosive, cutaneous irritant, respiratory irritant, eye irritant
Ethyl alcohol	70%, 96%	2 minutes	Protein action, membrane disruption	Surface disinfectant, mycobacterial disinfectants	Eye irritant
Hydrogen peroxide	3 - 10%	5 minutes	Free radicals, lipid and proteins action	Disinfectants	Corrosive, respiratory irritant
Iodophore	2 - 10 %, 75 ppm		Iodination and oxidation of proteins	Disinfectants	Cutaneous irritant, respiratory irritant, eye irritant, corrosive
Mix 1 : Iodine + ionophores, or ethyl alcohol	variable	variable	Iodination and oxidation of proteins	Efficient disinfectants	Cutaneous irritant, respiratory irritant, eye irritant, corrosive
Mix 2: paraformal dehyde + glutaraldeh yde or formalin solutions.	2- 5%	10 - 30 minutes	Protein alkylation, membrane disruption	Inactivation	Toxic, cutaneous irritant, respiratory irritant, eye irritant

Table 3. Summarizes the properties of some liquid germicides that are recommended again *M. tuberculosis*.

germicidal chemicals is: bacterial spores, Mycobacteria (especially *M. tuberculosis*), small viruses (Non lipid), fungi, vegetative bacteria, and medium size viruses (lipid) (Favero 1998, and 2001, and CDC 2003). Each laboratory must evaluate the efficacy of germicides. See table 3 (Kunz 1982, Best 1988, 1990, Rubin 1991, Rutala 1991, Sattar 1995, Schwebach 2001, and Blackwood 2005). Is essential that manufacturer's recommended use dilutions are followed.

Sterilization: susceptible to moist heat sterilization at 121°C for 15 minutes at 121 pounds of pressure.

6. Packing and shipping biological materials

The care and responsibility that one assumes when transporting infected material that contains live *M. tuberculosis* serve to guarantee the biosecurity of this important process. The transportation of infected material can occur within the same hospital (from the patient's room to the laboratory) or to an outside location (other institutions, cities, or countries). The transport of material infected with *M. tuberculosis* to areas within a hospital or laboratory should be made using resistant containers that can be easily disinfected; the container should have a hermetic seal capable of containing infectious substances during accidents, or until such time as the substances can be handled in a BSC. Currently, various organizations have prepared guidelines that should be used in order to reduce the risk of infection to personnel and the environment.

Current regulations governing the transport of hazardous items include obligatory actions that apply to the three parties involved in the process: the recipient (should receive import authorization and provide the appropriate documentation); the transporter or operator (should use a verification list, accept or reject the items to be transported, provide training, adequate documentation and instructions) and the shipper who should comply with packaging norms (should classify, identify and package the infected material, place markings and labels, document and have emergency plans in place).

The majority of the guidelines established for the transport of hazardous materials have been issued by the following agencies:

- International Civil Aviation Organization (ICAO), a specialized United Nations (UN) agency with the regulations entitled "Safe Transport of Dangerous Goods by Air"
- The IATA with regulations entitled "IATA Dangerous Goods Regulations"
- The U.S. Department of Transportation (DOT) with regulations entitled "United States Hazardous Material Uniform Safety Act."

These regulations are similar with respect to the following guidelines: classification and naming of diagnostic specimens and infectious substances; marking and labeling packaging material; training and certification of personnel; practical suggestions for classifying diagnostic specimens and infectious substances; resources for additional information and documentation; and instructions for completing a shipper's declaration for dangerous goods. This can change significantly as a result of investigations or sudden pandemics which may necessitate new measures and regulations.

IATA requirements and DOT regulations provide minimum requirements for packing and shipping diagnostic specimens and infectious substances. These provisions include:

- Classification and naming of the substances to be shipped: select the appropriate IATA packing instructions; in the case of category A material, submit the necessary information to complete the shipper's declaration. The substance or material should be classified in one of the nine IATA specified classes (1- explosivos, 2- gases, 3- flammable liquids, 4- flammable solids, 5- oxidizing substances and organic peroxides, 6- toxic and infectious substances, division 6.1: toxic substances, division 6.2 infectious substances, 7- radioactive materials, 8- corrosives and 9- miscellaneous dangerous goods) (IATA 2006).
- The classification 6.2 must be divided into one of nine IATA specific groups such as: - category A infectious substances, -category B infectious substances, -exempt human or animal specimens, -exempt substances, -patient specimens, -genetically modified organisms, -biological products, - infected animals, -medical waste.

The category A substances are specifically designated as pathogens which can be dangerous to both individual and public health. Category A pathogens and substances likely to contain category A pathogens must be assigned UN number UN2814 for infectious substances that affect humans or UN2900 for infectious substances affecting animals.

- The selection of package and packing the shipment correctly: after having classified the infectious substance, the shipper must officially name the Category A or B material; the substances must then be assigned one of the more than 3,000 IATA specified, and internationally recognized, UN numbers accompanied by the proper shipping name as provided by IATA regulations. This list provides information about 14 items, identified in alphabetical order from A to N, for each of the proper shipping names; this data is required in order to complete the shipper's declaration.

The PI describes the minimum standards for the safe transport of various biological materials. Shippers are legally obligated to comply with the regulations. Materials must be packaged properly in order to ensure the safety of all personnel who handle the package before, during, and after shipment. Clinical laboratories transporting category A infectious substances should use PI 602; for category B infectious materials, PI 650 should be used.

The PI 602 used for the packaging of infectious material should comply with the following requirements: leakproof and pressure-resistant for the first and second containers; absorbent between the first and second containers; list of contents between the second container and outer package; rigid outer packaging; positively sealed first container; name, address, and telephone number of responsible person on outer package or air waybill; shipper declaration for dangerous goods; outer packaging marking and labels; and strict manufacturing specifications. See table 4:

- The outer package should include appropriate markings and labels: labeling is the act of placing informational labels or stickers onto the surface of an outer package. The shipper is responsible for the proper marking and labeling of the outer shipping container. The labels and markings include:

Column	Information		
A	United Nations number of the proper shipping name/description	2814	
B	Proper shipping name/description	Infectious Substance, Affecting Humans (Liquid)	Infectious Substance, Affecting Humans (Solid)
C	Class or division of dangerous good	6.2	6.2
D	NA		
E	Hazard label required on the outer package	Infectious substance	Infectious substance
F	NA		
G	NA		
H	NA		
I	PI to use for passenger and cargo aircraft	602	602
J	Maximum allowable amounts to be shipped in passenger and cargo aircraft	50 ml	50 g
K	PI to use for cargo aircraft only	602	602
L	Maximum allowable amounts to be shipped in cargo aircraft only	4 liters	4 kg
M	Applicable special provisions and exceptions	A81 A140	A81 A140
N	Emergency response code	11Y	11Y

Table 4. Shipping requirements for infection substances (*M. tuberculosis*).

- Name and direction of the shipper, name, address and telephone number of the responsible party, as provided by IATA regulations. The diamond-shaped infectious substances label should be used when shipping contaminated material, accompanied by a label which shows the proper shipping name, UN number, and quantity of the substances; the package orientation label must also be included and placed on opposite

sides of the entire package. Additional markings may be included as required. The “cargo aircraft only” label indicates that the package may be shipped only in cargo, and not on passenger, aircraft; this label is used if the infectious substance is over 50 ml but less than 4 liters per the outer package being shipped. The “overpack” markings indicate that an overpack is used and that inner packages comply with the regulations. Packaging that meets UN specifications is marked by a “UN” inside of a circle and a series of letters and numbers which indicate the type of package, class of goods the package is designed to carry, the manufacturer, authorizing agency, and manufacturing date. The designation “Class 6.2” indicates that the container is approved for shipping infectious substances. (see figure 3)

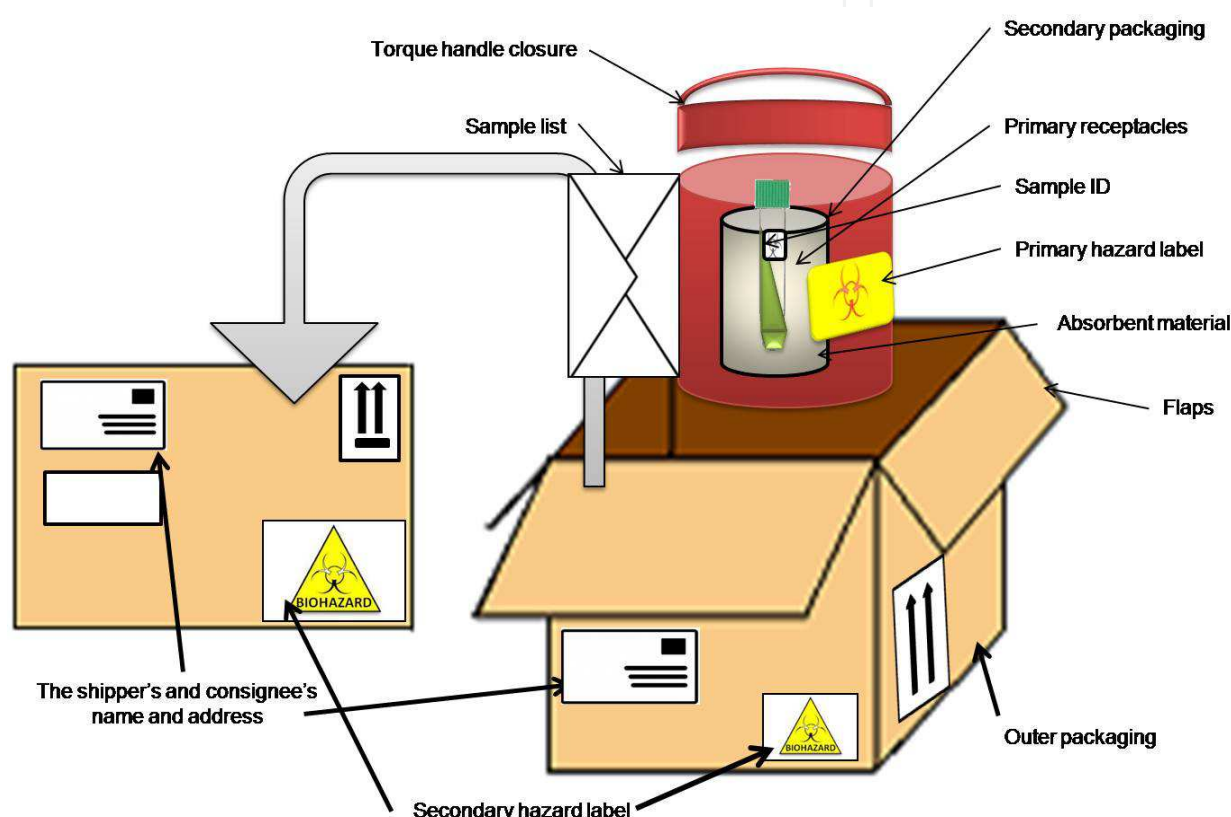


Fig. 3. Biomedical packaging for infectious substances, marking and labeling packages established by agencies governing transportation of dangerous goods.

- The relevant document: the preparation and delivery of a shipper's declaration is necessary in order to formalize a legal contract; this document is required for category A substances; however, it is not required for category B material. These documents should be completed in their entirety.
- Training for personnel: competent authorities such as the CDC offer training in the transport of infectious substances to laboratory personnel, members of biosecurity committees, and professionals in different areas. (IATA 2006, U.S. Department of Transportation 2004, and 2006, and WHO 2004, and 2005) Additional information concerning these regulations is available at <http://www.iata.org> or http://www.who.int/crs/resourceces/publications/biosafety/WWHO_CDS-EPR-2007_2/en/index.html

7. Conclusion

Is buying biosafety and biocontainment the best option?

The growing number of professionals infected by *M. tuberculosis*, many of whom have died due to this without knowing for certain the source of infection or exposure (whether the community or their workplace), and the dramatic figures of deaths caused by TB around the world among people infected or not with HIV confront us with a question to which we have no answer: Did the person get infected in a hospital waiting room, in an international flight? When did it happen? We don't know, and that is why it is so important to enhance community awareness on these issues.

Risk assessment and the adoption of biosecurity and biocontainment measures with the participation of academic institutions, scientists, designers and community at large represent paying the right price and obtaining the expected impact.

8. Self evaluation

Self evaluation:

1. What is *M. tuberculosis*?
 - a. Fungi
 - b. Bacteria
 - c. Virus
 - d. Other
2. Which is *M. tuberculosis* mode of transmission?
 - a. Airborne
 - b. Sexual contact
 - c. Vertical transmission.
3. How is *M. tuberculosis* spread person to person?
 - a. Inhalation of infectious aerosol or infected droplets
 - b. Water
 - c. Foods
 - d. Clothes
4. What is a N95?
 - a. Respirator
 - b. Surgical mask
 - c. HEPA filter
5. Is the infectious dose of *M. tuberculosis* less than 1000 bacilli by inhalation route in humans?
 - a. Yes
 - b. No
 - c. Is unknown
6. What are the tests for TB infection?
 - a. PPD or IGRAs
 - b. BCG
 - c. PPE

7. What is the meaning of microorganisms RG 3
 - a. no or low individual and community risk
 - b. high individual and community risk
 - c. high individual risk, low community risk
 - d. moderate individual risk, low community risk
8. What IATA category is *M. tuberculosis*?
 - a. Category A
 - b. Category B
 - c. Category PI
9. The sterilization conditions for *M. tuberculosis* included?
 - a. 121°C for 15 minutes at 121 pounds of pressure
 - b. 121°C for 90 minutes at 140 pounds of pressure
 - c. 15°C for 15 minutes at 121 pounds of pressure
 - d. Unknown

9. References

- Advisory Committee on Dangerous Pathogens. 1998. The Large Scale Contained Use of Biological Agents. Her Majesty's Stationery Office, London, England.
- American Institute of Architects, Committee on Architecture for Health. 2001. Guidelines for Construction and Equipment of Hospital and Medical Facilities. American Institute of Architects Press, Washington, D.C.
- American National Standard Institute. 1992. American National Standard for Respirator Protection (ANSI Z88.2). American National Standards Institute, New York, N.Y.
- American Thoracic Society. 1983. Levels of laboratory services for mycobacteria diseases. Am. Rev. Dis. 128:213.
- Best M, Sattar S.A., Springthorpe V.S., Kennedy M.E. 1988. Comparative mycobactericidal efficacy of chemical disinfectants in suspension and carrier test. Appl Environ Microbiol. 54:2856-8.
- Best M, Sattar S.A., Springthorpe V.S., Kennedy M.E. 1990. Efficacies of selected disinfectants against *Mycobacterium tuberculosis*. J Clin Microbiol 28:2234-9.
- Blackwood K.s., Burdz T.V., Turenne C.Y., Sharma M.K., Kabani A.M. Wolfe J.N. 2005. Viability testing of material derived from *Mycobacterium tuberculosis* prior to removal from a containment Level-III Laboratory as part of a laboratory risk Assessment program. BMC Infectious Diseases, 5, (4): 1-7.
- Boa, E., J. Lynch, and D.R. Liliquist. 2000. Risk Assessment Resource. American Industrial Hygiene Association, Fairfax, Va.
- Burgos M.V., Mendez J.C., Ribón W. 2004. Molecular epidemiology of tuberculosis: methodology and applications. Biomédica. 24(Supl.):188-201
- Castro C., González., Roza J., Puerto G., Ribón W. 2009. Biosafety evaluation of the DNA extraction protocol for *Mycobacterium tuberculosis* complex species, as implemented at the Instituto Nacional de Salud, Colombia. Biomédica. 29:561-6
- Centers for Disease Control and Prevention and National Institutes of Health. 1999. Biosafety in Microbiological and Biomedical laboratories, 4th ed. U.S. Government Printing Office, Washington, D.C. Centers for Disease Control and Prevention and

- National Institutes of Health. 2006. Biosafety in Microbiology and Biomedical laboratories, 5th ed. U.S. Government Printing Office, Washington, D.C.
- Centers for Disease Control and Prevention and the Healthcare Infection Control Advisory Committee. 2003. Guidelines for environmental infection control in health care facilities: recommendation of CDC and the Healthcare Infection Control Advisory Committee.
- Centers for disease Control and Prevention. 1994. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care facilities, 1994. Morb. Mortal. Wkly. Rep. 43(RR-13):1-132.
- Centers for Disease Control and Prevention. 1996. Guideline for isolation precautions in hospitals. Am. J. Infect. Control 42:24-45.
- Centers for Disease Control and Prevention. 1997. Goals Working safely with *Mycobacterium tuberculosis* in clinical, public health, and research laboratories.
- Centers for Disease Control and Prevention. 2002. Guideline for hand hygiene in health-care settings. Morb. Mortal. Wkly. Rep. 51(RR-16):1-44.
- Centers for Disease Control and Prevention. 2005. Possession, Use, and Transfer of Select Agents and Toxins. 42 CFR parts 72 and 73. U.S. Department of Health and Human Services. Fed. Regist. 70:13293-13325.
- Centers for Diseases Control and Prevention and National Institutes of Health. 2000. Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 2nd ed. J. R. Richmond and R.W. McKinney (ed.) U.S. Government Printing Office, Washington, D.C.
- Chen, S.-K., D. Vesley, L.M. Brosseau, and J.H. Vincent. 1994. Evaluation of single-use mask and respirators for protection of health care workers against mycobacterial aerosols. Am. J. Infect. Control. 22:65-74.
- Commission of the European Communities. 2000. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16 (1) of Directive 89/391/EEC). Official journal of the European Communities, L262/21-45, 17.10.2000.
- Cousins D.V., Bastida R., Cataldi A., Redrobe S., Dow s., Duignan P., Murray A., Dupont C., Ahmed N., Collins d. M. butler W.R., Dawson D., Rodriguez D., Loureiro J., Romano M.I., Alito A., Zumarraga M., Bernardelli A. 2003. Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipeddi* sp. Nov. Int Syst Evol Microbiol, 53,1305-1314.
- Favero, M. 1998. Developing indicators for sterilization, p.119-132. In W.A. Rutala (ed.), Disinfection, Sterilization and Antisepsis in Health Care. Association for Professionals in Infection Control and Epidemiology, Inc., Champlain, N.Y.
- Favero, M. 2001. Sterility assurance: concepts for patient safety, p.110-119. In W.A. Rutala (ed.), Disinfection, Sterilization and antisepsis: Principles and Practices in Healthcare Facilities. Association for Professionals in Infection Control and Epidemiology, Inc., Washintong, D.C.

- Favero, M., and W. Bond. 2001. Chemical disinfection of medical surgical material, p. 881-917. In S.S. Block (ed.) Disinfection, Sterilization, and Preservation, 5th ed. Lippincott, Williams and Wilkins, Philadelphia, Pa.
- Food and Drug Administration. 2004. 21 CFR Part 211. Current Good Manufacturing Practices for finished pharmaceuticals. U.S. Code of Federal Regulations.
- Garner, J.S., and the Hospital Infection Control Practices Advisory Committee. 1996. CDC guideline for isolation precautions in hospitals. Am. J. Infect. Control 24:24-52.
- Garner, J.S., and the Hospital Infection Control Practices Advisory Committee. 1996. CDC guideline for isolation precautions in hospitals. Am. J. Infect. Control 24:24-52.
- Ghosh J., Larsson P., Singh B., Pettersson B., Islam N., Sarkar S., Dasgupta S., and Kirsebom L. 2009. Sporulation in mycobacteria. PNAS 106: 10781-10.
- Grange J.M. Tuberculosis. In: Topley and Wilson Principles of Bacteriology, Virology and Immunology, 9th ed. Year book, 1990. vol.3,p.94-121.
- Hankenson, F. C., N.A. Johnson, B.J. Weigler, and R.F. Di Giacomo. 2003. Overview: Zoonoses of occupational health importance in contemporary laboratory animal research. Comp. Med. 53:570-601.
- Harrington J.M., Shannon H.S. Incidence of tuberculosis, hepatitis, brucellosis and shigellosis in British Medical Laboratory workers. Br Med J 1976;1:759-62.
- International Air Transport Association. 2006. IATA Dangerous Goods Regulations, 47th ed. International Air Transport Association, Montreal, Canada.
- International Air Transport Association. 2006. Infectious Substances Shipping Guidelines, 7th ed. Ref. N°9052-07. International Air Transport Association, Montreal, Quebec, Canada.
- Kent, P. T., and G. P. Kubica. 1985. Public Health Mycobacteriology. A guide for the level III Laboratory. Centers for Disease Control, Atlanta, Ga.
- Krauss, H., A. Weber, M. Appel, B. Enders, H.D. Isenberg, H.G. Schiefer, W. Slenczka, A. von Graevenitz, and Zahner. 2003. Zoonoses: Infectious Diseases Transmissible from Animals to humans, 3rd ed. ASM Press, Washintong, D.C.
- Kunz R., Gunderman KC. The survival of *Mycobacterium tuberculosis* on surfaces at different relative humidities. Zent Bakt Hyg 1982; 176. 105-115.
- Laboratory Center for Diseases Control, Health Protection Branch, Health Canada. 1996. Laboratory Biosafety guidelines, 2nd ed. Ministry of Supply and Services, Ottawa, Ontario, Canada.
- Lietman, T., and S. Blower. 1999. Tuberculosis vaccines. Science 286:1300-1301.
- Lindell, M. K., and R. W. Perry. 1996. Addressing gaps in environmental emergency planning hazardous materials releases during earthquakes. J. Environ. Planning Manag. 39:529-545.
- Miller C.D. Songer J.R., Sullivan J.F. 1987. A twenty-five year review of laboratory acquired human infections at the National Animal Disease Center. Am Ind hyg assoc J, 48,271-275.
- Muller H.E. 1988 Laboratory-acquired mycobacterial infection. Lancet, 2:331.
- National Institute of Health. 2002. NIH guidelines for research involving recombinant DNA molecules (NIH guidelines). Fed. Regist. 59:34496 (July 5, 1994) as amended. (http://www4.od.nih.gov/oba/rac/guidelines/guidelines_html).

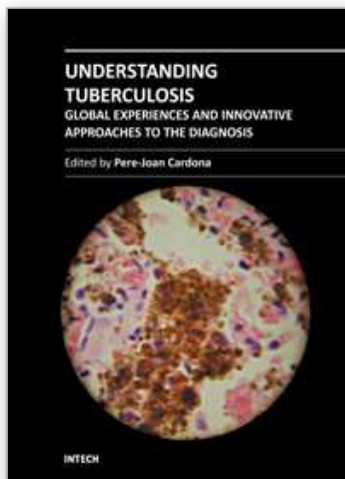
- National Institutes of Health. 2002. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), 59 FR 34496 (July 5, 1994), as amended.
- National Research Council. 1997. Occupational Health and Safety in the Care and Use of Research Animals. National Academy Press, Washington, D.C.
- National Research Council. 2003. Occupational Health and Safety in the Care and Use of Nonhuman Primates. National Academy Press, Washington, D.C.
- NSF International. 2002. Class II (Laminar Flow) Biosafety cabinetry. NSF/ANSI standard 49-2002. NSF International, Ann Arbor, Mich.
- Occupational Safe and Health Administration (OSHA). 2003. Occupational exposure to tuberculosis. Notice. Fed. Regist. 68:75767-75775.
- Occupational Safe and Health Administration (OSHA). 2003. Personal Protective Equipment. Publication 3151-12R. OSHA Publications Office, Washington, D.C.
- Occupational Safety and Health Administration. 1996. CPL 2.106 Enforcement Procedures and Scheduling for Occupational Exposure to tuberculosis. Occupational Safety and Health Administration, Washington, D.C.
- Occupational Safety and Health Administration. 1997. Occupational Exposure to tuberculosis; proposed rule. Fed. Regist. 62:54159-54309
- Occupational Safety and health Administration. 30 July 2004. Standard Interpretations- Tuberculosis and Respiratory Protection. R. Davis Layne, Deputy Assistant secretary.
- Pike R.M.1978. Past and present hazards of working with infectious agent. Arch. Pathol. Lab. Med. 102:333-36.
- Pike, R.M. 1976. Laboratory-associated infections: summary and analysis of 3921 cases. Health Lab. Sci.13:105-114.
- Pike.R.M. 1979. Laboratory-associated infections: incidence, fatalities, causes, and prevention. Annu. Rev. microbial. 33:41- 66.
- Reid DD. Incidence of tuberculosis among workers in medical laboratories. Br Med J 1957;2:10-4.
- Riley, R. 1961. Airborne pulmonary tuberculosis. Bacteriol. Rev. 25:243-248.
- Riley, R.L. 1957. Aerial dissemination of pulmonary tuberculosis. Am. Rev. Tuberc. 76:931-941.
- Rozo J., and Ribón W.2010. Molecular tools for *Mycobacterium tuberculosis* genotyping. Rev. salud pública. 12 (3): 510-521.
- Rubin J. Mycobacterial disinfection and control. In: Seymour S. Block Lea and Febiger editors. Disinfection, sterilization and preservation, 4th edition, Year book, 1991.377-83.
- Rutala W. A., Cole E.C., Wannamaker N.S. Weber D.J. 1991. Inactivation of *Mycobacterium tuberculosis* and *Mycobacterium bovis* by 14 hospitals disinfectants. Am J Med 91:267-271S.
- Sattar S.A., Best M., Springthorpe V.S., Sanani G.1995. Mycobacterial testing of disinfectants: an update. Hosp Inf, 30 suppl. 372-382.
- Schwebach J.R., Jacobs W.R. Jr, Casadevall A. 2001. Sterilization of *Mycobacterium tuberculosis* Erdman samples by antimicrobial fixation in biosafety level 3 laboratory. J Clin Microbiol, 39, 769-771.

- Sessler, S.M., and R.M. Hoover. 1983. Laboratory Fume Hood Noise, Heating Piping and Air Conditioning. Penton/PC Reinhold, Cleveland, Ohio.
- Standars Australia/Standars New Zealand. 2002. Safety in Laboratories. Part 3: Microbiological Aspects and Containment Facilities. Australia/New Zealand Standard AS/NZS2243.3:2002. Standards Australia International Ltd., Sydney, Australia.
- Traag B., Driks A., Stragier P., Bitter W., Broussard G., Hatfull G., Chu F., Adams K., Ramakrishnan L., and Losick R.2010. Do mycobacteria produce endospores? PNAS 12:878-881
- U.S. Department of transportation, Pipeline and Hazardous Materials Safety Administration. 2006. Hazardous materials: infectious substances; harmonization with the United Nations recommendations; proposed rule. Fed. Regist. 71:32244-32263.
- U.S. Department of transportation, Research and Special Programs Administration. 2004. Harmonization with the United Nations recommendations. International Maritime Dangerous Goods Code, and International Civil Aviation Organizations Technical Instructions; final rule. Fed Regist. 69:76043-76187.
- Verhagen, L. Van den Hof, S. Mycobacterial Factors Relevant for Transmission of Tuberculosis. Journal of Infectious Diseases Advance Access published March 4, 2011.
- Warren R. Koch M., Engelke E., Myburgh R., van pittus N., Victor T., and van Helden P. 2006. Safe *Mycobacterium tuberculosis* DNA extraction method that does not compromise integrity. *Journal of Clinical Microbiology*, 44: 254-256.
- Wayne L.G. Mycobacterial speciation. In Kubica G.P. Wayne LG. editors. The Mycobacteria. A sourcebook. New York: Marcel-Dekker: Year book, 1984. 26-65.
- Welch, D. F., A. P. Guruswamy, S. J. Sides, C. J. Shaw, and M. J. R. Gilchrist. 1993. Timely culture for mycobacteria which utilizes a microcolony method. J. Clin. Microbiol. 31:2178-2184.
- Wells, W. 1934. On air-borne infection. II. Droplets and droplet nuclei. Am. J. Hyg. 20:611-18.
- Wells, W. F. 1955. Airborne contagion and Air Hygiene. Harvard University Press, Cambridge, Mass.
- Wheelis, M.2002. Biological warfare at the 1346 Siegui of Caffa. Emerg. Infect. Dis. 8:971-975.
- World Health Organization. 1992. Expert Committee on Specifications for Pharmaceutical Preparations, Thirty-Second Report. World Health Organization, New York, N.Y.
- World Health Organization. 2004. Laboratory Biosafety Manual.3rd ed. World Health Organization, Geneva, Switzerland.
- World Health Organization. 2004. Transport of infectious substances. Background to the amendments adopted in the 13th revision of the United Nations Model Regulations guiding the transport of infectious substances.
- World Health Organizations. 2005. Guidance on Regulations for the Transport of Infectious Substances. World Health Organization, Geneva, Switzerland.

Young, S., L. Balluz, and J. Malilay. 2004. Natural and technologic hazardous material releases during and after natural disasters: a review: *Sci. Total Environ.* 322:3-20.

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Mycobacterium tuberculosis is a disease that is transmitted through aerosol. This is the reason why it is estimated that a third of humankind is already infected by Mycobacterium tuberculosis. The vast majority of the infected do not know about their status. Mycobacterium tuberculosis is a silent pathogen, causing no symptomatology at all during the infection. In addition, infected people cannot cause further infections. Unfortunately, an estimated 10 per cent of the infected population has the probability to develop the disease, making it very difficult to eradicate. Once in this stage, the bacilli can be transmitted to other persons and the development of clinical symptoms is very progressive. Therefore the diagnosis, especially the discrimination between infection and disease, is a real challenge. In this book, we present the experience of worldwide specialists on the diagnosis, along with its lights and shadows.

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