

Scope

This biosafety manual is relevant for all research activities that especially include handling biological material in the laboratories.

Introduction

The research personnel in biological laboratories are at risk of handling samples that may be pathogen infected or live organisms that pose a hazardous effect to humans as well as environment. Thus this manual will provide guidelines to adoption of appropriate methods to avoid hazards in microbiological laboratories, dangerous chemicals, liquid nitrogen etc.

Risk Assessment:

Before starting any research activity in the lab, risk assessment must be performed to identify the hazards and design possible control methods to prevent risk.

For risk assessment, the following steps are necessary:

A. Identification of risk related to cell culture related biological materials that includes pathogen and human or animal cells being handled

All material of human origin, including cell lines, tissue, and blood, must be considered potentially infectious for hepatitis and HIV and handled under Universal Precautions, which reasonably preclude cutaneous, oral, and parenteral exposure to personnel.

For risk identification of the pathogen, the researcher should be informed for the following:

- (i) *Routes of transmission:* The probable routes of transmission are
 - (a) Exposure of the agent through the skin, eyes and mucous membranes.
 - (b) Piercing by a syringe needle or other contaminated sharp.
 - (c) Bites, scratches from infected animals.
 - (d) Ingestion of liquid suspension of an infectious agent.
 - (e) By contact of the contaminated hand to mouth exposure.

(f) Inhalation of infectious aerosols

(ii) Infective dose

(iii) Stability in the environment

(iv) Host range and its endemic nature.

(v) Method of treatment available.

B. Identification of risk related to procedures carried in the laboratory

Safety procedures must be established for the following laboratory handling procedures:

(i) that generate aerosols as they are invisible and spread faster.

(ii) on animals as they can transmit pathogen through saliva, urine and faeces.

(iii) Genetic manipulation of microorganisms can lead to generation of higher virulent microorganisms or antibiotic resistance.

C. Classify the microorganisms into appropriate biosafety levels

Based on the above information, the laboratory can be assigned at various levels that would help in the containment of the pathogens/human/animal cells. The containment is achieved by defining the safety of the research by various biosafety levels. Overall following biosafety levels are followed:

Biosafety Level 1 (BSL1)

BSL1 is defined for experiments that include a pathogen that is unlikely to cause any disease in humans or animals. Thus special facility or equipments are not needed but still the laboratory personnel should following initial training for carrying out experiments in BSL1. Following are the standard practices for BSL1

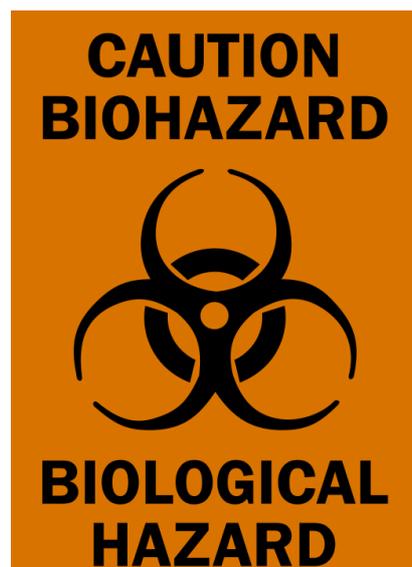
1. Lab coats, eye protection and toe-covered footwear to prevent contamination must be used.
2. Surgical masks should be used for handling carcinogens, chemicals or for biological agents if there is a risk of spread of infection through inhalation.
3. Protected glasses should be used during UV transilluminators.
4. Face shields must be used to protect the personnel from splashes.
5. Storage of food items or eating is not allowed in the laboratory
6. Mouth pipetting should not be practiced but pipetting devices should be used.
7. Broken glassware should be handled with dustpan and brush

8. In case of spills, contain the area and decontaminate the area using recommendations for that spill.
9. Waste material should be discarded in designated waste disposals.
10. The lab in charge should provide initial training for lab personnel about potential hazards and precautions to be taken and they are updated whenever there are changes in procedures.

Biosafety Level 2 (BSL2)

BSL2 is defined for experiments that include a pathogen that could cause disease in humans or animals but is unlikely a serious hazard. Also, effective treatment and preventive measures are available and the risk of spread of infection is limited. Thus BSL2 is a contained area where lab personnel requires specific training and are supervised by competent researcher.

- ⇒ Therefore, the lab doors must be self-closing type and there must be provision for locking the same.
- ⇒ The international biohazard-warning symbol must be displayed on the doors of the labs where microorganisms of Risk Group 2 or higher risk groups are handled. Name of the PI and contact details must also be displayed.



- ⇒ The pathogens must strictly be handled with biosafety cabinets BSLA2 type (described below). Also, the biosafety cabinet must be located away from doors and windows that could be opened which could create disruptions in the airflow.
- ⇒ Liquid disinfectant trap must be provided to vacuum lines.
- ⇒ The access to the laboratory is restricted to trained researchers.
- ⇒ Medical surveillance and immunization must be provided to the lab personnel.
- ⇒ Spills involving contaminated material must be decontaminated and cleaned up.

Biosafety Level 3 (BSL3)

BSL3 is defined for experiments that include a pathogen that has a potential to cause serious human or animal disease and is lethal through exposure by inhalation. Effective treatment and preventive measures are available

- ⇒ BSL3 is a strictly contained facility with special engineering and design features. Access is restricted and through two self-closing doors with a change room in between. The space between exit and window should be capable for sealing or fumigation.
- ⇒ Vacuum lines must be protected with high efficiency particulate air (HEPA) filters. The ventilation system must provide directional airflow by drawing air from clean areas to contaminated areas.
- ⇒ The decision to implement Biosafety Level 3 recommendations in the laboratory may only be made by the Institutional Biosafety Committee.

- ⇒ Entry into a BSL-3 facility is restricted to those individuals who have taken training and have demonstrated knowledge of BSL-3 Standard Operating Procedures and Safety Practices by means of a written exam.
- ⇒ All experiments involving the manipulation of infectious material are carried out in biological safety cabinets (BSCII or III) or other physical containment devices.
- ⇒ The personnel are required to wear appropriate personal protective clothing and devices to protect themselves from the exposure to pathogen. The personnel should be wrapped around in gowns or coveralls. The clothing needs to be decontaminated before discarding.
- ⇒ Eye and face protections should also be decontaminated.

Biosafety Level 4 (BSL4)

BSL4 is defined for experiments that include 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.

Since it is life threatening with no effective treatment, additional measures are followed for BSL4.

- ⇒ Access to the laboratory is strictly restricted and is through an airtight doors.
- ⇒ A shower is provided to decontaminate the surface of the pressure suit before the person leaves the work area.
- ⇒ The exhaust air from the suit area must be filtered by two sets of HEPA filters.
- ⇒ Double door autoclave for decontamination of disposable waste materials from the suit area to be used.

Summary of Classification of Biological Agents According to Risk

(Modified from *Biosafety in Microbiological and Biomedical Laboratories*, Centers for Disease Control and the National Institutes of Health, 3rd Ed., 1993.)

Biosafety Level 2: Bacterial Agents†

Bacillus anthracis

Bordetella pertussis

Campylobacter - all species

Chlamydia psittaci, *C. Pneumoniae*, *C. trachomatis*

Clostridium botulinum, *C. tetani*

Corynebacterium diphtheriae

Leptospira interrogans- all serovars

Legionella pneumophila; other Legionella-like agents

Mycobacteria except *M. bovis* or *M. leprae*

Neisseria gonorrhoeae, *N. Meningitidis*

Pseudomonas pseudomallei

Salmonella - all serotypes

Shigella - all species and all serotypes

Vibronic enteritis (*Vibrio cholerae*, *V. parahaemolyticus*)

† Additional primary containment and precautions, such as those described for work at the BSL-3 level are recommended for activities with high potential for droplet or aerosol production, for work with antibiotic - resistant strains and for activities involving production quantities or concentrations of infectious materials.

Biosafety Level 2: Fungal Agents

Blastomyces dermatitidis

Cryptococcus neoformans

Sporothrix schenckii

Pathogenic members of the genera *Epidermophyton*, *Microsporium* and *Trichophyton*

Miscellaneous molds

Cladosporium (Xylohypha) trichoides

Cladosporium bantianum

Penicillium marneffii

Exophiala (Wangiella) dermatitidis
Fonsecaea pedrosoi
Dactylaria gallopava (Ochroconis gallopavum)

Biosafety Level 2: Parasitic Agents

Nematode parasites of humans

Ascaris spp.

Strongyloides spp.

Hookworms *Enterobius* spp.

Protozoal Parasites of Humans

Toxoplasma spp.

Plasmodium spp.

Trypanosoma spp.

Entamoeba spp.

Coccidia spp.

Giardia spp.

Leishmania spp.

Sarcocystis spp.

Cryptosporidia spp.

Trematode Parasites of Humans

Schistosoma spp.

Fasciola spp.

Cestode Parasites of Humans

Echinococcus granulosus

Taenia solium (cysticercus cellulosae)

Hymenolepis nana.

Biosafety Level 2: Viral Agents ††

Hepatitis A, B, C, D, and E virus Herpes viruses - except Herpesvirus simiae (Monkey B virus) which is BSL-4

Influenza virus

Polioviruses Poxviruses - all types except variola which is restricted Rabies virus

Mouse hepatitis virus. For animal work it needs a separate contaminant suite.

†† Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for activities with potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials.

Biosafety Level 3: Bacterial Agents

Brucella - all species

Francisella tularensis

Mycobacterium bovis; *M. tuberculosis* *Yersinia pestis*

Biosafety Level 3: Fungal Agents

Coccidioides immitis

Histoplasma capsulatum

Biosafety Level 3: Viral and Rickettsial Agents

Lymphocytic choriomeningitis virus (LCM)

Rickettsiae - all species when used for transmission or animal inoculation experiments
Vesicular Stomatitis Virus

Retroviruses, including Human and Simian Immunodeficiency viruses (HIV and SIV)

Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob, kuru and related agents)

Arboviruses and Arenaviruses

Biosafety cabinets

Biosafety cabinets are one of the essential requirements for maintenance as well as containment of pathogen in a biosafety facility.

The selections of BSCs are decided on the type of protection needed. This includes

Lab personnel protection

- Sample protection
- Risk involved with the pathogen
- Risk involved with the chemicals

Following are the guidelines for biosafety cabinets to be considered for different biosafety levels;

Class I Biosafety cabinet

The Class I BSC protects the personnel and the environment from exposure to biohazards, however samples are vulnerable from coming in contact with airborne contaminant present in the room. Thus they are used for general microbiological research with low- and moderate-risk agents. The disadvantage is that it does not provide protection from contamination of the sample that is being handled as the room air passes over it.

Class II Biosafety cabinet

The Class II BSC provides protection both to personnel as well as the sample. These cabinets are suitable for BSL2, 3 and 4. The cabinet has an open front with a stream of inward air preventing the escape of aerosols outside. The air flowing downward over the working surface is HEPA filtered and thus the cabinet is free of airborne contaminant protecting the sample from contamination.

Class III Biosafety cabinet

BSC III cabinets provide an absolute safety where the cabinets are of welded metals construction and are gas tight. It has a non-opening window and the work is carried out glove ports in front of the cabinet. Thus they are working with highly infectious pathogenic agents and provide maximum protection for the environment and worker. Below are the details of different types of BSC cabinet suitable for different biosafety levels:

Source: **Laboratory Biosafety Manual**. Second Edition (revised). Interim guidelines. World Health Organization. p32.

Class	Inflow Velocity (m/s)	Recycle Air (%)	Exhaust Air (%)	Control Plenum Surrounded by	Exhaust Alternatives	Biosafety Level
I	US:0.38 EN:0.70	0	100	Outside Air	Inside room / Hard duct	1, 2 & 3
II Type A1	US:0.38 EN:0.40	70	30	Outside Air	Inside room / Thimble duct	1, 2 & 3
II Type A2	US:0.50 EN:0.40	70	30	Negative Plenum	Inside room / Thimble duct	1, 2 & 3
II Type B1	US:0.50 EN:0.40	30	70	Negative Plenum	Hard duct only	1, 2 & 3
II Type B2	US:0.50 EN:NA	0	100	Negative Plenum	Hard duct only	1, 2 & 3
III	Closed: * >0.5"WC	0	100	Negative Plenum	Inside room / Hard duct	1, 2, 3 & 4

* Pressure differential between chamber and environment.

Emergency responses

In case of spill inside biosafety cabinet

1. Contain aerosols by leaving BSC cabinet operating
2. Disinfect the cabinet by treating with 10% Bleach.
3. Clean the cabinet with water followed by 70% ethanol.
4. Operate the cabinet for 15min before starting work.

In case of spill outside biosafety cabinet

If the spill is of BSL1 or 2

1. Remove the contaminated clothing or gloves. Wear fresh gloves and if possible contain the spill by covering with paper towels. Decontaminate the area by addition of 10% bleach at least for 20min and mark the area of spillage.
2. Notify other lab personnel for spills.
3. Clean up the bleach
4. Dispose off all the materials in biohazard waste.

If the spill is of BSL3

1. Notify other lab personnel for spills.
2. Remove the contaminated clothing or gloves.
3. Wash body parts with disinfectant soap
4. Notify the emergency response team.

Biohazard Waste

Non-contaminated waste can be treated as non-infected waste and can be disposed off as general waste. The following waste can be treated as potentially biohazard waste and should be segregated and disposed off based on the waste type in a responsible manner.

Sharps Waste

All needles, syringes (with or without the attached needle), Pasteur pipettes, scalpel blades are collected in a puncture proof sharp containers for disposal.

Microbiological Waste

All materials containing or in contact with cultures of microbiological organisms, patient specimens sent for microbiological culture or items contaminated by patient

specimens are collected in autoclavable red bags with a biohazard sign. The materials are then autoclaved for decontamination and picked by the specified agency of the institute for incineration.

Biological Materials Waste

All discarded vaccines, immunoglobulin, plasma, albumin, blood or tissue fractionation products, enzyme preparations. Similar procedures are followed as for microbiological waste.

If the waste is liquid, then it is treated with 10% bleach solution overnight before discarding.

Animal Pathogen Contaminated Waste

All bedding and other materials contaminated with blood, excreta or secretions of animals infected with transmissible human or animal pathogens. Similar procedures are followed as for microbiological waste.

General Laboratory safety guidelines:

- **Wear approved lab coat at all the times.** If you have spilled chemical in your eyes, flush with water in an eye wash station for 10 to 15 minutes. Use safety shower in case of chemical spillage on body. Notify the incident to Supervisor and Safety In-Charge.
- **Confine long hair** whenever working in the laboratory.
- **Always wear full sleeves and a lab coat while working in the lab**
- **Wear Shoes while working in the lab.** Feet must be adequately covered. Open toed shoes or sandals are not permitted in the laboratory.
- **NO tobacco products in the laboratory.**
- **Food items and Eating is NOT allowed in the laboratory.**
- **Familiarize yourself with the lab** (equipment, chemicals).
- **Never mouth a pipette, use a rubber bulb.**
- **Report all spills** to Supervisor and Safety in Charge.
- **Do not use broken or chipped glassware** and dispose them in the glass disposal box.

- **There is a separate disposal for plasticware contaminated with organic solvents. Thus make sure that contaminated plasticware are discarded in the specified container present in each lab.**
- **Do not discard organic solvents in the sink but in the waste container for organic solvent placed in each lab.**