



PART I

Biosafety guidelines

2. Microbiological risk assessment

The backbone of the practice of biosafety is risk assessment. While there are many tools available to assist in the assessment of risk for a given procedure or experiment, the most important component is professional judgement. Risk assessments should be performed by the individuals most familiar with the specific characteristics of the organisms being considered for use, the equipment and procedures to be employed, animal models that may be used, and the containment equipment and facilities available. The laboratory director or principal investigator is responsible for ensuring that adequate and timely risk assessments are performed, and for working closely with the institution's safety committee and biosafety personnel to ensure that appropriate equipment and facilities are available to support the work being considered. Once performed, risk assessments should be reviewed routinely and revised when necessary, taking into consideration the acquisition of new data having a bearing on the degree of risk and other relevant new information from the scientific literature.

One of the most helpful tools available for performing a microbiological risk assessment is the listing of risk groups for microbiological agents (see Chapter 1). However, simple reference to the risk grouping for a particular agent is insufficient in the conduct of a risk assessment. Other factors that should be considered, as appropriate, include:

1. Pathogenicity of the agent and infectious dose
2. Potential outcome of exposure
3. Natural route of infection
4. Other routes of infection, resulting from laboratory manipulations (parenteral, airborne, ingestion)
5. Stability of the agent in the environment
6. Concentration of the agent and volume of concentrated material to be manipulated
7. Presence of a suitable host (human or animal)
8. Information available from animal studies and reports of laboratory-acquired infections or clinical reports
9. Laboratory activity planned (sonication, aerosolization, centrifugation, etc.)
10. Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment regimens (see Chapter 16)
11. Local availability of effective prophylaxis or therapeutic interventions.

On the basis of the information ascertained during the risk assessment, a biosafety level can be assigned to the planned work, appropriate personal protective equipment selected, and standard operating procedures (SOPs) incorporating other safety interventions developed to ensure the safest possible conduct of the work.

Specimens for which there is limited information

The risk assessment procedure described above works well when there is adequate information available. However, there are situations when the information is insufficient to perform an appropriate risk assessment, for example, with clinical specimens or epidemiological samples collected in the field. In these cases, it is prudent to take a cautious approach to specimen manipulation.

1. Standard precautions (2) should always be followed, and barrier protections applied (gloves, gowns, eye protection), whenever samples are obtained from patients.
2. Basic containment – Biosafety Level 2 practices and procedures should be the minimum requirement for handling specimens.
3. Transport of specimens should follow national and/or international rules and regulations.

Some information may be available to assist in determining the risk of handling these specimens:

1. Medical data on the patient
2. Epidemiological data (morbidity and mortality data, suspected route of transmission, other outbreak investigation data)
3. Information on the geographical origin of the specimen.

In the case of outbreaks of disease of unknown etiology, appropriate ad hoc guidelines may be generated and posted by national competent authorities and/or WHO on the World Wide Web (as was the case during the 2003 emergence of the severe acute respiratory syndrome (SARS)) to indicate how specimens should be consigned for shipment and the biosafety level at which they should be analysed.

Risk assessment and genetically modified microorganisms

A detailed discussion of risk assessment and genetically modified organisms (GMOs) is provided in Chapter 16.

3. Basic laboratories – Biosafety Levels 1 and 2

For the purposes of this manual, the guidance and recommendations given as minimum requirements pertaining to laboratories of all biosafety levels are directed at microorganisms in Risk Groups 1–4. Although some of the precautions may appear to be unnecessary for some organisms in Risk Group 1, they are desirable for training purposes to promote good (i.e. safe) microbiological techniques (GMT).

Diagnostic and health-care laboratories (public health, clinical or hospital-based) must all be designed for Biosafety Level 2 or above. As no laboratory has complete control over the specimens it receives, laboratory workers may be exposed to organisms in higher risk groups than anticipated. This possibility must be recognized in the development of safety plans and policies. In some countries, accreditation of clinical laboratories is required. Globally, standard precautions (2) should always be adopted and practised.

The guidelines for basic laboratories – Biosafety Levels 1 and 2 presented here are comprehensive and detailed, as they are fundamental to laboratories of all biosafety levels. The guidelines for containment laboratories – Biosafety Level 3 and maximum containment laboratories – Biosafety Level 4 that follow (Chapters 4 and 5) are modifications of and additions to these guidelines, designed for work with the more dangerous (hazardous) pathogens.

Code of practice

This code is a listing of the most essential laboratory practices and procedures that are basic to GMT. In many laboratories and national laboratory programmes, this code may be used to develop written practices and procedures for safe laboratory operations.

Each laboratory should adopt a safety or operations manual that identifies known and potential hazards, and specifies practices and procedures to eliminate or minimize such hazards. GMT are fundamental to laboratory safety. Specialized laboratory equipment is a supplement to but can never replace appropriate procedures. The most important concepts are listed below.

Access

1. The international biohazard warning symbol and sign (Figure 1) must be displayed on the doors of the rooms where microorganisms of Risk Group 2 or higher risk groups are handled.

Figure 1. *Biohazard warning sign for laboratory doors*



2. Only authorized persons should be allowed to enter the laboratory working areas.
3. Laboratory doors should be kept closed.
4. Children should not be authorized or allowed to enter laboratory working areas.
5. Access to animal houses should be specially authorized.
6. No animals should be admitted other than those involved in the work of the laboratory.

Personal protection

1. Laboratory coveralls, gowns or uniforms must be worn at all times for work in the laboratory.
2. Appropriate gloves must be worn for all procedures that may involve direct or accidental contact with blood, body fluids and other potentially infectious materials or infected animals. After use, gloves should be removed aseptically and hands must then be washed.
3. Personnel must wash their hands after handling infectious materials and animals, and before they leave the laboratory working areas.

4. Safety glasses, face shields (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes, impacting objects and sources of artificial ultraviolet radiation.
5. It is prohibited to wear protective laboratory clothing outside the laboratory, e.g. in canteens, coffee rooms, offices, libraries, staff rooms and toilets.
6. Open-toed footwear must not be worn in laboratories.
7. Eating, drinking, smoking, applying cosmetics and handling contact lenses is prohibited in the laboratory working areas.
8. Storing human foods or drinks anywhere in the laboratory working areas is prohibited.
9. Protective laboratory clothing that has been used in the laboratory must not be stored in the same lockers or cupboards as street clothing.

Procedures

1. Pipetting by mouth must be strictly forbidden.
2. Materials must not be placed in the mouth. Labels must not be licked.
3. All technical procedures should be performed in a way that minimizes the formation of aerosols and droplets.
4. The use of hypodermic needles and syringes should be limited. They must not be used as substitutes for pipetting devices or for any purpose other than parenteral injection or aspiration of fluids from laboratory animals.
5. All spills, accidents and overt or potential exposures to infectious materials must be reported to the laboratory supervisor. A written record of such accidents and incidents should be maintained.
6. A written procedure for the clean-up of all spills must be developed and followed.
7. Contaminated liquids must be decontaminated (chemically or physically) before discharge to the sanitary sewer. An effluent treatment system may be required, depending on the risk assessment for the agent(s) being handled.
8. Written documents that are expected to be removed from the laboratory need to be protected from contamination while in the laboratory.

Laboratory working areas

1. The laboratory should be kept neat, clean and free of materials that are not pertinent to the work.
2. Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of the working day.
3. All contaminated materials, specimens and cultures must be decontaminated before disposal or cleaning for reuse.
4. Packing and transportation must follow applicable national and/or international regulations.
5. When windows can be opened, they should be fitted with arthropod-proof screens.

Biosafety management

1. It is the responsibility of the laboratory director (the person who has immediate responsibility for the laboratory) to ensure the development and adoption of a biosafety management plan and a safety or operations manual.
2. The laboratory supervisor (reporting to the laboratory director) should ensure that regular training in laboratory safety is provided.
3. Personnel should be advised of special hazards, and required to read the safety or operations manual and follow standard practices and procedures. The laboratory supervisor should make sure that all personnel understand these. A copy of the safety or operations manual should be available in the laboratory.
4. There should be an arthropod and rodent control programme.
5. Appropriate medical evaluation, surveillance and treatment should be provided for all personnel in case of need, and adequate medical records should be maintained.

Laboratory design and facilities

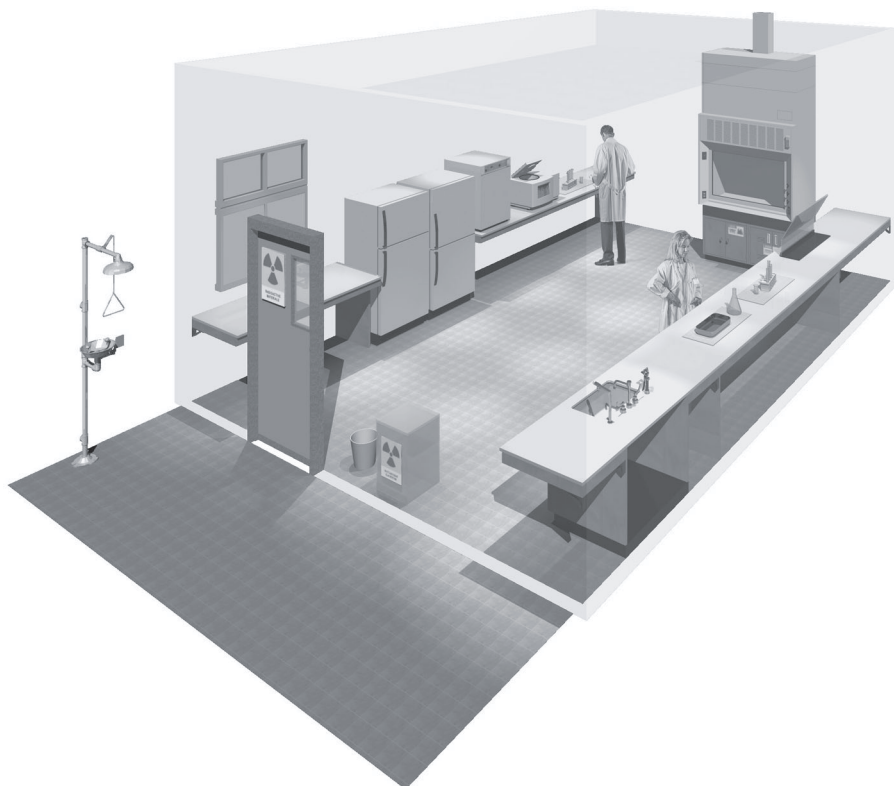
In designing a laboratory and assigning certain types of work to it, special attention should be paid to conditions that are known to pose safety problems. These include:

1. Formation of aerosols
2. Work with large volumes and/or high concentrations of microorganisms
3. Overcrowding and too much equipment
4. Infestation with rodents and arthropods
5. Unauthorized entrance
6. Workflow: use of specific samples and reagents.

Examples of laboratory designs for Biosafety Levels 1 and 2 are shown in Figures 2 and 3, respectively.

Design features

1. Ample space must be provided for the safe conduct of laboratory work and for cleaning and maintenance.
2. Walls, ceilings and floors should be smooth, easy to clean, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floors should be slip-resistant.
3. Bench tops should be impervious to water and resistant to disinfectants, acids, alkalis, organic solvents and moderate heat.
4. Illumination should be adequate for all activities. Undesirable reflections and glare should be avoided.
5. Laboratory furniture should be sturdy. Open spaces between and under benches, cabinets and equipment should be accessible for cleaning.
6. Storage space must be adequate to hold supplies for immediate use and thus prevent clutter on bench tops and in aisles. Additional long-term storage space, conveniently located outside the laboratory working areas, should also be provided.



*Figure 2. A typical Biosafety Level 1 laboratory
(graphics kindly provided by CUH2A, Princeton, NJ, USA)*

7. Space and facilities should be provided for the safe handling and storage of solvents, radioactive materials, and compressed and liquefied gases.
8. Facilities for storing outer garments and personal items should be provided outside the laboratory working areas.
9. Facilities for eating and drinking and for rest should be provided outside the laboratory working areas.
10. Hand-washing basins, with running water if possible, should be provided in each laboratory room, preferably near the exit door.
11. Doors should have vision panels, appropriate fire ratings, and preferably be self-closing.
12. At Biosafety Level 2, an autoclave or other means of decontamination should be available in appropriate proximity to the laboratory.
13. Safety systems should cover fire, electrical emergencies, emergency shower and eyewash facilities.
14. First-aid areas or rooms suitably equipped and readily accessible should be available (see Annex 1).

15. In the planning of new facilities, consideration should be given to the provision of mechanical ventilation systems that provide an inward flow of air without recirculation. If there is no mechanical ventilation, windows should be able to be opened and should be fitted with arthropod-proof screens.
16. A dependable supply of good quality water is essential. There should be no cross-connections between sources of laboratory and drinking-water supplies. An anti-backflow device should be fitted to protect the public water system.
17. There should be a reliable and adequate electricity supply and emergency lighting to permit safe exit. A stand-by generator is desirable for the support of essential equipment, such as incubators, biological safety cabinets, freezers, etc., and for the ventilation of animal cages.
18. There should be a reliable and adequate supply of gas. Good maintenance of the installation is mandatory.
19. Laboratories and animal houses are occasionally the targets of vandals. Physical and fire security must be considered. Strong doors, screened windows and restricted issue of keys are compulsory. Other measures should be considered and applied, as appropriate, to augment security (see Chapter 9).

Laboratory equipment

Together with good procedures and practices, the use of safety equipment will help to reduce risks when dealing with biosafety hazards. This section deals with basic principles related to equipment suitable for laboratories of all biosafety levels. Requirements for laboratory equipment pertinent to higher biosafety levels are dealt with in the relevant chapters.

The laboratory director should, after consultation with the biosafety officer and safety committee (if designated), ensure that adequate equipment is provided and that it is used properly. Equipment should be selected to take account of certain general principles, i.e. it should be:

1. Designed to prevent or limit contact between the operator and the infectious material
2. Constructed of materials that are impermeable to liquids, resistant to corrosion and meet structural requirements
3. Fabricated to be free of burrs, sharp edges and unguarded moving parts
4. Designed, constructed and installed to facilitate simple operation and provide for ease of maintenance, cleaning, decontamination and certification testing; glassware and other breakable materials should be avoided, whenever possible.

Detailed performance and construction specifications may need to be consulted to ensure that the equipment possesses the necessary safety features (see also Chapters 10 and 11).

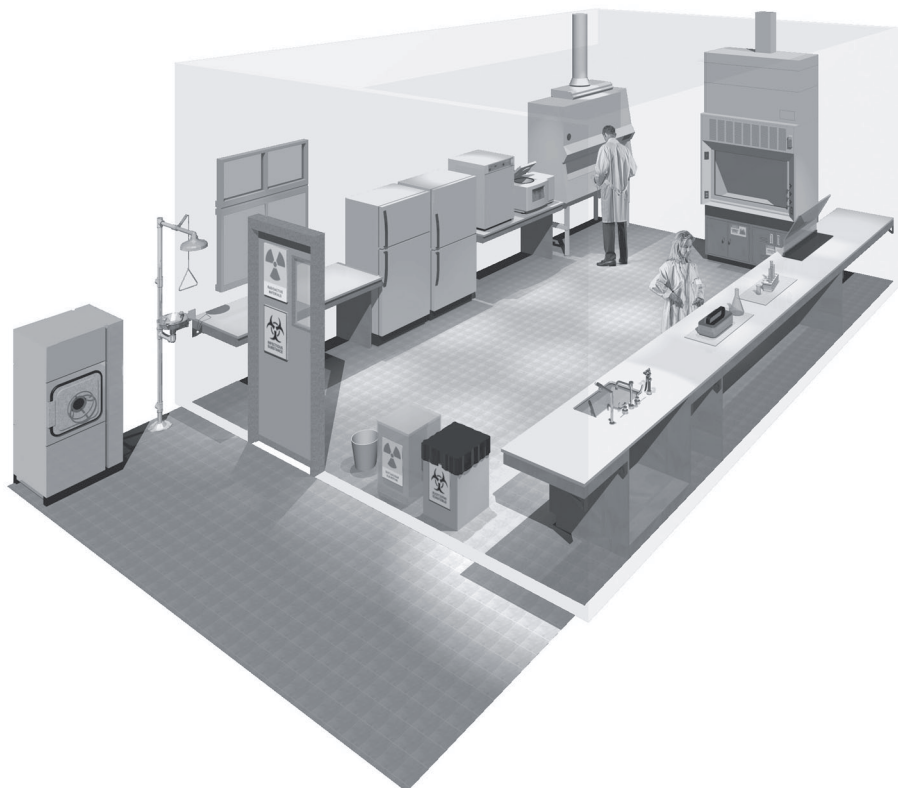


Figure 3. A typical Biosafety Level 2 laboratory (graphics kindly provided by CUH2A, Princeton, NJ, USA). Procedures likely to generate aerosols are performed within a biological safety cabinet. Doors are kept closed and are posted with appropriate hazard signs. Potentially contaminated wastes are separated from the general waste stream.

Essential biosafety equipment

1. Pipetting aids – to avoid mouth pipetting. Many different designs are available.
2. Biological safety cabinets, to be used whenever:
 - infectious materials are handled; such materials may be centrifuged in the open laboratory if sealed centrifuge safety cups are used and if they are loaded and unloaded in a biological safety cabinet
 - there is an increased risk of airborne infection
 - procedures with a high potential for producing aerosols are used; these may include centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption, opening of containers of infectious materials whose internal pressure may be different from the ambient pressure, intranasal inoculation of animals, and harvesting of infectious tissues from animals and eggs.
3. Plastic disposable transfer loops. Alternatively, electric transfer loop incinerators may be used inside the biological safety cabinet to reduce aerosol production.

4. Screw-capped tubes and bottles.
5. Autoclaves or other appropriate means to decontaminate infectious materials.
6. Plastic disposable Pasteur pipettes, whenever available, to avoid glass.
7. Equipment such as autoclaves and biological safety cabinets must be validated with appropriate methods before being taken into use. Recertification should take place at regular intervals, according to the manufacturer's instructions (see Chapter 7).

Health and medical surveillance

The employing authority, through the laboratory director, is responsible for ensuring that there is adequate surveillance of the health of laboratory personnel. The objective of such surveillance is to monitor for occupationally acquired diseases. Appropriate activities to achieve these objectives are:

1. Provision of active or passive immunization where indicated (see Annex 2)
2. Facilitation of the early detection of laboratory-acquired infections
3. Exclusion of highly susceptible individuals (e.g. pregnant women or immunocompromised individuals) from highly hazardous laboratory work
4. Provision of effective personal protective equipment and procedures.

Guidelines for the surveillance of laboratory workers handling microorganisms at Biosafety Level 1

Historical evidence indicates that the microorganisms handled at this level are unlikely to cause human disease or animal disease of veterinary importance. Ideally, however, all laboratory workers should undergo a pre-employment health check at which their medical history is recorded. Prompt reporting of illnesses or laboratory accidents is desirable and all staff members should be made aware of the importance of maintaining GMT.

Guidelines for the surveillance of laboratory workers handling microorganisms at Biosafety Level 2

1. A pre-employment or preplacement health check is necessary. The person's medical history should be recorded and a targeted occupational health assessment performed.
2. Records of illness and absence should be kept by the laboratory management.
3. Women of childbearing age should be made aware of the risk to an unborn child of occupational exposure to certain microorganisms, e.g. rubella virus. The precise steps taken to protect the fetus will vary, depending on the microorganisms to which the women may be exposed.

Training

Human error and poor technique can compromise the best of safeguards to protect the laboratory worker. Thus, a safety-conscious staff, well informed about the recognition and control of laboratory hazards, is key to the prevention of laboratory-

acquired infections, incidents and accidents. For this reason, continuous in-service training in safety measures is essential. An effective safety programme begins with the laboratory managers, who should ensure that safe laboratory practices and procedures are integrated into the basic training of employees. Training in safety measures should be an integral part of new employees' introduction to the laboratory. Employees should be introduced to the code of practice and to local guidelines, including the safety or operations manual. Measures to assure that employees have read and understood the guidelines, such as signature pages, should be adopted. Laboratory supervisors play the key role in training their immediate staff in good laboratory techniques. The biosafety officer can assist in training and with the development of training aids and documentation (see also Chapter 21).

Staff training should always include information on safe methods for highly hazardous procedures that are commonly encountered by all laboratory personnel and which involve:

1. Inhalation risks (i.e. aerosol production) when using loops, streaking agar plates, pipetting, making smears, opening cultures, taking blood/serum samples, centrifuging, etc.
2. Ingestion risks when handling specimens, smears and cultures
3. Risks of percutaneous exposures when using syringes and needles
4. Bites and scratches when handling animals
5. Handling of blood and other potentially hazardous pathological materials
6. Decontamination and disposal of infectious material.

Waste handling

Waste is anything that is to be discarded.

In laboratories, decontamination of wastes and their ultimate disposal are closely interrelated. In terms of daily use, few if any contaminated materials will require actual removal from the laboratory or destruction. Most glassware, instruments and laboratory clothing will be reused or recycled. The overriding principle is that all infectious materials should be decontaminated, autoclaved or incinerated within the laboratory.

The principal questions to be asked before discharge of any objects or materials from laboratories that deal with potentially infectious microorganisms or animal tissues are:

1. Have the objects or materials been effectively decontaminated or disinfected by an approved procedure?
2. If not, have they been packaged in an approved manner for immediate on-site incineration or transfer to another facility with incineration capacity?
3. Does the disposal of the decontaminated objects or materials involve any additional potential hazards, biological or otherwise, to those who carry out the immediate disposal procedures or who might come into contact with discarded items outside the facility?

Decontamination

Steam autoclaving is the preferred method for all decontamination processes. Materials for decontamination and disposal should be placed in containers, e.g. autoclavable plastic bags, that are colour-coded according to whether the contents are to be autoclaved and/or incinerated. Alternative methods may be envisaged only if they remove and/or kill microorganisms (for more details see Chapter 14).

Handling and disposal procedures for contaminated materials and wastes

An identification and separation system for infectious materials and their containers should be adopted. National and international regulations must be followed. Categories should include:

1. Non-contaminated (non-infectious) waste that can be reused or recycled or disposed of as general, “household” waste
2. Contaminated (infectious) “sharps” – hypodermic needles, scalpels, knives and broken glass; these should always be collected in puncture-proof containers fitted with covers and treated as infectious
3. Contaminated material for decontamination by autoclaving and thereafter washing and reuse or recycling
4. Contaminated material for autoclaving and disposal
5. Contaminated material for direct incineration.

Sharps

After use, hypodermic needles should not be recapped, clipped or removed from disposable syringes. The complete assembly should be placed in a sharps disposal container. Disposable syringes, used alone or with needles, should be placed in sharps disposal containers and incinerated, with prior autoclaving if required.

Sharps disposal containers must be puncture-proof/-resistant and must not be filled to capacity. When they are three-quarters full they should be placed in “infectious waste” containers and incinerated, with prior autoclaving if laboratory practice requires it. Sharps disposal containers must not be discarded in landfills.

Contaminated (potentially infectious) materials for autoclaving and reuse

No precleaning should be attempted of any contaminated (potentially infectious) materials to be autoclaved and reused. Any necessary cleaning or repair must be done only after autoclaving or disinfection.

Contaminated (potentially infectious) materials for disposal

Apart from sharps, which are dealt with above, all contaminated (potentially infectious) materials should be autoclaved in leakproof containers, e.g. autoclavable, colour-coded plastic bags, before disposal. After autoclaving, the material may be placed in transfer containers for transport to the incinerator. If possible, materials deriving from health-care activities should not be discarded in landfills even after decontamination. If an

incinerator is available on the laboratory site, autoclaving may be omitted: the contaminated waste should be placed in designated containers (e.g. colour-coded bags) and transported directly to the incinerator. Reusable transfer containers should be leakproof and have tight-fitting covers. They should be disinfected and cleaned before they are returned to the laboratory for further use.

Discard containers, pans or jars, preferably unbreakable (e.g. plastic), should be placed at every work station. When disinfectants are used, waste materials should remain in intimate contact with the disinfectant (i.e. not protected by air bubbles) for the appropriate time, according to the disinfectant used (see Chapter 14). The discard containers should be decontaminated and washed before reuse.

Incineration of contaminated waste must meet with the approval of the public health and air pollution authorities, as well as that of the laboratory biosafety officer (see section on Incineration in Chapter 14).

Chemical, fire, electrical, radiation and equipment safety

A breakdown in the containment of pathogenic organisms may be the indirect result of chemical, fire, electrical or radiation accidents. It is therefore essential to maintain high standards of safety in these fields in any microbiological laboratory. Statutory rules and regulations for each of these will normally be laid down by the competent national or local authority, whose assistance should be sought if necessary. Chemical, fire, electrical and radiation hazards are considered in greater detail in Part VI of this manual (Chapters 17 and 18).

Additional information regarding safety equipment is presented in Chapter 11.

4. The containment laboratory – Biosafety Level 3

The containment laboratory – Biosafety Level 3 is designed and provided for work with Risk Group 3 microorganisms and with large volumes or high concentrations of Risk Group 2 microorganisms that pose an increased risk of aerosol spread. Biosafety Level 3 containment requires the strengthening of the operational and safety programmes over and above those for basic laboratories – Biosafety Levels 1 and 2 (set out in Chapter 3).

The guidelines given in this chapter are presented in the form of additions to those for basic laboratories – Biosafety Levels 1 and 2, which must therefore be applied before those specific for the containment laboratory – Biosafety Level 3. The major additions and changes are in:

1. Code of practice
2. Laboratory design and facilities
3. Health and medical surveillance.

Laboratories in this category should be registered or listed with the national or other appropriate health authorities.

Code of practice

The code of practice for basic laboratories – Biosafety Levels 1 and 2 applies except where modified as follows.

1. The international biohazard warning symbol and sign (see Figure 1) displayed on laboratory access doors must identify the biosafety level and the name of the laboratory supervisor who controls access, and indicate any special conditions for entry into the area, e.g. immunization.
2. Laboratory protective clothing must be of the type with solid-front or wrap-around gowns, scrub suits, coveralls, head covering and, where appropriate, shoe covers or dedicated shoes. Front-buttoned standard laboratory coats are unsuitable, as are sleeves that do not fully cover the forearms. Laboratory protective clothing must not be worn outside the laboratory, and it must be decontaminated before it is laundered. The removal of street clothing and change into dedicated laboratory clothing may be warranted when working with certain agents (e.g. agricultural or zoonotic agents).

3. Open manipulations of all potentially infectious material must be conducted within a biological safety cabinet or other primary containment device (see also Chapter 10).
4. Respiratory protective equipment may be necessary for some laboratory procedures or working with animals infected with certain pathogens (see Chapter 11).

Laboratory design and facilities

The laboratory design and facilities for basic laboratories – Biosafety Levels 1 and 2 apply except where modified as follows:

1. The laboratory must be separated from the areas that are open to unrestricted traffic flow within the building. Additional separation may be achieved by placing the laboratory at the blind end of a corridor, or constructing a partition and door or access through an anteroom (e.g. a double-door entry or basic laboratory – Biosafety Level 2), describing a specific area designed to maintain the pressure differential between the laboratory and its adjacent space. The anteroom should have facilities for separating clean and dirty clothing and a shower may also be necessary.
2. Anteroom doors may be self-closing and interlocking so that only one door is open at a time. A break-through panel may be provided for emergency exit use.
3. Surfaces of walls, floors and ceilings should be water-resistant and easy to clean. Openings through these surfaces (e.g. for service pipes) should be sealed to facilitate decontamination of the room(s).
4. The laboratory room must be sealable for decontamination. Air-ducting systems must be constructed to permit gaseous decontamination.
5. Windows must be closed, sealed and break-resistant.
6. A hand-washing station with hands-free controls should be provided near each exit door.
7. There must be a controlled ventilation system that maintains a directional airflow into the laboratory room. A visual monitoring device with or without alarm(s) should be installed so that staff can at all times ensure that proper directional airflow into the laboratory room is maintained.
8. The building ventilation system must be so constructed that air from the containment laboratory – Biosafety Level 3 is not recirculated to other areas within the building. Air may be high-efficiency particulate air (HEPA) filtered, reconditioned and recirculated within that laboratory. When exhaust air from the laboratory (other than from biological safety cabinets) is discharged to the outside of the building, it must be dispersed away from occupied buildings and air intakes. Depending on the agents in use, this air may be discharged through HEPA filters. A heating, ventilation and air-conditioning (HVAC) control system may be installed to prevent sustained positive pressurization of the laboratory. Consideration should be given to the installation of audible or clearly visible alarms to notify personnel of HVAC system failure.

9. All HEPA filters must be installed in a manner that permits gaseous decontamination and testing.
10. Biological safety cabinets should be sited away from walking areas and out of cross-currents from doors and ventilation systems (see Chapter 10).
11. The exhaust air from Class I or Class II biological safety cabinets (see Chapter 10), which will have been passed through HEPA filters, must be discharged in such a way as to avoid interference with the air balance of the cabinet or the building exhaust system.
12. An autoclave for the decontamination of contaminated waste material should be available in the containment laboratory. If infectious waste has to be removed from the containment laboratory for decontamination and disposal, it must be transported in sealed, unbreakable and leakproof containers according to national or international regulations, as appropriate.
13. Backflow-precaution devices must be fitted to the water supply. Vacuum lines should be protected with liquid disinfectant traps and HEPA filters, or their equivalent. Alternative vacuum pumps should also be properly protected with traps and filters.
14. The containment laboratory – Biosafety Level 3 facility design and operational procedures should be documented.

An example of laboratory design for Biosafety Level 3 is shown in Figure 4.

Laboratory equipment

The principles for the selection of laboratory equipment, including biological safety cabinets (see Chapter 10) are the same as for the basic laboratory – Biosafety Level 2. However, at Biosafety Level 3, manipulation of all potentially infectious material must be conducted within a biological safety cabinet or other primary containment device. Consideration should be given to equipment such as centrifuges, which will need additional containment accessories, for example, safety buckets or containment rotors. Some centrifuges and other equipment, such as cell-sorting instruments for use with infected cells, may need additional local exhaust ventilation with HEPA filtration for efficient containment.

Health and medical surveillance

The objectives of health and medical surveillance programmes for basic laboratories – Biosafety Levels 1 and 2 also apply to containment laboratories – Biosafety Level 3, except where modified as follows:

1. Medical examination of all laboratory personnel who work in containment laboratories – Biosafety Level 3 is mandatory. This should include recording of a detailed medical history and an occupationally-targeted physical examination.
2. After a satisfactory clinical assessment, the examinee may be provided with a medical contact card (e.g. as shown in Figure 5) stating that he or she is employed in a facility with a containment laboratory – Biosafety Level 3. This card should include

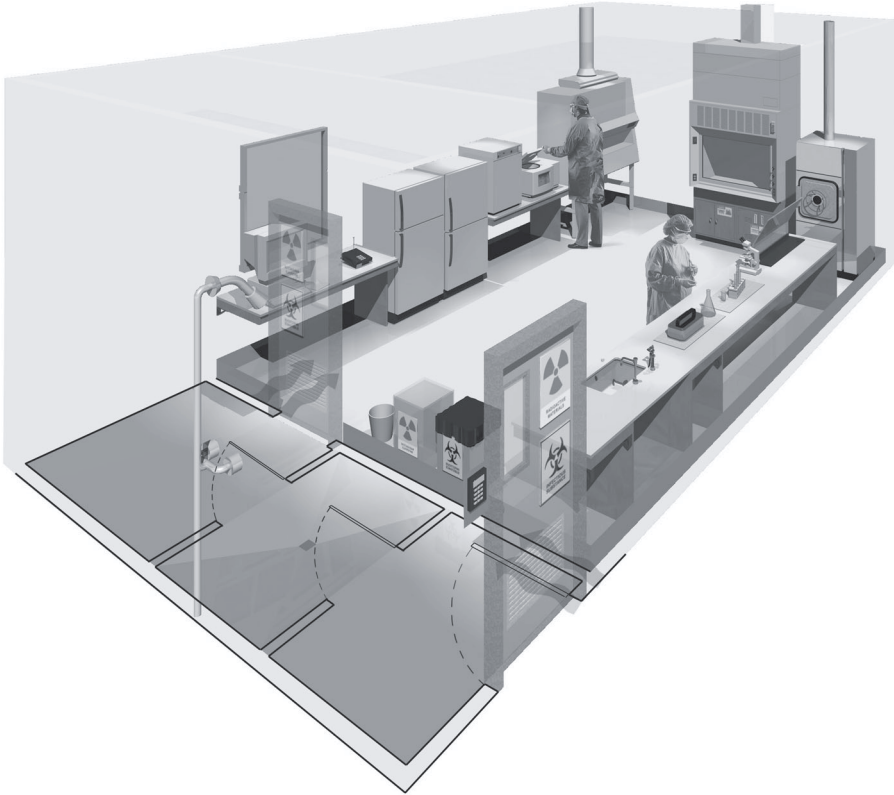


Figure 4. A typical Biosafety Level 3 laboratory

(graphics kindly provided by CUH2A, Princeton, NJ, USA). The laboratory is separated from general traffic flow and accessed through an anteroom (double door entry or basic laboratory – Biosafety Level 2) or an airlock. An autoclave is available within the facility for decontamination of wastes prior to disposal. A sink with hands-free operation is available. Inward directional airflow is established and all work with infectious materials is conducted within a biological safety cabinet.

a picture of the card holder, be wallet-sized, and always be carried by the holder. The name(s) of the contact persons to be entered will need to be agreed locally but might include the laboratory director, medical adviser and/or biosafety officer.

A. Front of card

ILLNESS SURVEILLANCE NOTICE	
Name _____ _____	<i>Card holder's picture</i>
TO THE EMPLOYEE Keep this card in your possession. In case of unexplained febrile illness, present the card to your physician and notify one of the following in the order listed.	
Dr _____	Tel (Work): _____
	Tel (Home): _____
Dr _____	Tel (Work): _____
	Tel (Home): _____

B. Back of card

TO THE PHYSICIAN The holder of this card works in an area at _____ in which pathogenic viruses, rickettsia, bacteria, protozoa or helminths are present. In the event of an unexplained febrile illness, please call the employer for information on agents to which this employee may have been exposed.
Name of laboratory: _____
Address: _____ _____ _____ _____
Tel: _____

Figure 5. Suggested format for medical contact card

5. The maximum containment laboratory – Biosafety Level 4

The maximum containment laboratory – Biosafety Level 4 is designed for work with Risk Group 4 microorganisms. Before such a laboratory is constructed and put into operation, intensive consultations should be held with institutions that have had experience of operating a similar facility. Operational maximum containment laboratories – Biosafety Level 4 should be under the control of national or other appropriate health authorities. The following information is intended only as introductory material. Entities working to pursue development of a Biosafety Level 4 laboratory should contact the WHO Biosafety programme for additional information.¹

Code of practice

The code of practice for Biosafety Level 3 applies except where modified as follows:

1. The two-person rule should apply, whereby no individual ever works alone. This is particularly important if working in a Biosafety Level 4 suit facility.
2. A complete change of clothing and shoes is required prior to entering and upon exiting the laboratory.
3. Personnel must be trained in emergency extraction procedures in the event of personnel injury or illness.
4. A method of communication for routine and emergency contacts must be established between personnel working within the maximum containment laboratory – Biosafety Level 4 and support personnel outside the laboratory.

Laboratory design and facilities

The features of a containment laboratory – Biosafety Level 3 also apply to a maximum containment laboratory – Biosafety Level 4 with the addition of the following.

1. **Primary containment.** An efficient primary containment system must be in place, consisting of one or a combination of the following.
 - *Class III cabinet laboratory.* Passage through a minimum of two doors prior to entering the rooms containing the Class III biological safety cabinet(s) (cabinet room) is required. In this laboratory configuration the Class III biological safety

¹ Biosafety programme, Department of Communicable Disease Surveillance and Response, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (<http://www.who.int/csr/>).

cabinet provides the primary containment. A personnel shower with inner and outer changing rooms is necessary. Supplies and materials that are not brought into the cabinet room through the changing area are introduced through a double-door autoclave or fumigation chamber. Once the outer door is securely closed, staff inside the laboratory can open the inner door to retrieve the materials. The doors of the autoclave or fumigation chamber are interlocked in such a way that the outer door cannot open unless the autoclave has been operated through a sterilization cycle or the fumigation chamber has been decontaminated (see Chapter 10).

- *Suit laboratory.* A protective suit laboratory with self-contained breathing apparatus differs significantly in design and facility requirements from a Biosafety Level 4 laboratory with Class III biological safety cabinets. The rooms in the protective suit laboratory are arranged so as to direct personnel through the changing and decontamination areas prior to entering areas where infectious materials are manipulated. A suit decontamination shower must be provided and used by personnel leaving the containment laboratory area. A separate personnel shower with inner and outer changing rooms is also provided. Personnel who enter the suit area are required to don a one-piece, positively pressurized, HEPA-filtered, supplied-air suit. Air to the suit must be provided by a system that has a 100% redundant capability with an independent source of air, for use in the event of an emergency. Entry into the suit laboratory is through an airlock fitted with airtight doors. An appropriate warning system for personnel working in the suit laboratory must be provided for use in the event of mechanical system or air failure (see Chapter 10).
2. **Controlled access.** The maximum containment laboratory – Biosafety Level 4 must be located in a separate building or in a clearly delineated zone within a secure building. Entry and exit of personnel and supplies must be through an airlock or pass-through system. On entering, personnel must put on a complete change of clothing; before leaving, they should shower before putting on their street clothing.
 3. **Controlled air system.** Negative pressure must be maintained in the facility. Both supply and exhaust air must be HEPA-filtered. There are significant differences in the ventilating systems of the Class III cabinet laboratory and suit laboratory:
 - *Class III cabinet laboratory.* The supply air to the Class III biological safety cabinet(s) may be drawn from within the room through a HEPA filter mounted on the cabinet or supplied directly through the supply air system. Exhaust air from the Class III biological safety cabinet must pass through two HEPA filters prior to release outdoors. The cabinet must be operated at negative pressure to the surrounding laboratory at all times. A dedicated non-recirculating ventilating system for the cabinet laboratory is required.
 - *Suit laboratory.* Dedicated room air supply and exhaust systems are required. The supply and exhaust components of the ventilating system are balanced to provide directional airflow within the suit area from the area of least hazard to

the area(s) of greatest potential hazard. Redundant exhaust fans are required to ensure that the facility remains under negative pressure at all times. The differential pressures within the suit laboratory and between the suit laboratory and adjacent areas must be monitored. Airflow in the supply and exhaust components of the ventilating system must be monitored, and an appropriate system of controls must be used to prevent pressurization of the suit laboratory. HEPA-filtered supply air must be provided to the suit area, decontamination shower and decontamination airlocks or chambers. Exhaust air from the suit laboratory must be passed through a series of two HEPA filters prior to release outdoors. Alternatively, after double HEPA filtration, exhaust air may be recirculated, but only within the suit laboratory. Under no circumstances shall the exhaust air from the Biosafety Level 4 suit laboratory be recirculated to other areas. Extreme caution must be exercised if recirculation of air within the suit laboratory is elected. Consideration must be given to the types of research conducted, equipment, chemicals and other materials used in the suit laboratory, as well as animal species that may be involved in the research.

All HEPA filters need to be tested and certified annually. The HEPA filter housings are designed to allow for in situ decontamination of the filter prior to removal. Alternatively, the filter can be removed in a sealed, gas-tight primary container for subsequent decontamination and/or destruction by incineration.

4. **Decontamination of effluents.** All effluents from the suit area, decontamination chamber, decontamination shower, or Class III biological safety cabinet must be decontaminated before final discharge. Heat treatment is the preferred method. Effluents may also require correction to a neutral pH prior to discharge. Water from the personnel shower and toilet may be discharged directly to the sanitary sewer without treatment.
5. **Sterilization of waste and materials.** A double-door, pass-through autoclave must be available in the laboratory area. Other methods of decontamination must be available for equipment and items that cannot withstand steam sterilization.
6. **Airlock entry ports** for specimens, materials and animals must be provided.
7. **Emergency power** and dedicated power supply line(s) must be provided.
8. **Containment drain(s)** must be installed.

Because of the great complexity of the engineering, design and construction of Biosafety Level 4 facilities, in either cabinet or suit configuration, schematic representations of such facilities have not been included.

Because of the great complexity of the work in the Biosafety Level 4 laboratory, a separate detailed work manual should be developed and tested in training exercises. In addition, an emergency programme must be devised (see Chapter 13). In the preparation of this programme, active cooperation with national and local health authorities should be established. Other emergency services, e.g. fire, police and designated receiving hospitals, should also be involved.

6. Laboratory animal facilities

Those who use animals for experimental and diagnostic purposes have a moral obligation to take every care to avoid causing them unnecessary pain or suffering. The animals must be provided with comfortable, hygienic housing and adequate wholesome food and water. At the end of the experiment they must be dealt with in a humane manner.

For security reasons, the animal house should be an independent, detached unit. If it adjoins a laboratory, the design should provide for its isolation from the public parts of the laboratory should such need arise, and for its decontamination and disinfection.

Table 4. Animal facility containment levels: summary of practices and safety equipment

RISK GROUP	CONTAINMENT LEVEL	LABORATORY PRACTICES AND SAFETY EQUIPMENT
1	ABSL-1	Limited access, protective clothing and gloves.
2	ABSL-2	ABSL-1 practices plus: hazard warning signs. Class I or II BSCs for activities that produce aerosols. Decontamination of waste and cages before washing.
3	ABSL-3	ABSL-2 practices plus: controlled access. BSCs and special protective clothing for all activities.
4	ABSL-4	ABSL-3 plus: strictly limited access. Clothing change before entering. Class III BSCs or positive pressure suits. Shower on exit. Decontamination of all wastes before removal from facility.

ABSL, animal facility Biosafety Level; BSCs, biological safety cabinets

Animal facilities, like laboratories, may be designated according to a risk assessment and the risk group of the microorganisms under investigation, as Animal facility Biosafety Level 1, 2, 3 or 4.

With respect to agents to be used in the animal laboratory, factors for consideration include:

1. The normal route of transmission

2. The volumes and concentrations to be used
3. The route of inoculation
4. Whether and by what route these agents may be excreted.

With respect to animals to be used in the animal laboratory, factors for consideration include:

1. The nature of the animals, i.e. their aggressiveness and tendency to bite and scratch
2. Their natural ecto- and endoparasites
3. The zoonotic diseases to which they are susceptible
4. The possible dissemination of allergens.

As with laboratories, the requirements for design features, equipment and precautions increase in stringency according to the animal biosafety level. These are described below and summarized in Table 4. These guidelines are additive, so that each higher level incorporates the standards of the lower levels.

Animal facility – Biosafety Level 1

This is suitable for the maintenance of most stock animals after quarantine (except nonhuman primates, regarding which national authorities should be consulted), and for animals that are deliberately inoculated with agents in Risk Group 1. GMT are required. The animal facility director must establish policies, procedures and protocols for all operations, and for access to the vivarium. An appropriate medical surveillance programme for the staff must be instituted. A safety or operations manual must be prepared and adopted.

Animal facility – Biosafety Level 2

This is suitable for work with animals that are deliberately inoculated with micro-organisms in Risk Group 2. The following safety precautions apply:

1. All the requirements for animal facilities – Biosafety Level 1 must be met.
2. Biohazard warning signs (see Figure 1) should be posted on doors and other appropriate places.
3. The facility must be designed for easy cleaning and housekeeping.
4. Doors must open inwards and be self-closing.
5. Heating, ventilation and lighting must be adequate.
6. If mechanical ventilation is provided, the airflow must be inwards. Exhaust air is discharged to the outside and should not be recirculated to any part of the building.
7. Access must be restricted to authorized persons.
8. No animals should be admitted other than those for experimental use.
9. There should be an arthropod and rodent control programme.
10. Windows, if present, must be secure, resistant to breakage and, if able to be opened, must be fitted with arthropod-proof screens.
11. After use, work surfaces must be decontaminated with effective disinfectants (see Chapter 14).

12. Biological safety cabinets (Classes I or II) or isolator cages with dedicated air supplies and HEPA-filtered exhaust air must be provided for work that may involve the generation of aerosols.
13. An autoclave must be available on site or in appropriate proximity to the animal facility.
14. Animal bedding materials must be removed in a manner that minimizes the generation of aerosols and dust.
15. All waste materials and bedding must be decontaminated before disposal.
16. Use of sharp instruments should be restricted whenever possible. Sharps should always be collected in puncture-proof/-resistant containers fitted with covers and treated as infectious.
17. Material for autoclaving or incineration must be transported safely, in closed containers.
18. Animal cages must be decontaminated after use.
19. Animal carcasses should be incinerated.
20. Protective clothing and equipment must be worn in the facility, and removed on leaving.
21. Hand-washing facilities must be provided. Staff must wash their hands before leaving the animal facility.
22. All injuries, however minor, must be treated appropriately, reported and recorded.
23. Eating, drinking, smoking and application of cosmetics must be forbidden in the facility.
24. All personnel must receive appropriate training.

Animal facility – Biosafety Level 3

This is suitable for work with animals that are deliberately inoculated with agents in Risk Group 3, or when otherwise indicated by a risk assessment. All systems, practices and procedures need to be reviewed and recertified annually. The following safety precautions apply:

1. All the requirements for animal facilities – Biosafety Levels 1 and 2 must be met.
2. Access must be strictly controlled.
3. The facility must be separated from other laboratory and animal house areas by a room with a double-door entrance forming an anteroom.
4. Hand-washing facilities must be provided in the anteroom.
5. Showers should be provided in the anteroom.
6. There must be mechanical ventilation to ensure a continuous airflow through all the rooms. Exhaust air must pass through HEPA filters before being discharged to the atmosphere without recirculation. The system must be designed to prevent accidental reverse flow and positive pressurization in any part of the animal house.
7. An autoclave must be available at a location convenient for the animal house where the biohazard is contained. Infectious waste should be autoclaved before it is moved to other areas of the facility.

8. An incinerator should be readily available on site or alternative arrangements should be made with the authorities concerned.
9. Animals infected with Risk Group 3 microorganisms must be housed in cages in isolators or rooms with ventilation exhausts placed behind the cages.
10. Bedding should be as dust-free as possible.
11. All protective clothing must be decontaminated before it is laundered.
12. Windows must be closed and sealed, and resistant to breakage.
13. Immunization of staff, as appropriate, should be offered.

Animal facility – Biosafety Level 4

Work in this facility will normally be linked with that in the maximum containment laboratory – Biosafety Level 4, and national and local rules and regulations must be harmonized to apply to both. If work is to be done in a suit laboratory, additional practices and procedures must be used over and above those described here (see Chapter 5).

1. All the requirements for animal facilities – Biosafety Levels 1, 2 and 3 must be met.
2. Access must be strictly controlled; only staff designated by the director of the establishment should have authority to enter.
3. Individuals must not work alone: the two-person rule must apply.
4. Personnel must have received the highest possible level of training as microbiologists and be familiar with the hazards involved in their work and with the necessary precautions.
5. Housing areas for animals infected with Risk Group 4 agents must maintain the criteria for containment described and applied for maximum containment laboratories – Biosafety Level 4.
6. The facility must be entered by an airlock anteroom, the clean side of which must be separated from the restricted side by changing and showering facilities.
7. Staff must remove street clothing when entering and put on special, protective clothing. After work they must remove the protective clothing for autoclaving, and shower before leaving.
8. The facility must be ventilated by a HEPA-filtered exhaust system designed to ensure a negative pressure (inward directional airflow).
9. The ventilation system must be designed to prevent reverse flow and positive-pressurization.
10. A double-ended autoclave with the clean end in a room outside the containment rooms must be provided for exchange of materials.
11. A pass-through airlock with the clean end in a room outside the containment rooms must be provided for exchange of non-autoclavable materials.
12. All manipulations with animals infected with Risk Group 4 agents must take place under maximum containment – Biosafety Level 4 conditions.
13. All animals must be housed in isolators.
14. All animal bedding and waste must be autoclaved before removal from the facility.

15. There must be medical supervision of staff.

Invertebrates

As with vertebrates, the animal facility biosafety level will be determined by the risk groups of the agents under investigation or when otherwise indicated by a risk assessment. The following additional precautions are necessary with certain arthropods, particularly with flying insects:

1. Separate rooms should be provided for infected and noninfected invertebrates.
2. The rooms should be capable of being sealed for fumigation.
3. Insecticide sprays should be readily available.
4. “Chilling” facilities should be provided to reduce, where necessary, the activity of invertebrates.
5. Access should be through an anteroom containing insect traps and with arthropod-proof screens on the doors.
6. All exhaust ventilation ducts and openable windows should be fitted with arthropod-proof screens.
7. Waste traps on sinks and sluices should not be allowed to dry out.
8. All waste should be decontaminated by autoclaving, as some invertebrates are not killed by all disinfectants.
9. A check should be kept on the numbers of larval and adult forms of flying, crawling and jumping arthropods.
10. Containers for ticks and mites should stand in trays of oil.
11. Infected or potentially infected flying insects must be contained in double-netted cages.
12. Infected or potentially infected arthropods must be handled in biological safety cabinets or isolators.
13. Infected or potentially infected arthropods may be manipulated on cooling trays.

For further information see references (3–6).

7. Guidelines for laboratory/facility commissioning

Laboratory/facility commissioning may be defined as the systematic review and documentation process signifying that specified laboratory structural components, systems and/or system components have been installed, inspected, functionally tested and verified to meet national or international standards, as appropriate. The respective building system's design criteria and design function establish these requirements. In other words, laboratories designated as Biosafety Levels 1–4 will have different and increasingly complex commissioning requirements. Geographical and climatic conditions, such as geological fault lines or extreme heat, cold or humidity may also affect the laboratory design and therefore the commissioning requirements. Upon the completion of the commissioning process, the pertinent structural components and support systems will have been subjected to the various operating conditions and failure modes that can be reasonably expected, and will have been approved.

The commissioning process and acceptance criteria should be established early, preferably during the programming phase of the construction or renovation project. By acknowledging the commissioning process early in the project, architects, engineers, safety and health personnel and ultimately the laboratory occupants understand the performance requirements of the specific laboratory and set uniform expectations for laboratory and/or facility performance. The commissioning process provides the institution and the surrounding community with a greater degree of confidence that the structural, electrical, mechanical and plumbing systems, containment and decontamination systems, and security and alarm systems will operate as designed, to assure containment of any potentially dangerous microorganisms being worked with in a particular laboratory or animal facility.

Commissioning activities generally begin during the programming phase of the project and proceed through the construction and subsequent warranty period for the laboratory/facility. Warranty periods should generally extend for one year following occupancy. It is recommended that a commissioning agent is retained who is independent of the architectural, engineering and construction firms involved in the design and construction. The commissioning agent serves as an advocate for the institution constructing or renovating the laboratory and should be considered as a member of the design team; involvement of the agent in the early programming phase of the project is essential. In some cases, the institution may act as its own commissioning agent. In the case of more complex laboratory facilities (Biosafety

Levels 3 or 4), the institution may wish to retain an outside commissioning agent who has demonstrated experience and success in the commissioning of complex biosafety laboratory and animal facilities. When an independent commissioning agent is used, the institution should still be a member of the commissioning team. It is recommended that, in addition to the commissioning agent, the institution's Safety Officer, Project Officer, Programme Manager and a representative of the Operations and Maintenance staff are also part of the team.

The following is a list of laboratory systems and components that may be included in a commissioning plan for functional testing, depending on the containment level of the facility being renovated or constructed. The list is not exhaustive. Obviously, the actual commissioning plan will reflect the complexity of the laboratory being planned.

1. Building automation systems including links to remote monitoring and control sites
2. Electronic surveillance and detection systems
3. Electronic security locks and proximity device readers
4. Heating, ventilation (supply and exhaust) and air-conditioning (HVAC) systems
5. High-efficiency particulate air (HEPA) filtration systems
6. HEPA decontamination systems
7. HVAC and exhaust air system controls and control interlocks
8. Airtight isolation dampers
9. Laboratory refrigeration systems
10. Boilers and steam systems
11. Fire detection, suppression and alarm systems
12. Domestic water backflow prevention devices
13. Processed water systems (i.e. reverse osmosis, distilled water)
14. Liquid effluent treatment and neutralization systems
15. Plumbing drain primer systems
16. Chemical decontaminant systems
17. Medical laboratory gas systems
18. Breathing air systems
19. Service and instrument air systems
20. Cascading pressure differential verification of laboratories and support areas
21. Local area network (LAN) and computer data systems
22. Normal power systems
23. Emergency power systems
24. Uninterruptible power systems
25. Emergency lighting systems
26. Lighting fixture penetration seals
27. Electrical and mechanical penetration seals
28. Telephone systems

29. Airlock door control interlocks
30. Airtight door seals
31. Window and vision-panel penetration seals
32. Barrier pass-through penetration
33. Structural integrity verification: concrete floors, walls and ceilings
34. Barrier coating verification: floors, walls and ceilings
35. Biosafety Level 4 containment envelope pressurization and isolation functions
36. Biological safety cabinets
37. Autoclaves
38. Liquid nitrogen system and alarms
39. Water detection systems (e.g. in case of flooding inside containment zone)
40. Decontamination shower and chemical additive systems
41. Cage-wash and neutralization systems
42. Waste management.

8. Guidelines for laboratory/facility certification

Laboratories are complex and dynamic environments. Today's biomedical research and clinical laboratories must be able to adapt quickly to continuously increasing public health needs and pressures. An example of this is the need for laboratories to adjust priorities to meet the challenges of emerging or re-emerging infectious diseases. In order to assure that adaptation and maintenance are undertaken promptly and in an appropriate and safe manner, all biological research and clinical laboratories should be regularly certified. Laboratory certification helps to ensure that:

1. Proper engineering controls are being used and are functioning adequately as designed
2. Appropriate site and protocol specific administrative controls are in place
3. Personal protective equipment is appropriate for the tasks being performed
4. Decontamination of waste and materials has been adequately considered and proper waste management procedures are in place
5. Proper procedures for general laboratory safety, including physical, electrical and chemical safety are in place.

Laboratory certification differs from laboratory commissioning activities (Chapter 7) in several important ways. Laboratory certification is the systematic examination of all safety features and processes within the laboratory (engineering controls, personal protective equipment and administrative controls). Biosafety practices and procedures are also examined. Laboratory certification is an on-going quality and safety assurance activity that should take place on a regular basis.

Adequately trained safety and health or biosafety professionals may conduct laboratory certification activities. Institutions may employ personnel having the appropriate skill-set required for conducting audits, surveys or inspections (these terms are used interchangeably) associated with the certification process. However, institutions may consider engaging or be required to engage a third party to provide these services.

Biomedical research and clinical laboratory facilities may develop audit, survey or inspection tools to help ensure consistency in the certification process. These tools should be flexible enough to allow for the physical and procedural differences between laboratories necessitated by the type of work being conducted, while at the same time providing a consistent approach within the institution. Care must be taken to ensure

that these tools are used only by appropriately trained personnel, and that they are not used as a substitute for a sound professional biosafety assessment. Examples of such tools are provided in Tables 5–7.

Findings of the audit, survey or inspection should be discussed with laboratory personnel and management. Within the laboratory, an individual should be identified and made responsible for ensuring that corrective actions are taken for all deficiencies identified during the audit process. Certification of the laboratory should not be completed, and the laboratory should not be declared functional, until deficiencies have been adequately addressed.

The complexity of Biosafety Level 4 laboratory operations goes beyond the scope of this manual. For details and further information, please contact the WHO Biosafety programme¹ (see also Annex 3).

¹ WHO Biosafety programme, Department of Communicable Disease Surveillance and Response, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (<http://www.who.int/csr/>).

Table 5. Basic Laboratory – Biosafety Level 1: laboratory safety survey

Location Date
 Person in charge of laboratory

CHECKED ITEM (ENTER DATE OF CHECK)	YES	NO	N/A	COMMENTS
Laboratory				Biosafety Level: <i>Attach the appropriate Biosafety Level Survey Form</i>
Proper signage: ultraviolet light, laser, radioactive material, etc.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Appropriate biosafety guidelines available and followed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Laboratory equipment properly labelled (biohazardous, radioactive, toxic, etc.) ..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Laboratory design				
Designed for easy cleaning	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Room ultraviolet lights on interlock switch	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
All shelves secured	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Bench-tops waterproof and resistant to acids, alkali, organic solvents and heat .	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Adequate illumination provided	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Adequate storage space available and appropriately used	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Gas cylinders				
All cylinders secured	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Caps on reserve cylinders	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Asphyxiating and hazardous gases only in ventilated rooms	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Excess or empty cylinders present	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Chemicals				
Flammables stored in flammable storage cabinet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Peroxide formers double-dated (received and opened)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Chemicals properly segregated	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Hazardous chemicals stored above eye level	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Chemicals stored on the floor	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Chemical containers left open	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
All solutions properly labelled	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Mercury thermometers in use	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Refrigerators/freezers/cold rooms				
Food for human consumption present	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Flammables in explosion-proof/-safe units	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Labelled externally if containing carcinogens, radioactivity and/or biohazards	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Cold-room has emergency release	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

8. GUIDELINES FOR LABORATORY/FACILITY CERTIFICATION

CHECKED ITEM (ENTER DATE OF CHECK)	YES	NO	N/A	COMMENTS
Electrical equipment				
Extension cords present	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Outlets earthed/grounded and with proper polarity	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Connections by sinks, under showers, etc.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Equipment with frayed or damaged wiring	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Overloaded outlets or electrical strips	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Power strips mounted off the floor	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Proper fuses in conduits	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Electrical outlets near water sources meet local codes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Earths/grounds present on electrical cords	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Portable space heaters	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Personal protective equipment				
Eyewash available in laboratory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Safety shower available	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Personal protective equipment available (gloves, gowns, goggles, etc.)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Occupants properly attired	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Laboratory coats, gowns, smocks, gloves and other personal protective clothing not worn outside the laboratory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Personal protective equipment available for cryogenic storage	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Waste management				
Evidence of improper waste disposal	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Wastes segregated in proper containers	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Chemical waste containers tagged, labelled, dated and kept closed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Chemical waste containers appropriately handled and stored	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Sharps containers used and disposed of properly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
No trash on floor	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Waste disposal procedures posted in laboratory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Occupational health and safety programmes available				
Hazard communication	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Respiratory protection	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Hearing conservation	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Formaldehyde monitoring	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Ethylene oxide monitoring	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Anaesthetic gas monitoring	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

CHECKED ITEM (ENTER DATE OF CHECK)	YES	NO	N/A	COMMENTS
General engineering controls				
Laboratory airflow is negative to general occupancy, corridor and office areas	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Cup sinks or drains acting as vents	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Sink available for hand-washing	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Exposed machine parts (pulleys, gears)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Vacuum line has filters and traps on laboratory benches	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Backflow hazards to water supply	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Distilled water systems in good condition ..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Active and effective arthropod and rodent control programme	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
General practices and procedures				
Food for human consumption stored outside the laboratory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Microwave oven(s) clearly labelled "No Food Preparation, Laboratory Use Only"	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Eating, drinking, smoking and/or applying of cosmetics occurring in the laboratory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Pressurized glass containers taped or shielded (i.e. vacuum traps)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Mouth pipetting prohibited	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Mechanical pipetting devices available and used	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Protective laboratory clothing stored separately from street clothing	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
General laboratory housekeeping				
Glass containers stored on the floor	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Trip hazards evident	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Clean absorbent pads on work surfaces	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Broken glassware handled by mechanical means (brush and dustpan, tongs, etc.)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Fire protection				
Sprinkler heads free and unobstructed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Open penetrations in walls, ceiling, floor, etc.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Wiring or tubing through door openings	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Minimum passage width of 1 m in laboratory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Storage observed on ductwork or light fixtures	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Excess combustibles stored in laboratory ..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Heated constant temperature baths				
Equipped with low water level and overheat shutoff	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Constructed of noncombustible materials ..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

Safety surveyor's signature: Date survey completed:

Table 6. **Basic laboratory – Biosafety Level 2: laboratory safety survey.**

This form is used in conjunction with the Biosafety Level 1 laboratory safety survey form

Location Date

Person in charge of laboratory

CHECKED ITEM (ENTER DATE OF CHECK)	YES	NO	N/A	COMMENTS
Biological safety cabinet (BSC)				Date:
Certification within last year	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Location:
BSC surface wiped down with appropriate disinfectant at beginning and end of each procedure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Brand:
Front grill and exhaust filter unobstructed ..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Type:
Open flames used inside cabinet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Serial no.:
Vacuum lines have in-line filters and disinfectant traps in use	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
BSC compromised by room air or location	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
BSC used when there is potential for creating aerosols	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Laboratory				
Access limited and restricted to authorized personnel	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Entry limited to personnel advised of all potential hazards	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Biohazard sign posted on laboratory door as appropriate	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
• Information on sign accurate and current	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
• Sign legible and not defaced	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
All doors closed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Decontamination				
Decontaminant specific to the organism(s) in use	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
All spills and accidents involving infectious materials reported to the laboratory supervisor	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Appropriate decontaminant used during spill clean-ups	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Work surfaces decontaminated before and after each procedure, daily and after spills	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Handling of contaminated waste				
Infectious waste containers properly used	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Containers not overfilled	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Containers properly labelled and closed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Culture stocks and other regulated waste properly decontaminated before disposal	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

CHECKED ITEM (ENTER DATE OF CHECK)	YES	NO	N/A	COMMENTS
Materials decontaminated outside the laboratory transported in closed, durable, leakproof containers according to local rules and regulations	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Mixed waste biologically decontaminated prior to disposal as chemical or radiological waste	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Personal protection				
Laboratory personnel reminded of appropriate immunizations/tests for agents handled	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Appropriate medical services contacted for medical evaluations, surveillance and treatment of occupational exposures	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Gloves worn when handling infectious material or contaminated equipment	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Face protection provided when working outside the BSC with infectious material	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Hands washed after removing gloves, after working with infectious agents, before leaving the laboratory	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Antimicrobial agent available for immediate first aid	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Practices				
BSC used when potential for creating infectious aerosols/splashes exists	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Biosafety manual prepared and adopted	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Personnel read, review and follow the instructions on practices and procedures, including safety or operations manual (required for all personnel annually)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Procedures performed so as to minimize aerosols/splashes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Needle-locking syringes/single-use needle-syringe units used with infectious agents	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Centrifuge cups and rotors opened only in a BSC	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Infectious specimens transported outside a BSC in approved containers following approved transport regulations	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
Facility				
Hand-washing sink available near laboratory exit	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	

Safety surveyor's signature: Date survey completed:

Table 7. Containment laboratory – Biosafety Level 3: laboratory safety survey.
This form is used in conjunction with the Bioafety Level 1 and Biosafety Level 2 laboratory safety survey forms

Location Date

Person in charge of laboratory

CHECKED ITEM (ENTER DATE OF CHECK)	YES	NO	N/A	COMMENTS
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Facility

- Laboratory separated from unrestricted traffic flow in building YES NO N/A
- Access to laboratory through an anteroom with self-closing doors YES NO N/A
- All penetrations in laboratory sealed or sealable for decontamination YES NO N/A
- Room exhaust air single-pass and exhausted away from occupied areas YES NO N/A
- Controlled ventilation system to monitor directional airflow available YES NO N/A

Personal protection

- Closed-front gowns worn in laboratory YES NO N/A
- Protective laboratory clothing worn only in laboratory areas YES NO N/A
- Hand-washing sink foot, elbow or automatically controlled YES NO N/A

Hand protection

- Double gloves worn when handling infectious material, potentially contaminated equipment and work surfaces YES NO N/A

Respiratory protection

- Respiratory protection worn by all personnel in the laboratory when aerosols are not safely contained in a BSC YES NO N/A

Practices

- Mucous membrane protection provided when working with infectious material outside a BSC YES NO N/A
- Personnel advised of special hazards associated with the agent(s) YES NO N/A
- Personnel required to read and follow all instructions on practices and procedures, including safety or operations manual ... YES NO N/A
- Personnel receive annual updates/additional training for procedural changes YES NO N/A
- All contaminated waste autoclaved prior to disposal YES NO N/A

Safety surveyor's signature: Date survey completed: