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REQUIREMENTS AND PROCEDURES IN MICROBIOLOGICAL LABORATORIES FOR WORK WITH CONTAGIOUS INFECTIOUS AGENTS

Tamaš Petrović*[1], Sava Lazić[1], Diana Lupulović[1], Gospava Lazić[1], Sara Savić[1], Živoslav Grgić[1], Dubravka Milanov[1], Vladimir Polaček[1]

[1] Scientific Veterinary Institute „Novi Sad“, Rumenački put 20, 21000 Novi Sad
e-mail: tomy@niv.ns.ac.rs

Abstract: Requirements, necessary technical and technological conditions and procedures that should be established in diagnostic and research laboratories highly depend on the infectious agents that are, or plan to be detected or tested in them. Laboratories are as well as pathogens divided into four levels of biosafety: Biosafety Level 1 (BSL1) or the basic level of the safety, Biosafety Level 2 (BSL2) or the second basic level of biosafety, Biosafety Level 3 (BSL3) or „containment“ and the Biosafety Level 4 (BSL4) or maximal „containment“. Most frequently the laboratory level of biosafety corresponds to the level of the risk group of the pathogens that are to be work with in the laboratory. However, this is not always the rule. Frequently, and from the safety reasons, the work with the pathogen from the lower risk group is done in the higher biosafety level laboratory. In this paper, propositions related to biosafety levels in the microbiological laboratories, prescribed by the World Health Organization (WHO), are presented. A detailed explanation of the demands and procedures are given, as well as the description of necessary technical and technological conditions for different biosafety levels in laboratories, starting with Biosafety Level 1 up to Biosafety Level 4. A special attention is given to the demands regarding access to the laboratory, the protection of personnel, rule book on behaviour, working procedures, characteristics of laboratory equipment, laboratory area and its design, and handling with laboratory waste.

Keywords: *biosafety levels, requirements, procedures, design.*

INTRODUCTION

Published reports of laboratory associated infections (LAIs) first appeared around the start of thetwentieth century. According to the literature data 4079 LAIs resulting in 168 deaths occurring between 1930 and 1978 have been described (Sulkin and Pike, 1951; Pike and Sulkin,

1965; Pike, 1978, 1979). The ten most common causative agents of overt infections among laboratory workers found in these studies were Brucella spp., *Coxiella burnetii*, hepatitis B virus (HBV), *Salmonella typhi*, *Francisella tularensis*, *Mycobacterium tuberculosis*, *Blastomyces dermatitidis*, Venezuelan equine encephalitis virus, *Chlamydia psittaci* and *Coccidioides immitis*. During the following 20 years, 1267 overt infections with 22 deaths were described. The diagnostic and research laboratories accounted for respectively 45% and 51% of the total LAIsreported (Harding and Byers, 2000). *Mycobacterium tuberculosis*, *Coxiella burnetii*, hantavirus, arboviruses, HBV, *Brucella* spp., *Salmonella* spp., *Shigella* spp., hepatitis C virus, and *Cryptosporidium* spp. accounted for 1074 out of the total of 1267 infections. The authors also identified an additional 663 cases that presented as sub-clinical infections.

There are many published classifications, requirements and instructions from number of international organizations (WHO, OIE, FAO etc.) and national organizations and legislative bodies as well as organizations from experts in the field of biosafety (International Veterinary Biosafety Group etc.) that are responsible for the field of biosafety.

To understand this technique, it is necessary to explain the basic terminology. The term biosafety levels applies to the level of biosafety of the work process in diagnostic and research laboratories, and means safety of laboratory staff during work and safety of the environment i.e. entire surroundings from accidents that might occur in the laboratory. Biosecurity is considered as the safety of laboratory staff, especially the safety of environment i.e. live world outside the laboratory from the accidents that might occur in the laboratory with the purpose. Term biosecurity is recent and connected to various form of bioterrorism. Term „containment‟ means to preserve the content i.e. all that is in the laboratory stays inside the space, meaning that environment will be protected from the conditions in the lab and term isolation means that all that is outside the laboratory stays out from the laboratory i.e. the laboratory is protected from the outside world (this apply for instance to the laboratory for cell culture and sterile conditions). The fundamentals of „containment‟ include the microbiological practices, safety equipment and facility safeguards that protect laboratory workers, the environment and the public from exposure to infectious microorganisms that are handled and stored in the laboratory.

In this paper, propositions connected with biosafety levels in the microbiological laboratories given by World Health Organization (WHO) and by International Veterinary Biosafety Working Group are described.

CLASSIFICATION OF MICROORGANISMS INTO THE RISK GROUPS

Classification of microorganisms, given by WHO, considered only to the biosafety levels in laboratory work. There are four levels of risk groups such as:

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

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

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

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

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

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

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

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

THE CLASSIFICATION OF THE LABORATORIES ACCORDING TO THE BIOSAFETY LEVEL

Laboratories are as pathogens divided to four levels of biosafety: Biosafety Level 1 (BSL1) or the basic level of the safety, Biosafety Level 2 (BSL2) or the second basic level of biosafety, Biosafety Level 3 (BSL3) or "containment" and the Biosafety Level 4 (BSL4) or "maximal containment". The most frequently, level of biosafety corresponds to the level of the risk group of the pathogen that are to be work with in the laboratory. However, this is not always the rule. Often and from the safety reasons, the work with the pathogen from the lower risk group is done in the higher biosafety level laboratory. As for an example, if the production of the high quantities of certain pathogen (vaccine production or excretion from the infected animals) from the risk group 2 and if at that occasion much of the aerosol particles are produced or if such pathogen is exotic in certain area, the work with it is done in the Biosafety Level 3 laboratory.

Besides international classification it is very important that each country or its region establish the classification of the microorganisms by risk groups, taking care of:

1. Pathogenicity of the organism;

2. Mode of transmission and host range of the organism. These may be influenced by existing levels of immunity in the local population, density and movement of the host population, presence of appropriate vectors, and standards of environmental hygiene;
3. Local availability of effective preventive measures. These may include: prophylaxis by immunization or administration of antisera (passive immunization), sanitary measures, e.g. food and water hygiene and control of animal reservoirs or arthropod vectors;
4. Local availability of effective treatment. This includes passive immunization, post exposure vaccination and use of antimicrobials, antivirals and chemotherapeutic agents, and should take into consideration the possibility of the emergence of drug-resistant strains.

The classification of certain pathogens in different groups must be done according to the risk analysis for which, except at some points, possible outcome after exposure must be taken into consideration, none natural infection pathway and transfer that occur during laboratory work, stability of the pathogen in the environment, concentration of the pathogen and the quantity of the concentrated pathogen to be manipulated with, as well as genetic manipulations that might lead to the susceptibility of the other (none natural) hosts or if sensibility is changeable to until then effective antimicrobial drugs. Therefore to establish the laboratory biosafety level, the pathogen itself that will be used for the work is taken in to account, the available specific equipment and houses, as well as indispensable procedures and availability of the measures taken to secure the work in the laboratory.

REQUIREMENTS PROCEDURES AND MINIMAL TECHNOLOGICAL CONDITIONS FOR BIOSAFETY LEVELS 1 AND 2 LABORATORIES

Laboratories of the Biosafety Level 1 (BSL1) are laboratories of the lowest level of biosafety. These laboratories are mostly foreseen to conduct lectures and studding (schools, University laboratory used for training etc.), as well as for certain research. In these laboratories the work is done with known microorganisms and they by the rule do not accept samples for examination and their basic concept is to apply good laboratory practice. 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, national and international standard precautions should always be adopted and practised.

Code of practice:

Each laboratory, according to national and international standards must build and apply the „How to behave Manuel" or „Code of good practice" as safety operational procedures that identify known and potential hazards and define procedures for the prevention, elimination and minimization of these hazards. Based on this „Code" of minimal requirements that must be full field by such laboratories are:

Access to laboratory

1. The international biohazard warning symbol and sign must be displayed on the doors of the rooms where microorganisms of risk group 2 or higher risk groups are handled;
2. Only authorized persons should be allowed to enter the laboratory working areas;
3. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements;
4. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory;
5. Laboratory doors should be kept closed;
6. Children should not be allowed to enter laboratory working areas;
7. Access to animal houses should be specially authorized;
8. 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;

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 of work

1. Pipetting by mouth must be strictly forbidden;
2. All technical procedures should be performed in a way that minimizes the formation of aerosols and droplets;
3. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices;
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. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers;
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. A written procedure for the clean-up of all spills must be developed and followed;
6. Contaminated liquids must be decontaminated (chemically or physically) before discharge to the sanitary sewer;
7. Broken glassware must not be handled directly. Instead it must be removed using a brush and dustpan, or forceps. Plastic ware should be substituted for glassware whenever possible;
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor;
9. 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 designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate;
2. Animal and plants not associated with the work being performed must not be permitted in the laboratory;

3. The laboratory should be kept neat, clean and free of materials that are not pertinent to the work;
4. Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of the working day;
5. All contaminated materials, specimens and cultures, and other potentially infectious materials must be decontaminated before disposal or cleaning for reuse. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations;
6. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory;
7. 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;
1. The laboratory supervisor (reporting to the laboratory director) should ensure that regular training in laboratory safety is provided;
2. 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;
3. There should be an arthropod and rodent control program;
4. 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

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. Illumination should be adequate for all activities. Undesirable reflections and glare should be avoided;
4. Laboratory furniture should be sturdy and must be capable of supporting anticipated loads and uses. Open spaces between and under benches, cabinets and equipment should be accessible for cleaning. Bench tops must be impervious to water and resistant

to heat, organic solvents, acids, alkalis, and other chemicals and chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated;

5. 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;
6. Space and facilities should be provided for the safe handling and storage of solvents, radioactive materials, and compressed and liquefied gases;
7. Facilities for storing outer garments and personal items should be provided outside the laboratory working areas;
8. Facilities for eating and drinking and for rest should be provided outside the laboratory working areas;
9. Hand-washing basins, with running water if possible, should be provided in each laboratory room, preferably near the exit door;
10. An eyewash station must be readily available;
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;
15. The provision of mechanical ventilation systems that provide an inward flow of air without recirculation instead windows should be considered. If there is no mechanical ventilation, windows should be able to be opened and should be fitted with arthropod-proof screens;
16. 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.

Laboratory equipment

Together with good procedures and practices, the use of safety equipment will help to reduce risks when dealing with biosafety hazards. 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.

Essential biosafety equipment

1. Pipetting aids – to avoid mouth pipetting;
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; when there is an increased risk of airborne infection and when 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.

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. 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 color-coded according to whether the contents are to be autoclaved and/or incinerated.

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.

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 „infectiouswaste“ containers and incinerated, with prior autoclaving if laboratory practice requires it. Sharps disposal containers must not be discarded in landfills. No pre-cleaning 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. Apart from sharps, which are dealt with above, all contaminated (potentially infectious) materials should be autoclaved in leak proof containers, e.g. autoclavable, color-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 healthcare activities should not be discarded in landfills even after decontamination. Reusable transfer containers should be leak proof 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. 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.

REQUIREMENTS, PROCEDURES AND MINIMAL TECHNOLOGICAL CONDITIONS FOR BIOSAFETY LEVEL 3 LABORATORIES

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, as well as work with microorganisms that is considered as exotic for region/country. 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 laboratory supervisor must enforce the institutional policies that control access to the laboratory;
2. A laboratory-specific biosafety manual must be prepared and adopted as policy, and must be available and accessible;
3. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements;
4. The international biohazard warning symbol and sign 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;
5. 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;
6. Open manipulations of all potentially infectious material must be conducted within a biological safety cabinet or other primary containment device;
7. Respiratory protective equipment may be necessary for some laboratory procedures or working with animals infected with certain pathogens;
8. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory;
9. Laboratory personnel should receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must

receive annual updates or additional training when procedural or policy changes occur;

10. 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;
11. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor.

Laboratory design

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;
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 rooms;
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 should be installed;
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. 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 crosscurrents from doors and ventilation systems;
11. The exhaust air from Class I or Class II biological safety cabinets, 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 leak proof 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.

Laboratory equipment

The principles for the selection of laboratory equipment, including biological safety cabinets 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.

REQUIREMENTS, PROCEDURES AND MINIMAL TECHNOLOGICAL CONDITIONS FOR BIOSAFETY LEVEL 4 LABORATORIES

The maximum containment laboratory – Biosafety Level 4 is designed for work with risk group 4 and exotic microorganisms. Operational “maximum containment laboratories” – Biosafety Level 4

should be under the control of national or international appropriate health authorities.

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. All manipulations of infectious agents must be performed within a BSC or other primary barrier system;
4. Appropriate communication systems must be provided between the laboratory and the outside (e.g., voice, fax, and computer);
5. A visual and camera monitoring system of the work done in the laboratory must be installed;
6. Practical and effective protocols for emergency situations must be established. These protocols must include plans for medical emergencies, facility malfunctions, fires, escape of animals within the laboratory, and other potential emergencies;
7. Personnel must be trained in emergency extraction procedures in the event of personnel injury or illness;
8. A method of communication for routine and emergency contacts must be established between personnel working within the Biosafety Level 4 and support personnel outside the laboratory.

Laboratory design

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:

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 cabinets (cabinet room) is required. In this laboratory configuration the Class III biological safety 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.

— *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.

The BSL-4 facility design parameters and operational procedures must be documented. The facility must be tested to verify that the design and operational parameters have been met prior to operation. Facilities must also be re-verified annually.

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.

Controlled air system

Negative pressure must be maintained in the facility. Both supply and exhaust air must be HEPA-filtered. The ventilation system must be monitored and alarmed to indicate malfunction or deviation from design parameters. 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 cabinets 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 areas of greatest potential hazard. Redundant exhaust fans are required to ensure that the facility remains under negative pressure at all times. 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 and under no circumstances shall the exhaust air from the Biosafety Level 4 suit laboratory be recirculated to other areas. 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.

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. There is a possibilities for chemical decontamination by acids and alkaline. Decontamination of all liquid wastes must be documented. The decontamination process for liquid wastes must be validated physically and biologically. Biological validation must be performed annually or more often if required by institutional policy.Water from the personnel shower and toilet may be discharged directly to the sanitary sewer without treatment.

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 (like with formaldehyde).

Airlock entry ports for specimens, materials and animals must be provided, and ***Emergency power*** and dedicated power supply lines must be provided. In table 1 is the summary of the requirements for different biosafety levels.

Table 1: Summary of biosafety level requirements

BIOSAFETY LEVEL REQUIREMENTS	BIOSAFETY LEVEL			
	1	2	3	4
Isolation[a] of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
— inward airflow	No	Desirable	Yes	Yes
— controlled ventilating system	No	Desirable	Yes	Yes
— HEPA-filtered exhaust air	No	No	Yes/No[b]	Yes
Double-door entry	No	No	Da	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	Da	—
Anteroom with shower	No	No	Yes/No[c]	No
Effluent treatment	No	No	Yes/No[c]	Yes
Autoclave:				
— on site	No	Desirable	Yes	Yes
— in laboratory room	No	No	Desirable	Yes
— double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability[d]	No	No	Desirable	Yes
[a] Environmental and functional isolation from general traffic.				
[b] Dependent on location of exhaust.				
[c] Dependent on agents used in the laboratory.				
[d] For example, window, closed-circuit television, two-way communication.				

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