

CHAPTER 1.1.3.

BIOSAFETY AND BIOSECURITY IN THE VETERINARY MICROBIOLOGY LABORATORY AND ANIMAL FACILITIES

INTRODUCTION

Laboratory work of the type described in this Terrestrial Manual should be carried out with a minimum of risk to the health of the staff (biosafety) and the environment (biocontainment). This requires careful consideration of the risks involved in a particular procedure, followed by appropriate measures to minimise the risk of human disease and of possible release into the environment. This is a complex subject that can only be considered in outline in an introductory chapter. This chapter is concerned almost exclusively with risks from infectious agents, but physical and chemical injuries in microbiology laboratories must also be prevented. Risks from infection are reduced by good laboratory techniques and secure facilities, which aid in the containment of pathogens. It is important to understand that containment of pathogens can be used for two purposes. One is to prevent disease in humans in the laboratory; the other is to prevent the release of the pathogen into the environment and causing disease in animals or humans. Often the same methods of containment are used for both preventing laboratory-acquired infection in humans and for preventing escape of pathogens that could cause an outbreak of animal diseases. Although the methods, techniques and facilities required may be the same, the list of pathogens and categorisation into levels of risk will differ depending on whether it is human or animal diseases control that is the primary objective.

Existing national and international reference laboratories have considerable experience in the operation of safe working practices and provision of appropriate facilities. When new laboratories are being established, it would be prudent to seek advice from the relevant regulatory authorities and the competent authorities at established institutes. It is important to comply with legislative requirements.

A. ASSESSMENT OF RISK FROM PATHOGENS

It is necessary first to assess the risk from a pathogen, so that it can be assigned to a Risk Group. A further risk assessment can be conducted, based on the proposed work, to determine the appropriate containment level. To assess the risk to humans and animals from a particular pathogen it is necessary to know whether infection with that organism can cause clinical disease and/or mortality in humans and animals, and whether it could then spread to cause disease in the general human and/or animal population. There are additional requirements related to the containment of animal pathogens and the prevention of the spread of infection to animals. To assess these risks it is necessary to know the epidemiological background of the organism and also such attributes of the organism as infectivity for humans and animals, stability in the environment, ability to infect by different routes of exposure, and susceptibility to specific treatments or prophylaxis (Acha & Szyfres, 2001; Advisory Committee on Dangerous Pathogens, 1995; Bell *et al.*, 1988; Beran & Steele, 1994). It is relatively easy to obtain this information when working with a known pathogen, but the problem is more complex in a diagnostic laboratory receiving clinical material that may be infected with a variety of unknown pathogens, some of which could be extremely hazardous to human health or pose a significant threat to animal populations. Some of the considerations to take into account when evaluating risk are:

1. Known occurrence of human and animal infection with the organism or related organisms with similar characteristics, any history of laboratory-acquired infection, infective dose and disease severity; production of toxins or allergens.

2. The volume of culture to be handled and the concentration of the organism likely to be present. (Procedures such as antigen or vaccine production that require large quantities of organisms usually carry a higher risk than attempted isolation procedures.)
3. The origin of the sample, for example samples from wildlife species may contain human or animal pathogens not normally encountered.
4. The history of the isolate being handled. Pathogens on primary isolation or of low passage level are often more dangerous than pathogens of high passage level. In some cases, pathogenicity may be enhanced by passage or subculture using different media.
5. The possibility of aerosol formation should be especially taken into consideration when handling fluid samples or, for example, during grinding, homogenisation and centrifugation.
6. The threat that the organism may pose to food-producing or companion animals or to wildlife, irrespective of the threat to laboratory personnel. Additional precautions for handling and storage are required for animal disease agents from foreign countries.
7. The physical state of the employees. For example, in the case of pregnancy, immunodeficiency or allergy, special precautions may be required. Sometimes certain individuals have to be excluded from particular types of work that would be especially hazardous to them.
8. A higher level of risk may arise when agents such as *Brucella* or *Mycobacterium* are inoculated into animals. To evaluate the impact of animal inoculation, a risk assessment should be conducted and the following factors should be considered:
 - i) Host species versus inoculated species;
 - ii) Strain/treatment and concentration of the inoculum;
 - iii) Route of inoculation;
 - iv) Animal housing;
 - v) Types of sampling during the experiment.
9. Some pathogens need to be transmitted by specific vectors or require intermediate hosts to complete their life cycles before they can infect animals and cause disease. In countries where such vectors or intermediate hosts do not occur, or where climatic or environmental factors mitigate against their survival, the pathogen poses a lower risk to animal health than in countries where such vectors or intermediate hosts occur naturally or could survive.

B. GROUPING OF MICROORGANISMS BY HUMAN AND ANIMAL HEALTH RISK

The considerations outlined above have been used by several national authorities to designate microorganisms into four Risk Groups (Advisory Committee on Dangerous Pathogens, 1995; Barbeito *et al.*, 1995) representing increasing risks to human health. Such categorisation of pathogens makes no allowance for people who are particularly susceptible, for example due to pre-existing disease, a compromised immune system or pregnancy. The four Groups may be summarised thus:

- Group 1 – Organisms that are unlikely to cause human or animal disease and are disease-producing organisms in animals that are enzootic but not subject to official control.
- Group 2 – Organisms that may cause human or animal disease but are unlikely to be spread in the community or animal population and for which effective prophylaxis and treatment are available; examples of Group 2 animal pathogens:
 - i) They do not depend on vectors or intermediate hosts for transmission.
 - ii) There is very limited or no transmission between different animal species.
 - iii) Geographical spread if released from the laboratory is limited.
 - iv) Direct animal to animal transmission is relatively limited.
 - v) Mode of transmission is primarily through ingestion, inoculation or mucus membrane route.
 - vi) The need to confine diseased or infected nondiseased animals is minimal.
 - vii) The disease is of limited economic and/or clinical significance.
 - viii) Short-term survival in the environment and effective treatment or prevention is available.
 - ix) May be either exotic or enzootic but are subject to official control and have a low risk of spread from the laboratory.
- Group 3 – Organisms that can cause severe human or animal disease and may spread in the community and/or animal population but for which there is usually effective prophylaxis and treatment; examples of Group 3 animal pathogens:
 - i) They may depend on vectors or intermediate hosts for transmission.
 - ii) Transmission between different animal species may readily occur.
 - iii) Geographical spread if released from the laboratory is moderate.
 - iv) Direct animal to animal transmission occurs relatively easily.

- v) The statutory confinement of diseased, infected and in-contact animals is necessary.
 - vi) The disease is of severe economic and/or clinical significance.
 - vii) Prophylactic and/or therapeutic treatments are not readily available or of limited benefit.
 - viii) Mode of transmission may be through the airborne route or direct contact.
 - ix) Are either exotic or enzootic but are subject to official control and that have a moderate risk of spread from the laboratory.
- Group 4 – Organisms that cause severe human or animal disease, may represent a high risk of spread in the community or animal population and for which there is usually no effective prophylaxis or treatment.
 - i) They may depend on vectors or intermediate hosts for transmission.
 - ii) Transmission between different animal species may occur very readily.
 - iii) Geographical spread if released from the laboratory is widespread.
 - iv) Direct animal to animal transmission occurs very easily.
 - v) Can be transmitted through casual contact or indirectly.
 - vi) The statutory confinement of diseased, infected and in-contact animals is necessary.
 - vii) The statutory control of animal movements over a wide area is necessary.
 - viii) The disease is of extremely severe economic and/or clinical significance.
 - ix) No satisfactory prophylactic and/or therapeutic treatments are available.
 - x) Have a high risk of spread from the laboratory into the environment and the national animal population.

Infectious organisms that might be encountered in laboratory work have been assigned to Risk Groups 1–4 by authorities in several countries (Advisory Committee on Dangerous Pathogens, 1995; Barbeito *et al.*, 1995). Some examples of pathogens that may cause disease in humans, and also may be found in a veterinary laboratory, are listed in Table 1. Also, some very serious Group 4 agents, including Hendra and Nipah, have been isolated from diagnostic specimens in veterinary laboratories.

Table 1. Examples of some of the microorganisms in Risk Groups 2 and 3 that are capable of causing human disease and that may be present in a veterinary laboratory

Group 2

Viruses: Influenza viruses types A, B, C other than notifiable avian influenza (NAI); Newcastle disease virus; Orf (parapox virus)

Bacteria: *Alcaligenes* spp.; *Arizona* spp.; *Campylobacter* spp.; *Chlamydophila psittaci* (nonavian); *Clostridium tetani*; *Clostridium botulinum*; *Corynebacterium* spp.; *Erysipelothrix rhusiopathiae*; *Escherichia coli*; *Haemophilus* spp.; *Leptospira* spp.; *Listeria monocytogenes*; *Moraxella* spp.; *Mycobacterium avium*; *Pasteurella* spp.; *Proteus* spp.; *Pseudomonas* spp.; *Salmonella* spp.; *Staphylococcus* spp.; *Yersinia enterocolitica*; *Yersinia pseudotuberculosis*

Fungi: *Aspergillus fumigatus*; *Microsporum* spp.; *Trichophyton* spp.

Group 3

Viruses: Rabies virus; Equine encephalomyelitis virus (Eastern, Western and Venezuelan); Japanese B encephalitis virus; Louping ill virus

Bacteria: *Bacillus anthracis*; *Burkholderia mallei* (*Pseudomonas mallei*); *Brucella* spp.; *Chlamydia psittaci* (avian strains only); *Coxiella burnetii*; *Mycobacterium bovis*

C. REQUIREMENTS FOR WORK WITH INFECTIOUS AGENTS

1. Known pathogens

Having decided the risk level of certain work, it is then possible to decide the appropriate 'containment level' that is needed to minimise the risk of human disease and the risk of spread of disease to animals and the environment. The containment level is defined by a combination of the physical facilities and working practices employed. Organisms of the four Risk Groups indicated above may be placed into containment levels appropriate for safe working, see below. Laboratories usually appoint a Biological Safety Officer, responsible for ensuring that microorganisms are handled at the appropriate containment level. They should have sufficient expertise and be of sufficient seniority to oversee and advise on all safety matters. In large organisations with a network of laboratories, it is appropriate to appoint a central Safety Officer to advise on and coordinate safety matters of a corporate nature, which are implemented by local laboratory Safety Officers at each site. The working methods for a particular procedure or work station should be written out and readily available. Staff must be fully trained and fully aware of any health risks associated with their work and in procedures for reporting incidents or accidents. Staff should also be given a medical card indicating pathogens to which they might be exposed. In some cases, staff can be specially vaccinated to give additional protection, e.g. when working with the rabies virus; this should also be recorded on the medical card. Such information is useful for a medical practitioner in the event of illness occurring. Regular medical examinations of employees are recommended and, as appropriate, monitoring tests of employees working with the organisms that cause certain serious human diseases, such as brucellosis and tuberculosis.

Much information is available on containment of pathogens, and sophisticated apparatus and buildings may be constructed for containment of the more hazardous organisms as required by the guidelines, standards and regulations of each country. The requirements depend on the containment required, from the most basic to the highest level.

Essential requirements for all laboratory work. The essential requirements for any work with infectious agents, however innocuous they may seem, are as follows:

1. The laboratory should be easy to clean, with surfaces that are impervious to water and resistant to chemicals. There shall be a wash-hand basin and emergency shower, including an eye bath, in each laboratory suite as appropriate for the chemicals and other hazards present. Procedures shall be established for frequent cleaning and disinfection during and at the end of the work period;
2. Personnel access to the work area should be restricted; appropriate security measures such as controlled electronic access may be necessary with higher risk agents.
3. Personal protective equipment such as long-sleeved lab coats or gowns, closed-toe footwear, disposable gloves, masks, safety glasses, face shields, and oro-nasal respirators, as appropriate, shall be worn in the laboratory and removed when leaving the laboratory
4. The laboratory door should be closed when work is in progress and ventilation should be provided by extracting air from the room. (Where biosafety cabinets are used, care shall be taken to balance ventilation systems.);
5. Food (including chewing gum, candy, throat lozenges and cough drops) and/or drink shall not be stored or consumed in laboratories;
6. Smoking and/or application of cosmetics shall not take place in the laboratory;
7. Pipetting shall not be done by mouth;
8. Care shall be taken to minimise the production of aerosols;
9. Emergency response plans should be developed to deal with the biohazard of spills. Some of the items addressed in the plans should include having effective disinfectant available for cleaning spills, removal of and decontamination of contaminated protective clothing, washing of hands, and cleaning and disinfection of bench tops;
10. Used laboratory glassware and other contaminated material shall be stored safely. Materials for disposal shall be transported without spillage in strong containers. Waste material should be autoclaved, incinerated or otherwise decontaminated before disposal. Reusable material shall be decontaminated by appropriate means;
11. No infectious material shall be discarded down laboratory sinks or any other drain;
12. Any accidents or incidents shall be recorded and reported to the Safety Officer.

Containment level for Group 2 pathogens, in addition to the points given above, a Class I, II or III microbiological safety cabinet should be used when there is potential for generating aerosols or when handling large quantities of culture or where there is a real need to protect the biological product (see Section D). Appropriate signs are required at all entry doors to indicate the hazard present and the name and telephone number of the person(s) responsible. Emergency protocols should be posted within the laboratory to advise personnel of procedures to follow in case of a pathogen spill or the need to evacuate the laboratory in the event of a fire or other emergency.

Containment level for Group 3 pathogens, it is advisable that the laboratory be in an isolated location; access should be limited to appropriately trained level 3 staff. Emergency protocols should be posted within the laboratory to advise personnel of procedures to follow in case of a pathogen spill or the need to evacuate the laboratory in the event of a fire. OIE containment level for Group 3 pathogens surpasses biosafety level-3 (BSL-3) guidelines as outlined by the United States Department of Health and Human Services (DHHS) joint publication with CDC and NIH (2009) and the United States Department of Agriculture (USDA, 2002).

In addition to the previous requirements, the laboratory shall be under negative pressure and the pressure differentials should be monitored; a procedure should be developed to provide an alarm if there is a problem and personnel to respond to the alarm. A ventilation system is required that removes air from the laboratory through a high efficiency particulate air (HEPA) filter. HEPA filters shall be verified regularly (usually annually); this would include HEPA filters in biosafety cabinets and on room and equipment exhausts. The laboratory should be sealable for fumigation and contain an airlock entry. There is a requirement to treat effluent depending on the pathogen. Biological safety cabinets of Class I, II or III shall be used whenever the process to be undertaken is likely to generate an aerosol (DHHS/CDC/NIH, 2000). It may be necessary for staff to shower on exit from the laboratory and they must wear dedicated laboratory clothing that is left in the laboratory before leaving the building.

Note. Because of the link between bovine spongiform encephalopathy (BSE) and new variant Creutzfeldt-Jakob disease in humans, BSE and related agents are now categorised with the human transmissible spongiform encephalopathies in Risk Group 3. Consequently, veterinarians and laboratory workers conducting necropsies on BSE-suspect animals or handling tissues derived from such animals must conduct the work under appropriately strict containment conditions, sometimes with derogations allowed by the nature of the work and the results of local risk assessment. It is important that appropriate protective clothing be worn and that a strict code of practice be followed to prevent exposure to the agent. Laboratories conducting work on BSE must comply with national biocontainment and biosafety regulations (Advisory Committee on Dangerous Pathogens, 1998).

Containment level for Group 4 pathogens, the most stringent precautions are required, including access to the building through air locks, and the building being maintained under negative air pressure. Inlet air to the laboratory shall be filtered through a single HEPA filter and extracted air through double HEPA filters in series. All work with infective materials shall be conducted in a Class III cabinet or in a Class II cabinet in conjunction with the use of one-piece positive-pressure suits. All sewage from the laboratory, laboratory effluent and autoclave drain effluent shall be treated by appropriate means to ensure that all infectious material is destroyed before entering the sewerage outside the laboratory. Staff shall shower and change their clothing before leaving the building. Other precautions as described for Group 3 would also apply. The use of one-piece positive-pressure suits is now an internationally accepted way of providing additional protection at level 4.

OIE guidelines for the containment level for Group 4 pathogens are generally equal to the USDA's biosafety level 3 Ag guidelines (USDA, 2002). The primary difference between OIE level 4 and BSL-3 Ag is that the BSL-3 Ag guidelines specify that the laboratory will be airtight and shall pass a pressure decay test to confirm that it does not surpass the prescribed maximum leak rate.

2. Diagnostic specimens

Veterinary diagnostic centres readily receive specimens that are submitted because they are suspect for a variety of diseases. The infectious nature of the specimens is usually unknown, but they have the potential to contain biological agents that may cause disease in animals and humans. Practices and procedures need to be in place that will minimise the risk of occupational exposure of employees to such pathogens. Unless suspected of containing a pathogen requiring a higher containment level, it is advisable that initial processing of all unknown specimens should be carried out as though the material contained a Group 2 pathogen. The most important aspects are to prevent percutaneous, mucous membrane exposure, particularly inhalation and ingestion. Biological safety cabinets should be used for all manipulations that may generate aerosols. Class I or II are appropriate depending on the need for protection of the samples from contamination. Additionally, there should be no mouth pipetting, personal protective clothing shall be worn with, in some cases, eye and respiratory protection, depending on the anticipated level of exposure. Although initial diagnostic procedures may be carried out at level 2, once a Group 3 or 4 organism has been isolated (or suspected) further work must be carried out at the higher containment level.

D. MICROBIOLOGICAL SAFETY CABINETS

These are used at the different containment levels, as described in Section C above. They are of three types:

Class I: An open-fronted cabinet designed specifically to provide operator and environmental protection and not to give protection to the work being handled.

Class II: An open-fronted safety cabinet, sometimes referred to as a laminar flow recirculating cabinet. They are designed to give operator, product and environment protection.

Class III: These cabinets are closed, with glove ports at the front, and provide the highest degree of containment by complete separation of work and worker. Some cabinets have a removable glove port and are known as Class III/I cabinets, i.e. they can be used in either mode.

Descriptions of safety cabinets and safe working practices have been published (Collins, 1990; International Atomic Energy Agency [IAEA], 1994; DHHS/CDC/NIH, 2000).

E. STORAGE OF PATHOGENS

Storage of live pathogens requires appropriate containment and security to avoid risks due to breakage or unauthorised use of material. Storage facilities should be appropriately labelled to indicate the nature of the pathogens (e.g. their Group) and the contact information for the person(s) responsible for them. A complete inventory of the pathogens in storage should be kept up to date and available. Special care must be taken when

opening glass vials of freeze-dried pathogens, as these can sometimes shatter. Care must be taken when working with liquid nitrogen or rooms where asphyxiating gasses may be produced.

Many of the considerations given above relate not only to human safety but also to prevention of the spread of infection to animals. In a veterinary laboratory an important responsibility is to minimise any risk of escape of pathogens to animals, either wild or domestic, in the outside community. Close communication must be maintained with the veterinary authorities. There may be national requirements for special licences to work with certain microorganisms.

F. PHYSICAL AND CHEMICAL HAZARDS

Laboratory work involves many manipulations that are potentially dangerous, such as handling glassware and work with needles or other sharp instruments. There shall be appropriate procedures and equipment for the safe and proper disposal of needles and other 'sharps'.

Laboratory staff should be protected from the risk of receiving a burn from hot solids or liquids. Autoclaves shall be fitted with safety devices to prevent accidental opening of doors when under pressure, and be regularly serviced and tested. Heat-protective gloves, apron and face shields with brow and chin guards shall be provided. Extreme cold can also be a risk, for example when working with liquid nitrogen; splashes on exposed skin can be very damaging. Gloves should be worn that provide insulation from cold and that are also waterproof, to prevent penetration of the liquid nitrogen. Face shields with brow and chin guards and boots should also be worn when working with liquid nitrogen. Nitrogen evaporating from liquid nitrogen storage in poorly ventilated rooms can lead to depletion of oxygen with fatal consequences.

Irradiation is a serious health risk that may be present due to the use of X-ray machines, or use of gamma-emitters or other sources. Equipment shall be regularly serviced and tested. All use of radioactive material must be meticulously recorded. All staff must wear a personal radiation-monitoring device and have annual health checks. Local and national regulations must be followed (IAEA, 1994).

A wide range of chemicals are used in veterinary laboratories, many of which may be toxic or mutagenic, and some may be carcinogenic. It should be remembered that it is the dose that makes the poison. Vapours are especially hazardous, and some chemicals can be absorbed by penetration of intact skin. Steam sterilisation may make toxic chemicals volatile and endanger personnel who unload the autoclave/pressure steam steriliser. Procedures sufficient to protect pregnant laboratory workers should be followed at all times. A list of hazardous chemicals shall be maintained, and a file record kept of chemicals to which individual staff members could be exposed. This is now a legal requirement in some countries. Chemicals shall be correctly stored in appropriate containers and at the correct temperature. Those that are flammable shall be kept in a fireproof chemical store. A record must be maintained of the purchase and use of hazardous chemicals: how much, when used, by whom and for what purpose. Disposal of some chemicals is subject to official regulation.

Further information on physical and chemical safety precautions can be found in the literature (Office of Biosafety, Laboratory Centre for Disease Control, Health and Welfare Canada, 1996; Rayburn, 1990).

G. LABORATORY ANIMAL FACILITIES

Work with pathogens in laboratory animals poses special risks. Animal rooms have to be constructed to appropriate standards and containment levels, just as laboratories. Containment in animal houses is very important because of the large amount of infectious agents that they may generate. Similar considerations also apply regarding the training of staff, protective clothing and the recording of working procedures. Special care must be taken to avoid injury to staff, e.g. through animals biting and kicking or self inoculation accidents. Any such incidents must be recorded and wounds appropriately treated. There shall be provision for autoclaving steam sterilisation, incineration or rendering of carcasses and for the thorough cleansing and disinfection of animal rooms. The animal rooms should not only provide a suitable environment for the animals themselves but should be constructed and ventilated in such a way as to ensure comfort for the attending personnel. This is a large subject that can only be referred to briefly here (Barbeito et al., 1995; Canadian Food Inspection Agency, 1996). Also, an excellent book on health and safety in laboratory animal facilities is available (Wood & Smith, 1999).

H. EMERGENCY PROVISIONS

First-aid equipment should be readily available, but stored in a location that is unlikely to be contaminated by work conducted in the laboratory (for example, in the air-lock or ante-room). This equipment shall be appropriate to the

work and properly maintained. It shall be kept ready to hand for immediate emergency use by trained first aid personnel. Bandages and dressings should be available. Some staff shall receive training in safety and first aid from recognised authorities and shall possess a valid certificate as evidence of competence. Personnel working in Containment Level 4 facilities shall have advanced first aid competence. Their names and locations should be known to everyone and posted on notice boards. All staff should be aware of the importance of safety. There must be suitable procedures and equipment for dealing with spillages and decontamination. A record must be kept of all incidents and in some countries there may be a legal obligation to report incidents to the enforcing authority.

There must be written procedures for dealing with emergency failure of all safety and containment systems, for example in biosafety cabinets or biocontainment rooms, which can lead to loss of containment.

Many laboratories have a staff safety committee to increase safety awareness and to discuss safety issues with management. Personnel are responsible for their own safety and those around them. Managers are equally responsible for safety in their area of command and should not allow consideration of speed or cost of work to come before the safety of personnel or containment of animal disease agents.

There must be an emergency procedure for obtaining medical assistance if required, and for hospitalisation in appropriate infectious disease facilities when needed. Fire alarms shall be fitted, and tested regularly. The institute or laboratory must designate a warden to control and communicate in emergency situations and conduct periodic drills to make staff aware of what to do and where to assemble in the event of an emergency. The warden is responsible for checking that everyone is in a safe location. Procedures for natural disasters, such as hurricanes and earthquakes, should be in place where they present a risk. All these procedures should be written down and periodically reviewed.

I. TRANSPORT OF INFECTIOUS MATERIAL

Great care must be taken when preparing and packing diagnostic specimens, infectious materials and pathogens for transport, to ensure that there is no breakage of containers or leakage of contents that could put at risk personnel in the transport system or animals that may come in contact with contamination. Applicable local, national and international regulations for the transportation of dangerous goods (diagnostic or clinical sample and infectious materials) and importation of animal pathogens must be followed. These are summarised in Chapter 1.1.1 Collection and shipment of diagnostic specimens.

When categorising animal pathogens into specific Groups, the following criteria should be taken into account:

a) Group 1 animal pathogens

Disease-producing organisms that are enzootic but not subject to official control.

b) Group 2 animal pathogens

Disease-producing organisms that are either exotic or enzootic but subject to official control and that have a low risk of spread from the laboratory.

c) Group 3 animal pathogens

Disease-producing organisms that are either exotic or enzootic but subject to official control and that have a moderate risk of spread from the laboratory.

d) Group 4 animal pathogens

Disease-producing organisms that are either exotic or enzootic but subject to official control and that have a high risk of spread from the laboratory into the environment and the national animal population.

J. CONTAINMENT GROUPS

1. The principal purpose of containment is to prevent the escape of the pathogen from the laboratory into the national animal population. Some animal pathogens can infect humans. In these instances the risk to human health may demand additional containment than would otherwise be considered necessary from purely animal health considerations. The risk of human to animal transmission of disease must also be considered and controlled. In addition, other animals being used for experimental work on the pathogen should be held in the appropriate containment level.

2. The level of physical containment and biosafety procedures and practices should be not less than the Group into which the pathogen has been placed and the detailed requirements should be appropriate to the type of organism (i.e. bacterium, virus, fungus or parasite). The lowest containment level will be required for pathogens in Group 1 and the highest level for those in Group 4. Guidance on the containment requirements for Groups 2, 3 and 4 is provided in Section K.
3. Arthropods may be pathogens or vectors for pathogens. If they are a vector for a pathogen being used in the laboratory, the appropriate containment level for the pathogen will be necessary in addition to the containment facilities for the arthropod.

K. GUIDANCE ON THE LABORATORY/ANIMAL FACILITY REQUIREMENTS FOR THE DIFFERENT CONTAINMENT GROUPS

Requirements of the laboratory/animal facility	Containment Group		
	2	3	4
A) Laboratory/animal facility setting and structure			
1. It is advisable that the laboratory/animal facility be in an isolated location		Yes	Yes
2. Not next to known fire hazard	Yes	Yes	Yes
3. Workplace separated from other activities	Yes	Yes	Yes
4. Personnel access limited	Yes	Yes	Yes
5. Protected against entry/exit of rodents and insects	Yes	Yes	Yes
6. Liquid effluent must be sterilised and monitored		Yes	Yes
7. Liquid effluent from steam sterilisers shall be sterilised and monitored			Yes
8. Isolated by airlock. Continuous internal airflow		Yes	Yes
9. The laboratory/animal facility shall be under negative pressure and the pressure differentials should be monitored		Yes	Yes
10. Input air to be filtered using HEPA or equivalent such as gas tight damper; exhaust air to be single HEPA filtration for laboratories and double HEPA filtration for animal facilities.		Single on extract	Single for input, double for extract
A) Laboratory/animal facility setting and structure (cont.)			
11. HEPA filters shall be verified regularly (usually annually)		Yes	Yes
12. Mechanical air supply system with fail-safe system and an alarm provided if there is a problem		Yes	Yes
13. Laboratory/animal facility sealable to permit fumigation		Yes	Yes
14. Incinerator, pressure steam steriliser or renderer for disposal of carcasses and waste	Available	Yes	Yes on site
15. The laboratory/animal facility should be easy to clean, with surfaces that are impervious to water and resistant to chemicals. There shall be a wash-hand basin and emergency shower, including an eye bath, in each laboratory suite as appropriate for the chemicals and other hazards present. Procedures shall be established for frequent cleaning and disinfection during and at the end of the work period	Yes	Yes	Yes
B) Additional Laboratory facility requirements			
16. Class I or II biological safety cabinet available	Yes	Yes	Yes

Requirements of the laboratory/animal facility	Containment Group		
	2	3	4
17. Class III biological safety cabinet available		Yes	Yes
18 HEPA filters shall be verified regularly (usually annually)	Yes	Yes	Yes
19. Direct access to autoclave/pressure steam steriliser	Yes	Yes with double doors	Yes with double doors
20. Specified pathogens stored in laboratory	Yes	Yes	Yes
21. Double-ended dunk tank required		Preferable	Yes
22. Personal protective clothing and equipment not worn outside laboratory	Yes	Yes	Yes
23. Full body shower and change of clothing required before exiting laboratory		It may be necessary for staff to shower on exit from the laboratory and they must wear dedicated laboratory clothing that is left in the laboratory before leaving the building	Yes
24. Safety Officer responsible for containment	Yes	Yes	Yes
25. Staff receive special training and demonstrate competence in the requirements needed	Yes	Yes	Yes
C) Laboratory discipline			
26. Warning notices for containment area to indicate the hazard present and the name and telephone number of the person(s) responsible	Yes	Yes	Yes
27. Emergency protocols should be posted within the laboratory to advise personnel of procedures to follow in case of a pathogen spill or the need to evacuate the laboratory in the event of a fire or other emergency	Yes	Yes	Yes
28. Laboratory must be lockable	Yes	Yes	Yes
29. Authorised entry of personnel	Yes	Yes	Yes
30. Protective clothing, including gloves, masks, eye shields, and oro-nasal respirators, as appropriate, shall be worn in the laboratory and removed when leaving the laboratory	Yes	Yes	Yes
C) Laboratory discipline (cont.)			
31. The laboratory door should be closed when work is in progress and ventilation should be provided by extracting air from the room. (Where biosafety cabinets are used, care shall be taken to balance ventilation systems.)	Yes	Yes	Yes
32. Food and/or drink shall not be stored or consumed in laboratories	Yes	Yes	Yes
33. Smoking and/or application of cosmetics shall not take place in the laboratory	Yes	Yes	Yes
34. Pipetting shall not be done by mouth	Yes	Yes	Yes
35. Care shall be taken to minimise the production of aerosols	Yes	Yes	Yes
36. No infectious material shall be discarded down laboratory sinks or any other drain	Yes	Yes	Yes
37. Used laboratory glassware and other materials shall be stored safely before disinfection. Materials for disposal shall be transported without spillage in strong containers. Waste material should be autoclaved, incinerated or otherwise made safe before disposal. Reusable material shall be decontaminated by appropriate means	Yes	Yes	Yes

Requirements of the laboratory/animal facility	Containment Group		
	2	3	4
38. Any accidents or incidents shall be recorded and reported to the Safety Officer	Yes	Yes	Yes
39. On entering all clothing removed and clean clothes put on		Yes	Yes
40. On exiting all laboratory clothes removed, individual shall wash and transfer to clean side		Yes	
41. Individual shall shower prior to transfer to clean side			Yes
D) Handling of specimens			
42. Packaging requirements to be advised prior to submission	Yes	Yes	Yes
43. Incoming packages opened by trained staff in appropriately contained reception area	Yes	Yes	Yes
44. Movement of pathogens from an approved laboratory to another requires a licence	Yes	Yes	Yes
45. Standard Operating Procedures covering all areas must be available	Yes	Yes	Yes

1. Additional requirements for work in animal facilities

1. The animal facility should be easy to clean, with surfaces that are impervious to water and resistant to chemicals used in the area.
2. Personnel access to the work area should be restricted; appropriate security measures such as controlled electronic access may be necessary with higher risk agents.
3. Personal protective equipment such as coveralls, boots, disposable gloves, masks, safety glasses, face shields, and oro-nasal respirators, as appropriate, shall be worn in the animal facility and removed when leaving the animal facility.
4. The animal facility door should be closed when work is in progress and ventilation should be provided by extracting air from the room. (Where biosafety cabinets are used, care shall be taken to balance ventilation systems.)
5. Food (including chewing gum, candy, throat lozenges and cough drops) and/or drink shall not be stored or consumed in animal facilities.
6. Smoking and/or application of cosmetics shall not take place in the animal facility.
10. Used laboratory glassware and other materials shall be stored safely before disinfection. Materials for disposal shall be transported without spillage in strong containers. Waste material should be autoclaved, incinerated or otherwise made safe before disposal. Reusable material shall be decontaminated by appropriate means.
11. No infectious material shall be discarded down animal facility drains without appropriate waste treatment in place.
12. Any accidents or incidents shall be recorded and reported to the Safety Officer.

L. CONCLUSION

High standards of laboratory safety and containment that will ensure healthy working conditions for laboratory staff and protection of the environment must be of the greatest priority. They can only be achieved by careful study of the principles involved followed by practical application to premises, facilities, operating procedures and hygiene. Training of all laboratory personnel must be a high priority and no personnel should be allowed to work until appropriate training and competence has been demonstrated and documented. There is a large published literature on all aspects of the subject, and further reading is recommended (Beran & Steele, 1994; European Committee for Standardisation, 2000; Richmond, 1996–2002; Sewell, 1995; World Health Organization, 2004).

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