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Biosafety in laboratory animal facilities

A practical approach

Biosafety and Biotechnology Unit

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Introduction

This document aims to serve as guidance for biosafety officers, end-users, regulators and inspectors and to provide information on the correct implementation of the necessary containment criteria and other protective measures in laboratory animal facilities in order to guarantee an optimal protection of public and occupational health and of the environment. Quality aspects of animal care falling under laboratory animal welfare regulations will not be considered here.
To allow more clarity and a better understanding of the biosafety requirements and recommendations in laboratory animal facilities, various concerns on personal protection and biosafety equipment, facility design, working practices and waste management are addressed in this document, mainly with respect to small animal facilities. Several issues in risk management are discussed and check lists (in annex) describing the containment measures assigned to the different animal biosafety levels (see below) are provided and can be used for internal and external audits.

In Belgium, most activities involving animal experiments with transgenic animals or animals inoculated with either pathogens or genetically modified micro-organisms are subject to notification in the frame of regional legislation on contained use of genetically modified organisms (GMOs) and/or pathogens[1]. This legislation transposes European Directive 2009/41/EC, repealing Directive 90/219/EC (EC, 2009). Animal facilities should comply with the containment criteria defined in the legislation for each biosafety level.

Terminology

Directive 2009/41/EC makes a distinction between an animal unit and an animal facility. The animal unit is defined as “a building or separate area within a building containing facilities and other areas such as changing rooms, showers, autoclaves, food storage areas, etc”. The animal facility is defined as “a facility normally used to house stock, breeding or experimental animals or one which is used for the performance of minor surgical procedures”. In the Belgian contained use legislation a **laboratory animal facility** is defined as a building or a separate zone inside a building consisting of rooms or installations for the housing and manipulation of laboratory animals, including other rooms or installations such as changing rooms, showers, autoclaves, feed storage rooms.

The Centres for Disease Control and Prevention (CDC) have established animal biosafety levels for laboratory animal facilities and these are referred to as ABSL1, ABSL2, ABSL3 and ABSL4 depending on the risk proportional to the maximal risk level of the contained activity. Annex IV of Directive 2009/41/EC presents minimum requirements and measures necessary for each of the 4 containment levels in animal units, but no specific definition of each animal containment level is given as presented below. The containment levels for laboratory animal facilities in Belgium were set up based on the minimum requirements in the Directive and international guidelines such as these from the CDC. They are referred to as A1 to A4.

[1] Decree of the Flemish Government of 6 February 2004 amending the Decree of 6 February 1991 and the Decree of 1st June 1995.
Decree of the Government of the Brussels-Capital Region of 8 November 2001.
Decree of the Walloon Government of 5 June 2008 amending the Decree of the Walloon Government of 4 July 2002.

Animal biosafety level 1 (ABSL1)
…is suitable for work with laboratory animals involving well characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment…

Animal biosafety level 2 (ABSL2)
…is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure…

Animal biosafety level 3 (ABSL3)
…involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission and agents causing serious or potentially lethal disease…

Animal biosafety level 4 (ABSL4)
...is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission…

(CDC, BMBL 2009 5th edition)

Risk assessment

When conducting laboratory animal experiments, one of the first steps is to clearly identify the various hazards that are associated with the inherent characteristics of the animal species and/or with the agent used in experimental infection or inoculation.
Working with animals present special hazards not encountered in standard microbiological laboratories. They may bite, scratch, kick, disperse hair and dust from bedding and they may generate infectious aerosols. They can also escape.

If animals are experimentally infected with (zoonotic) **pathogens**, this can present a risk for humans and/or animals: a distinction must be made between animals infected with zoonotic agents and animals infected with micro-organisms that are only pathogenic to animals. Whereas zoonotic agents represent a health risk for the laboratory worker (e.g. generation of infectious aerosols, needle stick injury) non zoonotic animal pathogens represent only a risk for the environment (including animals). Hence the latter require other biosafety measures than zoonotic agents (Van Vaerenbergh *et al*, 2010).
It is important to keep in mind that zoonotic infections may also occur as a result of asymptomatic infection in laboratory animals that were not experimentally infected: a report from Harding and Byers (2006) on viral Laboratory Acquired Infections (LAIs) associated with animal activities cited 171 overt infections between 1979 and 2005. The majority of these infections were caused by Hantavirus. This underscores the importance of implementing a comprehensive pest control as well as regular

screening of laboratory animals to identify asymptomatic infections (Johnson, 2011). On the other hand, some species of laboratory animals are susceptible to diseases transmitted by humans (e.g. hepatitis A, measles, tuberculosis) warranting their protection as well (Virginia Department of Health, 2008).

Particular attention must also been given to **prion research** involving animals. Until recently, the transmission route for transmissible spongiform encephalopathy was considered to be limited mainly to oral or parenteral uptake. Recently it was shown that aerogenic infection could occur under certain experimental conditions (e.g. wildtype and transgenic mice overexpressing the cellular prion protein (PrP^c) in neurons as well as immunodeficient mice were susceptible to aerogenic exposure (nebulizer) to prions (Haybaeck *et al*, 2011; Stitz and Aguzzi, 2011)). So it should be recommended to apply adequate biosafety measures for manipulations generating prion-contaminated aerosols to protect laboratory personnel.

Consideration should also been given to indirect risks. This is illustrated by animal experiments with Influenza virus in the context of vaccine development. Even though vaccine strains are of risk class 1 they can cause subclinical infections. In the case of contamination of a lab worker who is already infected with a wild type influenza virus a reassortment might occur leading eventually to a potentially dangerous strain (reviewed in Pauwels *et al*, 2007). In that case additional personal protection should be considered, such as wearing the appropriate respiratory mask model to prevent that type of event.

Unlike work with experimentally infected animals, work with **transgenic animals** as such does not pose a major health risk for the personnel, but merely a risk for the environment in case of accidental escape. Another example is animals subcutaneously injected with mammalian cells (e.g. tumor cells) pose no risk to the personnel as the animal itself acts as a biological containment. However, special attention must be paid to animals inoculated with genetically modified micro-organisms (GMMs), for example viral vectors, since they still may shed recombinant viral particles (e.g. 3 days for rodents inoculated with lentiviral vectors (reviewed in Pauwels *et al*, 2007). On the other hand, mice inoculated with cells genetically modified by means of viral vectors represent no risk on the condition that the inoculum is free of viral particles after transduction (this can be achieved by including several washing steps, treatment of the cells with human serum or trypsine or prolonged culture (Pauwels *et al*, 2009)). Also, humanized mice models (e.g. mouse models engrafted with human hepatocytes (Meuleman *et al*, 2005) or grafted with cells permissive for HIV-replication (reviewed in Pauwels *et al*, 2009) represent a risk for the personnel since they can be infected with HCV and HIV respectively.

Although not under the scope of the contained use regulation, the use of **toxins**[2] of biological origin presents special risks and implies good understanding of chemical biohazards (e.g. diphtheria toxin which is harmful when inhaled or injected, but not when ingested as it is neutralized by gastric acid).

Finally, **allergens** present in animal bedding, hair and urine may cause a lot of discomfort and cause allergies and development of asthma. Allergic reactions to animals are among the most common problems encountered for workers involved in the care and use of animals. According to the literature, prevalence of allergic reactions has been rated between 10 and 33% (Aoyama *et al,* 1992*;* Bush *et al*, 1998). Approximately 10% of laboratory workers eventually develop occupation-related asthma (XQ, 2010). This may be overcome by good ventilation and wearing of mask, but the latter might cause discomfort to persons with allergenic rhinitis.

[2] Note that the Belgian contained use regulation covers only biosafety aspects related to *organisms* and not to *substances* of biological origin if used as such in an experiment. An organism is defined as any biological entity, including micro-organisms, capable of replication or of transferring genetic material.

Risk management

Based on biohazard identification, a risk assessment can be performed and the appropriate measures can be taken to control these hazards, e.g. housing and facility design, safety equipment, working practices, including personal protective equipment (PPE) for employees and waste management.

In the following part of this document, particular points concerning risk management in laboratory animal facilities are presented and several biosafety issues are discussed, but with a focus on small animals[3]. Specific containment requirements for large animals, special animal models (e.g. zebrafish) and insects will not be discussed in detail.

Facility design

Most laboratory animal facilities may include the following (Institute for Laboratory Animal Research. NRC, 2011):

- security features, such as card-key systems, electronic surveillance, and alarms to ensure access control;
- animal housing areas, possibly including special barrier facilities for housing of Specific Pathogen Free (SPF) animals[4];
- laboratories for specific manipulations (e.g. surgery, autopsy, experimental procedures, behavioural testing, imaging, …);
- space for washing and sterilizing equipment including mechanical cage washers;
- storage areas for food, bedding, supplies;
- storage areas for waste, cold storage or disposal of carcasses;
- room for administrative and animal care staff;
- sanitary facilities and break areas for personnel.

For small animals, a difference is made between a micro-environment and a macro-environment. The micro-environment is the physical environment immediately surrounding the animal: the primary enclosure with its own temperature, humidity, and gaseous and particulate composition of the air which constitutes the primary containment. The macro-environment is the physical environment of the secondary enclosure such as an animal room, including the micro-environment and constitutes the secondary containment. For large animals, the animal housing room constitutes the primary containment.

Barrier facilities are designed and constructed to ensure isolation and prevent introduction of adventitious infectious agents in areas where animals of a defined health status are housed and used. In this case, the aim is to protect the animals, not the laboratory workers. To achieve this, these facilities are under positive air pressure with respect to surrounding areas. Supply air is filtered (e.g., HEPA or 95% efficient filters), and flows from clean to potentially contaminated areas. They may be a

[3] By 'small' animals is meant :animals which can be housed and handled in a primary containment (e.g. a cage enclosed by solid walls, an isolator, a biosafety cabinet, a changing station, a fish tank).
[4] SPF animals are free of *certain* germs and other infectious agents that may not produce disease but nevertheless cause research interference.

part of a larger animal facility or a separate unit. They are used for immune deficient rodents, SPF animals and especially valuable genetically engineered animals.

Barrier facilities typically incorporate airlocks or special entries for staff and supplies. In general animal care takers wear dedicated lab clothing and footwear, disposable and sterile head and shoe covers, gloves, and sometimes face masks prior to entry. All consumables, such as drinking water, feed or bedding are sterilised and surface decontaminated on entry. Cages should be sterilized after washing before reuse. Strict operational procedures and unidirectional traffic flows must be followed to avoid contact between clean and soiled supplies and areas. Only animals of defined health status are received into the barrier, and once they leave they cannot re-enter without retesting. Personnel entry is restricted and employees are appropriately trained to avoid the introduction of contaminants. Specialized equipment such as isolator cages, individually ventilated cages, and animal changing stations provide an additional barrier.

Animals should be housed in animal facilities, not in laboratories for convenience. If animals must be maintained in a laboratory for some practical reason, that space should have the appropriate containment level[5] to house and care for the animals and its use should be limited to the duration of the experiment. Measures should be taken to minimize occupational hazards related to exposure to potentially infected animals in the laboratory and during transport to and from the laboratory.
However, in some cases, **decentralization** may be preferred or unavoidable for certain special research techniques involving complex equipment and support space such as magnetic resonance imaging. **Imaging techniques** offer non invasive methods for evaluating structure and function at the level of the whole animal, tissue, or cell and monitor biologic processes over time. The growing number of mouse and rat experiments, coupled with an increasing number of dedicated small animal imaging systems might necessitate a common technical centre for imaging small animals using these devices and shared by users from different facilities. An essential part of the imaging facility is an adjacent animal housing and preparation location. The majority of imaging experiments make use of immune deficient animals, primarily SCID and nude mice. To maintain the health of the animals over the course of imaging experiments, which can last several weeks, a pathogen barrier must be maintained around the animals at all times. Imaging chambers have been designed to house the animals during the imaging process. Mice are positioned and placed within the chamber using sterile techniques inside a biosafety cabinet.
But since animals infected with pathogens or inoculated with viral vectors might also be used for *in vivo* imaging, they pose a risk for contamination of equipment, personnel and other animals. Special consideration should be given to biosafety including housing, transportation to and from the site, careful decontamination of the equipment after use and occasionally personal protection equipment (Commissie Genetische Modificatie 2006). In that case, at least a Biosafety Level 2 containment level would be necessary. Imaging devices with components that are difficult to sanitize should be covered with disposable material or material that can be sterilised.
For work with highly pathogenic micro-organisms, special animal isolation imaging chambers with HEPA filtration have been designed and tested for leakage as the system operates under positive pressure with respect to the adjacent area (Alderman *et al*, 2010).

[5] The laboratory in which the animals are housed should comply with the criteria defined for an animal containment level 1 (e.g. transgenic mice) or 2 (e.g. infected mice).

Facility construction

Animal facilities have also particular requirements with respect to architectural aspects and building material (Institute for Laboratory Animal Research. NRC, 2011):

For safety, **doors** to areas where infected animals are housed should open inwards and should be self-closing. When the containment level requires restricted access doors should be equipped with locks or electronic security devices.
Doors with viewing windows are recommended. However, in some cases exposure to light can be undesirable (e.g., for animals requiring strict control of photoperiod), This can be solved by the use of red-tinted windows, proven useful for rodent holding rooms as both species have a limited ability to detect light in the red portions of the spectrum.

The presence of **external windows** should generally be avoided. If needed, for example for providing environmental enrichment for nonhuman primates in the frame of animal welfare legislation, they must be sealed and resistant to breakage.

Floors should be impervious to liquids and resistant to biological materials and chemicals. They may be textured in moist areas or for holding hoofed animals. Where floor drains are used, the floors should be sloped and drain traps kept filled with liquid and disinfectant to prevent migration of vermin and other contaminants. When drains are not in use for long periods, they should be capped and sealed to prevent backflow of sewer gases, vermin, and other contaminants; lockable drain covers may be advisable for this purpose in some circumstances.

Walls and ceilings should be smooth, impervious to water and chemical detergents, non absorbing and resistant to damage from impact (for example water under high pressure). Utility penetrations (e.g. ducts, cables) in floors, walls and ceiling should be sealed including opening around doorframes to facilitate pest control and cleaning. Exposed plumbing, ductwork, and light fixtures should be reduced to prevent accumulation of fomites[6] and facilitate cleaning. Wherever possible, utilities should be accessible via interstitial space or through access panels in corridors outside the animal rooms.

To prevent **accidental escape** of laboratory animals from the facility, it is necessary to leave the doors closed at all times e.g. by means of a self-closing system. Also door partitions or trenches can be installed and floor drains and vents can be covered with a suitable grill.

A **pest control program** to control and eliminate flying and crawling insects and wild rodents should be available. Cracks and holes in floors, walls and ceilings should be avoided. In addition to a thorough sanitary maintenance program, pesticides or traps can also be used provided that there is no interference with the animal experiments. As pesticides can have toxic effects, non toxic substances, such as insect growth regulators (Donahue *et al*. 1989; Garg and Donahue 1989; King and Bennett 1989; Verma 2002) and nontoxic substances (e.g. amorphous silica gel) should be used (Institute for Laboratory Animal Research. NRC, 2011).

[6] An inanimate object that may be contaminated with infectious organisms and serve in their transmission (Merriam Webster's Medical Dictionary, Merriam-Webster, Inc. 2007).

Ventilation

The primary purpose of ventilation is to provide appropriate air quality and a stable environment. Apart from removing odours, heat loads caused by the animals, personnel, lights, and equipment, and adjusting the moisture content, it will dilute contaminants including allergens and airborne pathogens.

10 to 15 fresh air changes per hour in animal housing rooms is an acceptable guideline to maintain air quality. However this may vary in function of different parameters such as species, size and number of animals, type of primary enclosure, cage changing frequency and room dimensions (Institute for Laboratory Animal Research. NRC, 2011). It is important to note that the positioning of air inlet and exhaust greatly determines the quality of airflow within a facility (EBSA Conference Workshop 2009).

A properly designed and functioning heating, ventilation, and air conditioning (HVAC) system is essential in controlling airborne contamination by providing directional airflow between adjoining spaces.
It is recommended that animal rooms have inward directional airflow. Exhaust air should not be re-circulated. It is generally preferable to connect the system directly into the building's exhaust system to prevent contamination.
Indeed, the use of recycled air to ventilate animal rooms may save energy but presents risks of cross-contamination with airborne pathogens or contaminated fomites (e.g. dust).

In the case of high containment animal facilities (A3 or ABSL3), a dedicated ventilation system is provided. The supply and exhaust components are designed to maintain a negative air pressure in the laboratory with respect to adjacent areas[7] and outgoing air is HEPA filtered. Supply and exhaust vans must be interlocked to avoid overpressure in case of an exhaust system failure. HEPA filter housings are equipped with valves which automatically close in case of overpressure. Monitoring and alarm systems must be present for verification and indication of malfunction.
The successful operation of any HVAC system requires regular preventive maintenance and evaluation.

Animal housing

In laboratory animal facilities, the animals should be housed in appropriate biocontainment enclosures, such as cages, pens, runs, stalls, aquaria, etc…). Specialized housing systems (e.g., isolation-type cages, Individually Ventilated Cages (IVCs), and gnotobiotic[8] isolators) are available for rodents and certain species (e.g. zebrafish). For species-specific housing and enrichment requirements we refer to Appendix A of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Council of Europe, 2006).

[7] The animal room should be sealable to ensure air tightness and allow decontamination with a gaseous disinfectant

[8] Gnotobiotic: germ-free animals or formerly germ-free animals in which the composition of any associated microbial flora, if present, is fully defined (Stedman's Electronic Medical Dictionary 2006. Lippincott Williams & Wilkins).

Because the bedding material may generate a lot of dust and aerosols that contain faecal residues and certain allergens, the air flow should be directed away from the personnel and towards the back of the cages. If airborne pathogens are being used to experimentally infect laboratory animals, the animals should be housed in HEPA filtered isolators in order to prevent infection of other animals and of the personnel that is handling the animals. These systems require the use of aseptic handling techniques, and specialized cleaning, disinfecting, or sterilization regimens to prevent microbial transmission.

Generally, it is advised to have separate rooms for separate manipulations, e.g. one room for animal housing and another room for animal handling. Also animal experiments with different biological agents should be conducted in different rooms to avoid cross contamination. All enclosures where the animals are housed should be labelled with information on the biological agent that is being used for infection, the date of exposure and the name and telephone number of the person responsible for the experiment. Also a biohazard sign should be present when working with experimentally infected animals.

For housing rodents, several types of cages exist:

Open cages

Open cages offer no specific protection, including against allergens. They can be used for animals which are not experimentally infected.

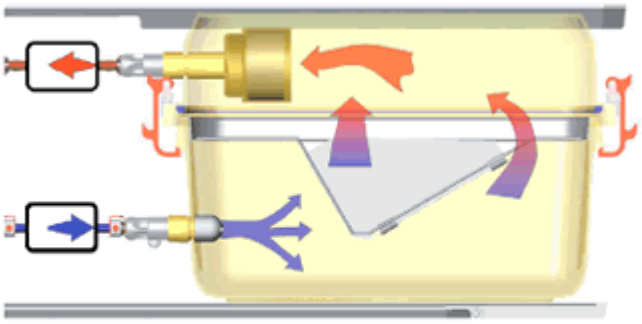
Filter top cages

Filter top cages (fig 1) are equipped with either HEPA or fine particle filters, but have no sealed joint between filter and cage, allowing passive air movement. Mainly they prevent dispersal of allergens. They merely offer protection for the animal, and only a limited protection for the animal care taker. These should not be used when there is a potential risk of shedding of the pathogen. They can be used for in house transport but they are not suitable for long term housing since moist, heat, ammonia, and carbon dioxide accumulates within the cages.

***Figure 1:** Filter top cage (Courtesy of Tecniplast)*

Individually Ventilated Cages (IVCs)

Individually Ventilated Cages (fig 2) protect both the animals and the researcher and ensure a better microenvironment for the animal. To ensure absolute isolation, the IVC cage must be totally sealed by means of durable gasket material between lid and base and sealed air supply and exhaust ports. Ingoing and exhaust air ventilated through HEPA filters and a negative air pressure is created inside the cage (Sidelsky, 2007). Optimizing animal room space is achieved by stacking IVC cages in a biocontainment cage rack. In that case, all connections between HEPA filters and the air delivery system must be sealed to create a system with sealed cages and a sealed rack. This can be realised by self-sealing ports on both the cage and the rack allowing removal of the cage from the rack without breaking the containment. Biocontainment racks with HEPA filtered airflow and proper sealing as well as an alarm system and battery back-up should ensure biosafety at all times. The enclosure can either be ventilated using filtered room air or can be ventilated independently of the room. Exhaust air may be returned to the animal room although it is generally preferable to connect the rack exhaust system directly into the building's exhaust system. However, since there is no procedure for testing the integrity of the HEPA filter, it is still necessary to wear (personal protection equipment (PPE) (Summermatter, EBSA Conference Workshop, 2011). IVCs are for use in an A3/ABSL3 and are often used in an A2/ABSL2 as no pressure requirements will then be needed for the animal room itself. Nevertheless, the macro-environment should be ventilated sufficiently to address heat loads, particulates, odours, and waste gases released from these primary enclosures. Also, the IVC should only be opened under a Class I or II biosafety cabinet (BSC).



BCU Airflow: Illustrates the movement of cage air in a sealed system.

***Figure 2:* Individually Ventilated Cage (Courtesy of Michael Sidelsky, RLATG)**

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***Figure 3:* isolator**

Isolators (fig 3) form an absolute barrier. They are comparable to Class III BSC as they are sealed boxes equipped with gloves authorizing manipulations inside. A pressure control system ensures a constant negative or positive pressure (for housing SPF- or germ free animals) and both ingoing and outgoing air is HEPA filtered. The pressure is monitored and alarmed. The enclosure can be decontaminated by means of a gaseous disinfectant. The isolator is equipped with a disinfection unit for in and out transfer of material and animals (e.g. a reservoir with an appropriate liquid disinfectant). Some types can also directly be connected to an autoclave or a BSC. The weak point in the system is the transfer of animals and material in and out the isolator. Special devices are available to bring products in and out the isolator without cross contamination: a bio-decontamination hatch, a bag-in/bag-out system and a Rapid Transfer Port. The bio-decontamination hatch is in fact an airlock for in and out transfer of materials which can be surface decontaminated with a vapour agent. The bag-in/bag-out system is used for handling toxic material in nuclear and pharmaceutical industries. It's not suitable for work with animals.

The Rapid Transfer Port is the preferred system (see fig 4). The system relies on a double door system, with a door (alpha unit) on the isolator and another door (beta unit) on the container transporting or receiving the item to be transferred. When docked, the two doors get attached and sealed together, entrapping the exposed surfaces of both doors. When the interlocked doors are opened, the entrapped air volume does not come in contact with the inside of the isolator. After transfer of material and closing of the doors, that volume is free of contamination, and the container can be safely disconnected and transferred to a BSC or sent to decontamination (Cloué *et al*, 2008).

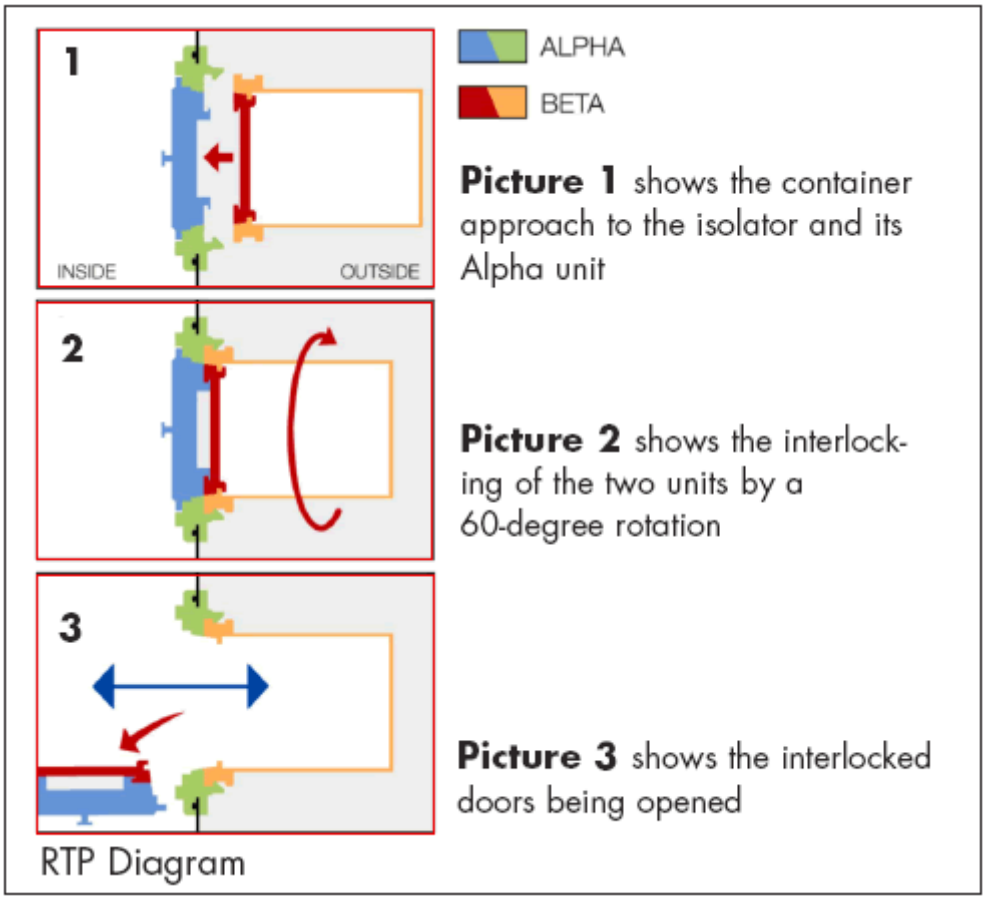

***Figure 4:* Rapid Transfer Port system (Courtesy of Randy Kray, AIA)**

Traffic patterns

Facilities should be efficiently designed to allow several one-way flows. Traffic should be always from the clean to the dirty area. Also different flows need to be considered:

- personnel flow;
- animal - material - feed flow;
- waste flow;
- animal carcass flow.

Adopting unidirectional traffic patterns within the animal facility minimises the risk of spread of pathogens and allergens in and around the facility and prevent cross contamination. This aspect should be taken into account in the animal facility design. One should be aware of the order of rooms one enters throughout the day, thereby always entering via the clean area and leaving via the dirty area. In particular SPF units would impose stringent procedures and control of the health status of the animals, and especially when new animal strains are acquired.

Animal transport

Internal transport (on-site)

Depending whether the animals are infected or not, different measures should be considered to ensure biosafety when animals are transported within the facility. A suitable transport box with the necessary biosafety requirements such as filters, seals, etc. should be used and an inspection window should be present to check the animals. When transporting infected animals, the transport box should be labelled with the biohazard sign.

External transport (by road, rail, shipping and air)

External transport of animals is done in compliance with the concerned national and international transport[9] regulations (Europe: Council of Europe Convention ETS 193 on the Protection of Animals during International Transport. International: IATA Live Animal Regulation).

Inventory monitoring and control

Inside the animal facility, a register must be kept of all housed animals and ongoing animal experiments, including also animal in and out transfer. This is currently realised by means of animal identification cards to detailed computerized records for individual animals (Field *et al*. 2007). Animal identification includes information on room, rack, pen, stall, and cage. Identification cards should include the source of the animal, the strain or stock, names and contact information for the responsible investigator(s), pertinent dates (e.g., arrival date, birth date, etc.), and protocol number when applicable. Genotype information, when applicable, should also be included.

[9] International transport means any movement from one country to another, but excludes, however, journeys of less than 50 km and movements between member States of the European Community (European Convention for the Protection of Animals during International Transport (Revised)).

In addition, the animals may wear collars, be marked by coloured stains, ear notches/punches and tags, tattoos, subcutaneous transponders, etc….

Biosafety equipment

Manipulations performed on laboratory animals that may create infectious aerosols (e.g. inoculations, necropsies, cage changing), should be conducted in a **Class II BSC**. After the examinations, appropriate disinfectants should be used to disinfect all instruments and working surfaces that have been in contact with animal tissues. But for easy handling of cages in a BSC, often the front window need to be opened quite far and the BSC losses his protective function. So, a BSC for dual use (as BSC and cage changing station) with a height adjustable working surface is advised. Alternatively, it might be more convenient to work in an animal containment workstation (cage change station) provided appropriate PPE is worn. If used for a contained use activity of risk class 2, proof of retention of aerosols emission has to be demonstrated (e.g. KI discus test). If used for a contained use activity of risk class 1, aerosol emission must be minimal, as demonstrated by the allergen containment test (Statement of the Central Committee for Biological Safety ZKBS, 2009).

There are 2 types of **animal containment workstations**, a single sided animal containment workstation (fig. 5) and a dual access animal containment workstation (fig. 6).
Both animal containment workstations provide operator, animal, and laboratory environment protection against allergens during animal handling. The usage of ULPA (Ultra Low Particulate Air) or HEPA filters to filter the downflow air can also filter out bio-hazardous agents. In this system, inflow air is pulled through the perforations at the front grille and travels through a return path toward the common air plenum at the top of the cabinet. A portion of air in the common plenum is exhausted through the filter to the room. The remaining air is passed through the downflow filter and into the work area. Near the work surface, the filtered downflow air stream splits with a portion moving toward the front air grille, and the remainder moving to the rear air grille. The combination of inflow and downflow air form an air barrier that prevents room air from entering the work zone, and (contaminated) air inside the cabinet from escaping the work zone.

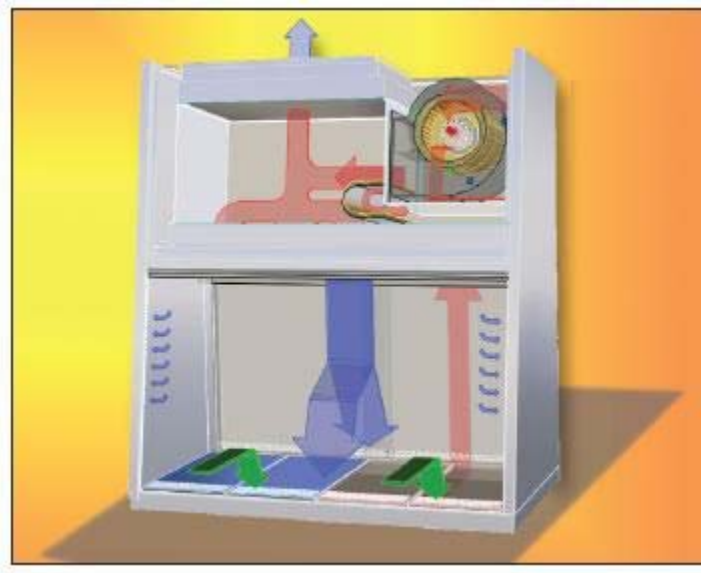

***Figure 5:** single sided animal containment workstation*
Blue arrows: HEPA filtered air
Red arrows: contaminated air
Green arrows: room air
(Courtesy of XQ Lin, Esco)

The large work area and ease of access makes a dual access animal containment workstation suited for handling small to medium sized laboratory animals. It usually employs three filters. The first one - usually an ULPA or HEPA filter - is located in the top portion of the workstation, on top of the first blower. It filters out the intake air from the laboratory environment. The second one – an activated carbon filter for removing odours - is located in the bottom portion of the workstation, on top of the second blower. It filters out the air pulled from the work zone. The third filter, also an ULPA or a HEPA filter, is located under the second blower and ensures the exhaust air is free of contaminants.

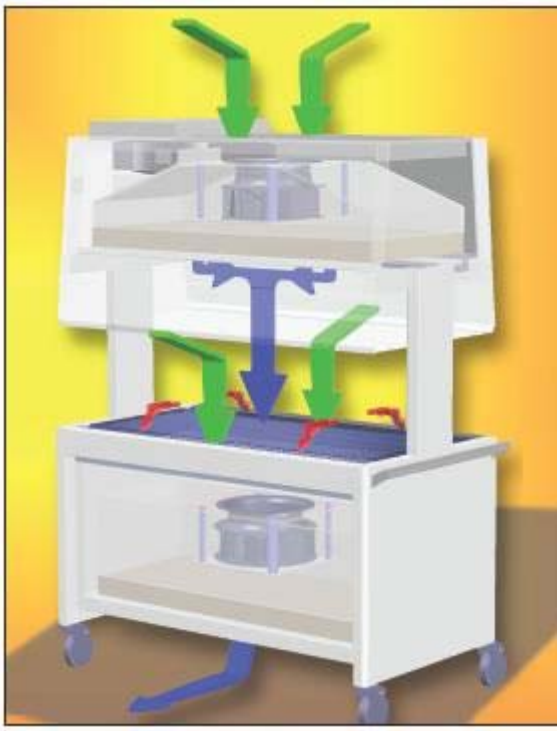

Figure 6: ***dual access animal containment workstation***
Blue arrows: HEPA filtered air
Red arrows: contaminated air
Green arrows: room air
(Courtesy of XQ Lin, Esco)

Specifically designed **animal bedding disposal workstations** (fig. 7) can be used for cage cleaning and bedding disposal. The airflow pattern of this type of workstations is comparable to that of a class I BSC since it provides protection of the operator and the environment, but not of the product. It typically uses two filters: usually a HEPA or ULPA filter and an activated carbon filter. Both are situated at the top of the workstation and ensure exhaust air is free from contaminants and odour. This type of workstation is also equipped with an integrated waste container to enables direct disposal of waste items within the work zone. However this type of work stations should only be used to protect the worker against allergens. Infected cages and bedding should be autoclaved before cleaning (see below). Also, since this type of workstations offers no product protection, it should only be used for cage cleaning and bedding disposal procedures and not for cage changing procedures or any other animal research activity (XQ, 2010).

Figure 7: ***animal bedding disposal workstations***
Blue arrows: HEPA filtered air
Red arrows: contaminated air
Green arrows: room air
(Courtesy of XQ Lin, Esco)

In some cases it is advisory to have an **autoclave** available, preferably a pass-through autoclave so that contaminated material can enter the autoclave at the 'dirty' side and leave the autoclave at the 'clean' side after sterilisation. The process need to be validated, preferentially by means of an appropriate bio-indicator (e.g. commercially available *Geobacillus stearothermophilus* spores) (A. Leunda *et al*, 2011).
The autoclave should be large enough for cages. Cages are piled up, put in special autoclave bags with built–in pores, the bags are closed and disinfected on the outside before transport to the autoclave. Cages can be autoclaved with faeces and bedding before washing (Note that cages need high resistance to heat and alkaline or acid detergents and that wood chip bedding can attack polycarbonate cages (Thomas, 2005). For this type of waste an autoclave cycle of 60' at 121°C would be recommended. Careful monitoring of temperature to ensure the required temperature is reached in centre of cage.

Personal Protection

Personnel working in animal facilities should be provided with clearly defined procedures and wear **Personnel Protective Equipment (PPE).** Depending on the risk assessment this may include the following:

- a lab coat or work suit;
- gloves;
- goggles;
- mouth mask;

- face shield;
- respiratory protection (e.g FFP3 mask);
- head cover, shoe covers,
- any other PPE required after a risk analysis.

The correct use of PPE should be ensured by proper training of the personnel wearing it. For example, if respiratory protection is necessary to protect against airborne pathogens or infectious aerosols, respiratory fit testing as well as training in the proper use and maintenance of the respirator is required (Pauwels *et al*, 2007). Note that personnel required to use respiratory protection may also require medical evaluation to ensure that they are physically and psychologically able to use the respirator properly.

As already mentioned in the risk assessment section, a distinction must be made between micro-organisms only pathogenic to animals and zoonotic pathogens. Since the former represent only a risk to the environment (including animals), PPE might be limited to a laboratory coat and gloves to prevent cross-contamination. However, since the lab worker can act as carrier, a shower might be required when leaving the containment area to prevent accidental spread in the environment. This is certainly the case for high containment animal facilities where experiments with animal pathogens of risk class 4 are conducted. Hence it is essential that personnel are well informed of the hazards and understand the proper selection and use of PPE.

Good personal hygiene is essential and will also reduce possible laboratory acquired infection and cross contamination. Hand washing basins should be present in the facility and personnel should wash and disinfect their hands as often as possible and also when leaving the animal room at the end of the activities.

In the frame of occupational health and safety, following **preventive actions** could be undertaken: pre-exposure immunization should be offered to people working with specific agents such as rabies virus (e.g., if working with species at risk for infection) or hepatitis B virus (e.g., if working with human blood or human tissues, cell lines, or stocks). It is also recommended to immunize animal care personnel against tetanus (NRC 1997). According to Directive 2000/54/EC (EC, 2000) vaccination is recommended when working with pathogens for which effective vaccines are available. Serum collection prior to exposure might be advisable in specific circumstances. In that case, the purpose for which the serum samples will be used must be in accordance with the concerned legislative requirements.
Finally, as laboratory animal allergy became an important issue for animal care personnel, early recognition and reporting (eventually of pre-existing allergies), and preventive control measures should be installed to mitigate health problems.

Training and Education

All personnel involved with the care and use of animals should receive a proper training in biosafety and a biosafety manual should be present in the facility. This includes not only the animal care takers and technicians, but also the researchers and visiting scientists.

The biosafety officer (person in charge or coordinating biosafety in a facility) and biosafety committee (as required in the Belgian legislation) of the institution are responsible for organizing training sessions and supervising the appropriate implementation of biosafety and biosecurity measures.

Personnel should be informed about the unique hazards present in the animal facility (including zoonoses and unusual risks such as those linked to the use of human tissue in immunocompromised animals). They should be provided with clearly defined procedures on work practices, personal protective equipment and waste management. To avoid pricking and biting accidents, personnel should be trained in handling and restraining the animals in a proper way. Incorrect handling of the animals will result in increased stress and injuries, eventually leading to laboratory acquired Infections. Restraining can either be done manually or by using plastic devices. Protocols on the handling and restraining of laboratory animals are available. (Donovan and Brown, 1995)

With respect to procedures for accidents employees should be aware of the importance of accident reporting as a means of 'lessons to learn': underreporting is a big problem and is often due to a perception of punishment as a consequence of the accident. As general rule, safety depends on trained personnel who rigorously follow the required biosafety measures. Every institution should implement a 'culture of safety' to ensure biosafety is integral part of the daily work and (research) goals (Pritt *et al*, 2007).

Decontamination and waste management

Room decontamination

Decontamination of the entire animal holding room by fumigation is required for an ABSL3 animal room in case of contamination, changes in usage, renovations or maintenance shutdowns (BMBL, 2009). This might also be used for animal rooms where large animals are kept, since the room itself constitutes the primary containment. Formaldehyde, vaporized hydrogen peroxide (VPH) or chlorine dioxide are effective compounds for room decontamination particularly following completion of studies with highly infectious agents or contamination with adventitious microbial agents (VPH is effective against anaerobic bacteria, enveloped and non-enveloped viruses, fungi and spores, also against *M. tuberculosis*) (Krause *et al*, 2001). The process needs to be validated, preferentially by means of an appropriate bio-indicator.

Waste treatment and disposal

In an animal facility, different types of waste are generated: solid waste such as bedding and faeces, liquid waste, manure and animal carcasses. Depending of the type of experiment and containment level, waste is treated in different ways. Also, appropriate procedures should exist for on-site packaging, labelling and storage of these wastes.

Generally speaking, waste from non-infected laboratory animals and transgenic animals can be treated as non-hazardous waste. A comparable situation is met when animals are subcutaneously grafted with cells (e.g. tumor cells). Carcasses, bedding and faeces can be collected and stored to be removed as non hazardous material for appropriate disposal in compliance with the legislative requirements concerning waste management (transport is achieved according to ADR regulation).

In contrast to that, waste from animals (experimentally) infected with pathogens requires inactivation and is treated as hazardous material. Inactivation is carried out via autoclaving, chemical treatment or incineration and the method needs to be validated. In a few cases, manure ensiling (storage in a dungpit) at elevated temperatures (50°C) for a certain time period can accelerate the inactivation process of eggs or cysts from certain intestinal parasites (protozoa) in faeces (Olson *et al*, 2004, Caballero-Hernandez *et al*, 2004). All wastes are collected in leak-proof, labelled (biohazard sign)

containers for hazardous waste equipped with tight-fitting lids for appropriate disposal in compliance with the legislative requirements concerning waste management. An exception can be made for bedding and faeces from animals infected with a pathogen that is not shed (e.g. certain intracellular parasites, for example alcelaphine herpesvirus 1 infecting lymphocytes of cattle).

Facilities with high numbers of animals may require centralized wastewater treatment systems: the use of laboratory animals - especially large animals – can generate huge quantities of fluids from the animals itself and wastewater from sinks, pipes, showers.

When selecting technologies for treating liquid animal wastes, issues such as waste type and waste quantity should be considered. Liquid animal wastes may include liquid manure, urine, blood, and other necropsy wastes and water from wash-down procedures. Other issues include the type of infectious agents as they may be resistant to chemical disinfectants. It's important to note that solid organic particles in wastewater can interfere with the effectiveness of both chemical inactivation and heat sterilization. Solids can react with chemical disinfectants and reduce their biocidal properties. Heat treatment is the most appropriate method for the sterilization[10] of wastewater that contains high levels of solids (Schultz, 2004). Wastewater treatment systems usually consist of one or more tanks where batches of wastewater are heated under temperatures and pressures typical of autoclaves. A liquid waste decontamination system should consist of at least one reactor or sterilizer plus a holding tank. An ideal system would consist of two or more sterilizers to provide redundancy. Heat treatment systems can be equipped with particle size reduction devices, storage tanks, and heat recovery systems. Autoclaves may be used for treating small quantities of liquid wastes generated in laboratories or small animal rooms.

Infectious **animal carcasses** can be processed in different ways. Carcasses of small animals are collected in biohazard bags, stored in a freezer and collected by a licensed contractor incineration. Autoclaving is not indicated because this would take too long to reach the required temperature in the centre of the animal. Large animal carcasses can be chopped in a shredder and autoclaved in a tissue autoclave providing shredding occurs in a closed system to avoid dispersal of infectious aerosols. They can also be treated by means of alkaline hydrolysis. This process involves the dissolving of animal tissue under conditions of high temperature, pressure and pH. When process is complete, a sterile hydrolysate is produced, consisting of sugars, amino acids and soaps. Only calcium based bone fragments and undigested cellulose are left in the reaction vessel. This system offers the advantage of disposing of carcasses in an environmental responsible way. Moreover, it has also been shown to effectively inactivate a great number of biological agents, including those causing transmissible spongiform encephalitis (TSE), (Richmond *et al*, 2003).

More information on biological waste treatment and inactivation methods can also be found on the Belgian Biosafety Server at the following address: http://www.biosafety.be/CU/EN/Tools_RA_RM.html.

Emergency plans

In the event of an emergency, institutional security personnel and fire brigade or police officials should be able to reach people responsible for the animals. Notification can be enhanced by prominently posting emergency procedures, names, or telephone numbers in animal facilities or by placing them in

[10] Sterilization is the use of a physical or chemical procedure to destroy all microorganisms including large numbers of bacterial spores.

the security department or telephone centre. Emergency procedures for handling special facilities or operations should be clearly posted and personnel trained in emergency procedures for these areas. A disaster plan that takes into account both personnel and animals should be prepared as part of the overall safety plan for the animal facility. The facility manager or veterinarian responsible for the animals should be members of the appropriate biosafety committee at the institution, and participate in the response to a disaster (Institute for Laboratory Animal Research. NRC, 2011).

Biosecurity

In comparison to biosafety, aiming at preventing accidental exposure to or release of biological agents within or from the laboratory, biosecurity aims at protecting biological agents from all kind of unauthorized intrusion into and intentional release from the laboratory (Rhodes, 2009).
Bioterrorism attacks with *Bacillus anthracis* spores in the US, in laboratory construction of pathogens (e.g. poliovirus and the influenza strain responsible for the Spanish flu), have revived the fear for use or development of biological agents for criminal purposes. As a consequence the United Nations[11] and Europe undertook initiatives to reduce these risks and introduced the concept of "biosecurity".
The World Health Organisation (WHO) defines biosecurity as 'the protection, control and accountability for valuable biological materials (VBM)[12] within laboratories, in order to prevent their unauthorized access, loss, theft, misuse[13], diversion or intentional release' (WHO. Laboratory Biosecurity Guidance, 2006).
Biosecurity has several points in common with Biosafety. Together with biosafety it is an integral part of Laboratory Biorisk Management Standard (CEN workshop Agreement (CWA) 15793: 2011[14]) outlining the requirements for managing risks associated with handling, storage and disposal of infectious biological material and toxins.
With regard to biosecurity in animal facilities, the first thing to do is to provide access control. Restricted access is already a standard biosafety measure to all animal facilities regardless of their containment level.
For an animal facility harbouring different units with different containment levels, the size of the facility and the nature of the activities carried out within will define the type of security systems needed. Security and access control would then be organized in zones, starting from the perimeter to individual animal rooms. Control measures may consist of security personnel, physical barriers, and control devices. Typically, electronic key cards and associated readers are used which control access, enable recording of the time, location, and personal identification. If a high degree of security is needed, thumb or palm readers or retinal scanners may be more suitable because key cards can be shared. Eventually electronic and video surveillance systems can be put in place to enhance security even more. (Institute for Laboratory Animal Research. NRC, 2011).

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[11] The United Nations resolution 1540 of 28 April 2004 concerning non-proliferation of nuclear, chemical and biological weapons
[12] Biological materials that require (according to their owners, users, custodians, caretakers or regulators) administrative oversight, control, accountability, and specific protective and monitoring measures in laboratories to protect their economic and historical (archival) value, and/or the population from their potential to cause harm. VBM may include pathogens and toxins, as well as non-pathogenic organisms, vaccine strains, foods, genetically modified organisms (GMOs), cell components, genetic elements, and extraterrestrial samples
[13] The misuse of valuable biological materials describes their inappropriate or illegitimate use, despite existing and subscribed agreements, treaties and conventions
[14] Available at http://www.cen.eu/CEN/sectors/technicalcommitteesworkshops/workshops/Pages/ws31.aspx

REFERENCES

Aoyama K., Ueda A., Manda F. Matsushita ., Ueda T., Yamauchi C. SO. Allergy to Laboratory Animals - An Epidemiologic study. Br J Ind Med 1992; 49: 41–47.

Alderman T. S., Frothingham R. and Sempowski G. D. Validation of an Animal Isolation Imaging Chamber for Use in Animal Biosafety Level-3 Containment. Applied Biosafety 2010;15 (2): 62-66.

Bush R. K., Wood R. A. and Eggleston P. A. Laboratory animal allergy. J Allergy Clin Immunol 1998;102: 99–112.

Caballero-Hernandez A. I., Castrejon-Pineda F., Martınez-Gamba R., Angeles-Campos S., Pérez-Rojas M., Buntinx S. E. Survival and viability of *Ascaris suum* and *Oesophagostomum dentatum* in ensiled swine faeces. Bioresource Technology 2004; 94: 137–142.

Cloué P., Apolinar R., Kray R. Isolation Technology as Applied to Lab Animal Research. Animal Lab News November 2008
Accessed online: August 2011
http://www.alnmag.com

Commissie Genetische Modificatie (NL) 2006, CGM/060516-01. Advies bioluminiscentie-experimenten met adenovirale vectoren (in Dutch).
Accessed on line: August 2011
http://www.cogem.net/index.cfm/nl/publicaties/publicatie/bioluminescentie-experimenten-met-knaagdieren-geinjecteerd-met-adenovirale-vectoren

Conner DA. Transgenic mouse colony management. Curr Protoc Mol Biol 2005; 23.10.1-23.10.8, suppl 71.

Department of Health and Human Services, Centers for Disease Control and Prevention & National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories 5th edition, 2009. Section V-Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities.

Donahue W.A., VanGundy D. N., Satterfield W. C., Coghlan L. J. Solving a tough problem. Pest Control 1989; 57:46-50.

Donovan J. and Brown P. Care and Handling of Laboratory Animals. Handling and Restraint. In: Current Protocols in Immunology1995; Supplement 14: 1.3.1-1.3.5 John Wiley & Sons.

Council of Europe. European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS NO. 123). Revised Appendix A. Guidelines for accommodation and care of animals (article 5 of the Convention) 2006.

EC (2000). Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. Official Journal L 262, 17.10.2000, p. 21).

EC (2009). Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. Official Journal L 125, 21.05.2009, p.75

Field K., Bailey M., Foresman L.L., Harris R. L., Motzel S. L., Rockar R. A., Ruble G., Suckow M. A. Medical records for animals used in research, teaching and testing: Public statement from the American College of Laboratory Animal Medicine. ILAR J 2007; 48:37-41.

Garg R. C., Donahue W. A. Pharmacologic profile of methoprene, an insect growth regulator, in cattle, dogs, and cats. JAVMA 1989; 194:410-412.

Haybaeck J., Heikenwalder M., Klevenz Petra Schwarz B., Margalith I., Bridel C., Mertz K., Zirdum E., Petsch B., Fuchs T. J., Stitz L., Aguzzi A.
Aerosols Transmit Prions to Immunocompetent and Immunodeficient Mice
PloSPathogens. 2011;7(1): e1001257.
http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1001257

Institute for Laboratory Animal Research, National Research Council. Guide for the Care and Use of Laboratory Animals, 8th edition (National Academy Press, Washington DC, 2011).
Accessed online: August 2011
http://oacu.od.nih.gov/regs/guide/guide.pdf

Johnson B., Animal bytes. Applied Biosafety 2011; 16 (2): 118-121.
Accessed online: August 2011
http://www.absa.org/

King J. E., Bennett G. W. Comparative activity of fenoxycarb and hydroprene in sterilizing the German cockroach (Dictyoptera: Blattellidae). J Econ Entomol 1989; 82: 833-838.

Krause J., McDonnell G., Riedesel H. Biodecontamination of animal rooms and heat-sensitive equipment with vaporized hydrogen peroxide. Contemp Top Lab Anim Sci 2001; 40: 8-21.

Leunda A., Do Thi C. D., Brosius B., Willemarck N., Verheust C., Van Vaerenbergh B. Modaliteiten van validatie en controle van autoclaven in het kader van inactivatie van afval afkomstig van het ingeperkte gebruik van genetisch gemodificeerde micro-organismen en/of pathogenen. Legal Depot 2011. D/2011/2505/46
http://www.biosafety.be/CU/PDF/Autoclaaf_SBB_D2011_2505_46_NL.pdf

Meuleman P., Libbrecht L., De Vos R., de Hemptinne B., Gevaert K., Vandekerckhove J. , Roskams T., Leroux-Roels G. Morphological and Biochemical Characterization of a Human Liver in a uPA-SCID Mouse Chimera. Hepatology 2005; 41 (4): 847-856.

NRC. 1997. Occupational Health and Safety in the Care and Use of Research Animals. Washington: National Academy Press.

Occupational Health & Safety Unit. Health Surveillance & Management of Laboratory Animal Allergy and Asthma , 2007, reviewed 2009.

Olson, M. E., O'Handley, R. M., Ralston, B. J., McAllister, T. A., Thompson, R. C. A.
Update on *Cryptosporidium* and *Giardia* Infections in Cattle. Trends
Parasitol 2004; 20: 185–191.

Pauwels K., Gijsbers R., Toelen J., Schambach A., Willard-Gallo K.,
Verheust C., Debyser Z., Herman P. State-of-the-Art Lentiviral Vectors for Research Use: Risk Assessment and Biosafety Recommendations. Current Gene Therapy 2009; 9*:* 459-474.
http://benthamscience.com/cgt/openaccessarticles/cgt9-6/0002Q.pdf

Pauwels K., Coppens F., Verheust C., Van Vaerenbergh B., Do ThI C. D., Herman P.
Gebruik van ademhalingsbeschermingsmiddelen bij het ingeperkt gebruik van genetisch gemodificeerde organismen en/of pathogenen. Legal Depot 2007. D2007/2505/64.
http://www.biosafety.be/CU/PDF/Ademhalingsbescherm_WIV_07_2505_64.pdf

Pritt S., Hankenson F. C., Wagner T., Tate M. The basics of animal biosafety and biocontainment training. Lab Animal 2007; 36 (6): 31-38.

Rhodes C. Consequences of Failure to Apply International Standards for Laboratory Biosafety and Biosecurity: The 2007 Foot-and-Mouth Disease Outbreak in the UK. Applied Biosafety 2009; 14 (3): 144-149.

Richmond J. Y., Hill R. H., Weyant R.S., Nesby-O'Dell S.I. and Vinson P.E. ILAR Journal 2003; 44 (1): 20-27.

Schultz, C. C. Liquid Waste Decontamination Systems. Animal Lab News January/February 2004.
Accessed online: August 2011
http://www.alnmag.com

Stedman's Electronic Medical Dictionary 2006. Lippincott Williams & Wilkins.
http://online.statref.com/TitleInfo/fxid-8.html

Sidelsky M. Laboratory Biosafety in Rodent Biocontainment. Animal Lab News March 2007.
Accessed online: August 2011
http://www.alnmag.com

Stitz L. and Aguzzi A. Aerosols. An underestimated vehicle for transmission of prion diseases? Landes Bioscience 2011; 5 (3): 1-4.

Summermatter K. and Mertsching J. Workshop Biosafety in laboratory animal facilities. 14th Annual Conference of the European BioSafety Association, Estoril, Portugal, 13-15 April 2011.
http://www.ebsaweb.eu/Events/Past+EBSA+Conferences/14th+EBSA+Conference+2011/Pre_Conference+Workshops.html#E

The Universities Federation for Animal Welfare. The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals, 8th edition.
Eds Robert Hubrecht & James Kirkwood (Wiley-Blackwell, 2010).
Accessed online: August 2011
http://eu.wiley.com/WileyCDA/WileyTitle/productCd-1405175230,descCd-google_preview.html

Thomas S. The ABCs of IVCs. Animal Lab News November/December 2005.
Accessed online: August 2011
http://www.alnmag.com

Van Vaerenbergh B., Koenen F., Pauwels K., Quanten K., Boyen F., Declercq K., Desmecht D., Thiry J., Herman P. Methodology of the biological risk classification of animal pathogens in Belgium. Scientific and Technical Review of the OIE 2010; 29 (3): 513-522.
http://web.oie.int/boutique/index.php?page=ficprod&id_prec=813&id_produit=1009&lang=en&fichrech=1&PHPSESSID=082c46f6265deb48704f2f4b88253143

Verma R. K. Advances on cockroach control. Asian J Microbiol, Biotech Environ Sci 2002; 4: 245-249.

Virginia Department of Health. Non-human primates 2008.
Assessed online : August 2011
http://www.vdh.state.va.us/epidemiology/DEE/otherzoonosis/nonhumanprimates.htm

WHO (2006). Biorisk management: Laboratory biosecurity guidance. Geneva: WHO.
Accessed online: December 2011
www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6.pdf

XQ L. Animal Containment Workstations: Minimising Cage Change Hazards. Animal Lab News World March/April 2010.
Accessed online: August 2011
http://www.alnmag.com

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ANNEX

Checklist for laboratory animal facilities

Animal biosafety level 1 (A1)

Date of inspection	
Goal of inspection	☐ routine inspection ☐ inspection after an incident ☐ ……
Institution	
Address	
Person in charge	

Part A: Facility design

Part A.1: Requirements

	Criteria	OK	not OK	Remarks
1	Entrance doors can be locked	☐	☐	
2	Hand-washing basins are available	☐	☐	
3	Coat-hooks or changing rooms for protective clothing are available	☐	☐	
4	Cages and work surfaces are impermeable, easy to clean and disinfectant-proof	☐	☐	
5	Cleaning area for cages	☐	☐	

Part A.2: Recommendations[1]

	Criteria	OK	not OK	Remarks
1	Facility measures to avoid accidental escape of the animals (e.g. partitions,…)	☐	☐	
2	Viewing window or equivalent system to check the presence of people in the facility	☐	☐	
3	Separate room for storage of clean cages, feed and litter	☐	☐	

Part A.3: Optional measures[2]

	Criteria	OK	not OK	Remarks
1	Ventilation	☐	☐	

Part B: Biosafety equipment

Part B.1: Requirements

	Criteria	OK	not OK	Remarks
1	An autoclave is available on site	☐	☐	

[1] To apply as a general rule unless the public health and the environment cannot be affected; to be specified in the Biosafety dossier and in the authorization delivered by the competent authority
[2] To apply case by case in function of the risk assessment ; to be specified in the Biosafety dossier and in the authorization delivered by the competent authority

Part B.2: Recommendations: /

Part B.3: Optional measures

	Criteria	OK	not OK	Remarks
1	Animals are housed in cages or equivalent containments (e.g. fenced area, aquarium,…)	☐	☐	

Part C: Working practices

Part C.1: Requirements

	Criteria	OK	not OK	Remarks
1	Restricted entrance	☐	☐	
2	Marked on the entrance door: Containment level Biological risk Name and phone number of the person in charge Entrance list of allowed people Entrance criteria	 ☐ ☐ ☐ ☐ ☐	 ☐ ☐ ☐ ☐ ☐	
3	Protective clothing, specifically for animal biosafety level A1	☐	☐	
4	Mitigation of splashes and aerosols	☐	☐	
5	Mechanical pipetting technique	☐	☐	
6	Forbidden to drink, eat or smoke, forbidden to apply cosmetics or contact lenses and to store food for human consumption	☐	☐	
7	Registration of all manipulations (import and export of laboratory animals, inoculation of GMOs,…)	☐	☐	
8	Check up of control measures and safety equipment	☐	☐	

9	Memo with user's guide of effective disinfectants	☐	☐	
10	Training of personnel	☐	☐	
11	Written instructions of biosafety procedures	☐	☐	
12	Isolation of laboratory animals during experimentations	☐	☐	

Part C.2: Recommendations

	Criteria	**OK**	**not OK**	**Remarks**
1	Effective vector control (e.g. for the detection of insects and rodents)	☐	☐	

Part C.3: Optional measures

	Criteria	**OK**	**not OK**	**Remarks**
1	Gloves	☐	☐	

Part D: Waste management

Part D.1: Requirements

	Criteria	**OK**	**not OK**	**Remarks**
1	Validated inactivation of biological waste and/or biological residues (contaminated cadavers, faeces, litter,…) according to an appropriate method before removal	☐	☐	
2	Validated inactivation of contaminated material (glass, cages, …) according to an appropriate method before cleaning, re-usage or destruction	☐	☐	

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Checklist for laboratory animal facilities

Animal biosafety level 2 (A2)

Date of inspection	
Goal of inspection	☐ routine inspection ☐ inspection after an incident ☐ ……
Institution	
Address	
Person in charge	

Part A: Facility design

Part A.1: Requirements

	Criteria	OK	not OK	Remarks
1	The laboratory animal facility is separated from other working areas in the same building or is situated in a separate building	☐	☐	
2	Entrance doors can be locked	☐	☐	
3	Entrance doors are self-closing	☐	☐	
4	Facility measures to avoid accidental escape of the animals (e.g. partitions,…)	☐	☐	
5	Hand-washing basins are available	☐	☐	
6	Coat-hooks or changing rooms for protective clothing are available	☐	☐	
7	Separate room for storage of clean cages, feed and litter	☐	☐	

8	Cages, work surfaces and floor are impermeable, easy to clean and disinfectant-proof	☐	☐	
9	Cleaning area for cages	☐	☐	

Part A.2: Recommendations

	Criteria	OK	not OK	Remarks
1	Entrance by means of a lock	☐	☐	
2	Closed windows during experimentation	☐	☐	
3	Viewing window or equivalent system to check the presence of people in the facility	☐	☐	
4	Hands-free hand-washing basins	☐	☐	

Part A.3: Optional measures

	Criteria	OK	not OK	Remarks
1	Facility can be hermetically sealed for decontamination with a gas	☐	☐	
2	Air removal system is separated from surrounding areas	☐	☐	
3	Air supply and air removal systems are connected to avoid accidental overpressure	☐	☐	
4	Air supply and air removal systems can be closed by means of lids	☐	☐	
5	Lower pressure in the controlled area as compared to surrounding areas	☐	☐	
6	HEPA filtration of the air	☐	☐	
7	Ventilation	☐	☐	

Part B: Biosafety equipment

Part B.1: Requirements

	Criteria	OK	not OK	Remarks
1	An autoclave is available in the building	☐	☐	

Part B.2: Recommendations

	Criteria	OK	not OK	Remarks
1	Fumigation system or decontamination bath	☐	☐	

Part B.3: Optional measures

	Criteria	OK	not OK	Remarks
1	Biosafety cabinet (class I or II)	☐	☐	
2	Animals are housed in cages or equivalent containments (e.g. fenced area, aquarium,…)	☐	☐	
3	Isolators with HEPA filters	☐	☐	

Part C: Working practices

Part C.1: Requirements

	Criteria	OK	not OK	Remarks
1	Restricted entrance	☐	☐	

2	Marked on the entrance door: Biosafety sign Containment level Biological risk Name and phone number of the person in charge Entrance list of allowed people Entrance criteria	☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐	
3	Protective clothing, specifically for animal biosafety level A1	☐ ☐	
4	Mitigation of splashes and aerosols	☐ ☐	
5	Mechanical pipetting technique	☐ ☐	
6	Forbidden to drink, eat or smoke, forbidden to apply cosmetics or contact lenses and to store food for human consumption	☐ ☐	
7	Registration of all manipulations (import and export of laboratory animals, inoculation of GMOs,…)	☐ ☐	
8	Check up of control measures and safety equipment	☐ ☐	
9	Memo with user's guide of effective disinfectants	☐ ☐	
10	Training of personnel	☐ ☐	
11	Written instructions of biosafety procedures	☐ ☐	
12	Effective vector control (e.g. for the detection of insects and rodents)	☐ ☐	
13	Isolation of laboratory animals during experimentations in a separate room	☐ ☐	

Part C.2: Recommendations

	Criteria	**OK**	**not OK**	**Remarks**
1	The animal facility has its own specific equipment	☐	☐	

2	Gloves	☐	☐	
3	Specific measures (including equipment) to control splashes and aerosol spreading	☐	☐	
4	Disinfectants in the siphons	☐	☐	

Part C.3: Optional measures

	Criteria	**OK**	**not OK**	**Remarks**
1	Respiratory mask	☐	☐	
2	Facial protection (eyes, mucosa)	☐	☐	

Part D: Waste management

Part D.1: Requirements

	Criteria	**OK**	**not OK**	**Remarks**
1	Validated inactivation of biological waste and/or biological residues (contaminated cadavers, faeces, litter,…) according to an appropriate method before removal	☐	☐	
2	Validated inactivation of contaminated material (glass, cages, …) according to an appropriate method before cleaning, re-usage or destruction	☐	☐	

WETENSCHAPPELIJK INSTITUUT VOLKSGEZONDHEID
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Checklist for laboratory animal facilities

Animal biosafety level 3 (A3)

Date of inspection	
Goal of inspection	☐ routine inspection ☐ inspection after an incident ☐ ……
Institution	
Address	
Person in charge	

Part A: Facility design

Part A.1: Requirements

	Criteria	OK	not OK	Remarks
1	The laboratory animal facility is separated from other working areas in the same building or is situated in a separate building	☐	☐	
2	Entrance by means of a lock	☐	☐	
3	Entrance doors can be locked	☐	☐	
4	Entrance doors are self-closing	☐	☐	
5	Closed windows	☐	☐	
6	Facility can be hermetically sealed for decontamination with a gas	☐	☐	
7	Facility measures to avoid accidental escape of the animals (e.g. partitions,…)	☐	☐	
8	Viewing window or equivalent system to check the presence of people in the facility	☐	☐	

9	Hands-free hand-washing basins are available near the exit or in the lock	☐ ☐	
10	Coat-hooks or changing rooms for protective clothing are available	☐ ☐	
11	Separate room for storage of clean cages, feed and litter	☐ ☐	
12	Cages, work surfaces, floor, walls and ceiling are impermeable, easy to clean and disinfectant-proof	☐ ☐	
13	Cleaning area for cages	☐ ☐	
14	Fire alarm system	☐ ☐	
15	Interphone, telephone or any other system to guarantee communication outside the contained area	☐ ☐	
16	Air supply and air removal systems are connected to avoid accidental overpressure	☐ ☐	
17	Air supply and air removal systems can be closed by means of lids	☐ ☐	
18	Lower pressure in the controlled area as compared to surrounding areas (control and alarm systems)	☐ ☐	
19	HEPA filtration of the outgoing air	☐ ☐	
19	System allowing filters to be changed without introducing contaminations	☐ ☐	
20	Ventilation	☐ ☐	

Part A.2: Recommendations

	Criteria	OK	not OK	Remarks
1	Shower	☐	☐	
2	Supply tubes for liquids with flow back prevention	☐	☐	
3	Autonomous electrical system in case of failure	☐	☐	
4	Ventilation air supply system is separated from surrounding areas	☐	☐	
5	Air removal system is separated from surrounding areas	☐	☐	

Part A.3: Optional measures

	Criteria	OK	not OK	Remarks
1	HEPA filtered air can be re-used	☐	☐	

Part B: Biosafety equipment

Part B.1: Requirements

	Criteria	OK	not OK	Remarks
1	Isolators with HEPA filters	☐	☐	
2	An autoclave is available in the laboratory animal facility or in a neighbouring room	☐	☐	
3	Fumigation system or decontamination bath	☐	☐	

Part B.2: Recommendations

	Criteria	OK	not OK	Remarks
1	A pass-through autoclave	☐	☐	

Part B.3: Optional measures

	Criteria	OK	not OK	Remarks
1	Biosafety cabinet (class I or II)	☐	☐	
2	Animals are housed in cages or equivalent containments (e.g. fenced area, aquarium,…)	☐	☐	

Part C: Working practices

Part C.1: Requirements

	Criteria	OK	not OK	Remarks
1	Restricted and controlled entrance	☐	☐	
2	Marked on the entrance door:			
	Biosafety sign	☐	☐	
	Containment level	☐	☐	
	Biological risk	☐	☐	
	Name and phone number of the person in charge	☐	☐	
	Entrance list of allowed people	☐	☐	
	Entrance criteria	☐	☐	
3	The animal facility has its own specific equipment	☐	☐	
4	Protective clothing, specifically for animal biosafety level A1	☐	☐	

5	Decontamination of clothing before it leaves the contained area	☐ ☐	
6	Gloves	☐ ☐	
7	Prevention of splashes and aerosols	☐ ☐	
8	Specific measures (including equipment) to control splashes and aerosol spreading	☐ ☐	
9	Mechanical pipetting technique	☐ ☐	
10	Forbidden to drink, eat or smoke, forbidden to apply cosmetics or contact lenses and to store food for human consumption	☐ ☐	
11	Registration of all manipulations (import and export of laboratory animals, inoculation of GMOs,…)	☐ ☐	
12	Check up of control measures and safety equipment	☐ ☐	
13	Memo with user's guide of effective disinfectants	☐ ☐	
14	Disinfectants in the siphons	☐ ☐	
15	Training of personnel	☐ ☐	
16	Written instructions of biosafety procedures	☐ ☐	
17	Effective vector control (e.g. for the detection of insects and rodents)	☐ ☐	
18	Isolation of laboratory animals during experimentations in a separate room	☐ ☐	

Part C.2: Recommendations

	Criteria	**OK** **not OK**	**Remarks**
1	When zoopathogens are manipulated: no contact with host animals for a certain time	☐ ☐	

Part C.3: Optional measures

	Criteria	OK	not OK	Remarks
1	Appropriate shoes	☐	☐	
2	Respiratory mask	☐	☐	
3	Facial protection (eyes, mucosa)	☐	☐	

Part D: Waste management

Part D.1: Requirements

	Criteria	OK	not OK	Remarks
1	Validated inactivation of biological waste and/or biological residues (contaminated cadavers, faeces, litter,…) according to a suitable method before removal	☐	☐	
2	Validated inactivation of contaminated material (glass, cages, …) according to a suitable method before cleaning, re-usage or destruction	☐	☐	

Part D.2: Recommendations

	Criteria	OK	not OK	Remarks
1	Validated inactivation of sink and shower effluents according to an appropriate method before removal	☐	☐	

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Risk assessment form for laboratory animal facilities

Date	
Institution	
Address	
Person in charge	

Title activity	
Pathogenic agent(s)	
Genetically modified (micro-) organism(s)	
Description	
Risk class	2 ☐ 3 ☐ 4 ☐
Containment level	2 ☐ 3 ☐ 4 ☐
Licence of the competent authority	Reference: valid until:
Environmental permit	Reference: valid until:

Biological material		**Remarks**
Highly virulent strain(s)?	Yes ☐ No ☐	

What is the infectious dose?		
Stadium of the pathogen handled?		
Critical stages?		
Mode of transmission?		
Zoonosis?	Yes ☐ No ☐	
Natural host(s)?		
Prophylactics available? If yes, which?	Yes ☐ No ☐	
Therapeutics available? If yes, which?	Yes ☐ No ☐	

Laboratory animals	**Remarks**
Which animals?	
Animal status (conventional, SPF,…)?	

Personal protection equipment		**Remarks**
Which personal protection equipment is being used?		
Specific requirements? If yes, which?	Yes ☐ No ☐	

Biosafety equipment		**Remarks**
Biosafety cabinet needed? If yes, which class?	Yes ☐ No ☐	
Underpressure?	Yes ☐ No ☐	
Ventilation?	Yes ☐ No ☐	

Possibility of aerosol generation?	Yes ☐ No ☐	
Centrifugation step(s)?	Yes ☐ No ☐	
Use of sharps?	Yes ☐ No ☐	
Which disinfectants are used?		
Specific requirements? If yes, which?	Yes ☐ No ☐	
Working practices		**Remarks**
Route of experimental infection?		
Experimental infection dose?		
Which type of animal housing (IVC, FTC,…)?		
Restricted entrance?	Yes ☐ No ☐	
Biosafety training for the personnel?	Yes ☐ No ☐	
Occasional refreshment of the biosafety training?	Yes ☐ No ☐	
Specific risks?	Yes ☐ No ☐	
Specific requirements? If yes, which?	Yes ☐ No ☐	

Waste management	**Remarks**
How is solid waste treated?	
How is liquid waste treated?	
How is re-usable waste treated?	
How is animal waste treated?	
How are animals discharged?	