

BIOSAFETY IN LABORATORY ANIMAL FACILITIES

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/ INTRODUCTION

In Belgium, most activities involving animal experiments with transgenic and experimentally infected animals are subject to notification in the framework of regional legislation on contained use of genetically modified organisms (GMOs) and/or pathogens. Animal facilities should comply with the containment criteria and other protective measures defined in this legislation in order to guarantee optimal protection of public health, the animals and the environment. This paper addresses various concerns on facility design, biosafety equipment, personal protection, working practices and waste management. Quality aspects of animal care falling under laboratory animal welfare regulations are not considered here.

This report summarises the full report referred to at the end of this article (1).

/ RISK ASSESSMENT

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

A distinction must be made between animals infected with zoonotic agents and animals infected with microorganisms 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 only represent a risk for the environment (including animals). Hence the latter require biosafety measures that are different to those for zoonotic agents.

Unlike work with experimentally infected animals, work with transgenic animals does not present a major health risk for the staff, but merely a risk for the environment in case of accidental escape. Animals can also be inoculated with genetically modified microorganisms (GMMs), for example viral vectors, which may shed recombinant viral particles.

/ RISK MANAGEMENT

Based on biohazard identification, a risk assessment is performed and appropriate biosafety measures must be taken to control these hazards.

In the following part of this document, particular points concerning risk management in laboratory animal facilities are presented and several biosafety issues are discussed.

Facility design

Most laboratory animal facilities may include the following:

- security features to ensure access control
- animal housing areas (Figure 1)
- laboratories for specific manipulations (e.g. surgery, autopsy, experimental procedures, etc.)
- space for washing and sterilizing equipment
- 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 staff

As a general rule, unidirectional traffic flows are recommended within the animal facility (from clean to dirty areas) to minimise the risk of spread of pathogens and allergens in and around the facility and to prevent cross-contamination.

Animal facilities may host 'barrier facilities'. These are designed to ensure isolation and prevent accidental infection of animals of a defined health status (e.g. immune deficient rodents, Specific Pathogen Free animals). 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 and the supply air is filtered (e.g. HEPA or 95% efficient filters).

Dedicated clothing, personal protection equipment and strict operational procedures must also be followed to avoid contact between clean and soiled supplies and areas.

Special research techniques involving complex equipment and support space such as magnetic resonance imaging necessitate a common technical centre for imaging small animals, 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. To maintain the health of the animals during imaging experiments, which can last several weeks, a pathogen barrier must be maintained around the animals by means of specially designed imaging chambers. Animals infected with pathogens or viral vectors might also be used for *in vivo* imaging. In that case, special consideration must be given to biosafety, including housing, transportation to and from the site, careful decontamination of the equipment after use and occasionally personal protection equipment.

Facility construction

Animal facilities have particular requirements with respect to architectural aspects and building material. For safety, doors of animal housing rooms should open inwards, be self-closing and equipped with viewing windows. Locks or electronic security devices might be required depending on the containment level. Floors should be impervious to liquids and resistant to biological materials and chemicals. Walls and ceilings should be smooth, impervious to water and chemical detergents, non-absorbing and resistant to damage from impact. Utility penetrations (e.g. ducts, cables) in floors, walls and ceiling should be sealed to facilitate pest control and cleaning and improve airtightness in high containment facilities. A pest control program to control and eliminate flying and crawling insects and wild rodents should be available.

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, staff, lights, and equipment and adjusting the moisture content, it will dilute contaminants including allergens and airborne pathogens.

A properly designed and functioning heating, ventilation and air conditioning (HVAC) system is essential in controlling airborne contamination. In the case of high containment animal facilities (A3), a dedicated ventilation system is provided and designed to maintain a negative air pressure in the laboratory with respect to adjacent areas, and outgoing air is HEPA filtered.

Animal housing

Laboratory animals should be housed in appropriate biocontainment enclosures, such as cages, pens, runs, stalls, aquaria, etc.). Animals experimentally infected with airborne pathogens should be housed in HEPA filtered isolators or individually ventilated cages to prevent infection of other animals and staff.

All enclosures should be labelled with information on the biological agent that is being used for the experiment (including a biohazard sign when animals are infected with pathogens), and the name and telephone number of the person responsible for the experiment. A register must be kept of all housed animals and ongoing animal experiments, including animal in and out transfer as well.

Animal transport

A suitable transport box with filters, seals, and an inspection window should be used for on-site transport of animals. When transporting infected animals, the transport box should be labelled with the biohazard sign. Transport of animals by road, rail, shipping and air is done in compliance with the national and international transport regulations concerned.

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 biological safety cabinet (BSC), ensuring protection for both the worker and the environment. For easy handling of cages, 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. Specifically designed animal bedding disposal workstations can be used for cage cleaning and bedding disposal. Contaminated cages can be autoclaved with faeces and bedding before washing, preferably in a pass-through autoclave.

Personal protection

Staff working in animal facilities should wear PPE. Depending on the risk assessment, this may include a lab coat or work suit, gloves, head and shoe covers, goggles, face shield and respiratory protection.

Correct use of PPE should be ensured by proper training of the staff wearing it (for example, respiratory fit testing and training on the proper use and maintenance of respiratory masks).

In the context of occupational health and safety, preventive actions such as vaccination should be offered to people working with specific agents. Finally, as laboratory animal allergies can become an important issue for animal care staff, early recognition, reporting and preventive control measures should be put in place to mitigate health problems.

Training and education

All staff involved in the care and use of animals (animal caretakers, technicians, researchers, visiting scientists) should receive proper training on biosafety and a biosafety manual should be present in the facility. Employees should also be aware of the importance of accident reporting as a means of 'lessons to learn' for improving biosafety measures.

Decontamination and waste management

Decontamination of the entire animal holding room by fumigation is required for high containment animal rooms (A3) in case of contamination, changes in usage, renovations or maintenance shutdowns. Fumigation might also be used for animal rooms for large animals, since the room itself constitutes the primary containment. Formaldehyde, vaporised hydrogen peroxide (VPH) or chlorine dioxide are effective compounds for room decontamination.

Different types of waste are generated in an animal facility: 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: waste from non-infected laboratory animals and transgenic animals can be treated as non-hazardous waste, whereas waste from animals (experimentally) infected with pathogens is treated as hazardous material and requires inactivation. Inactivation is carried out via autoclaving, chemical treatment or incineration, and the method needs to be validated.

Facilities with high numbers of animals may require centralised wastewater treatment systems, whereby heat treatment is the most appropriate method. 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 for incineration. Large animal carcasses can be chopped in a shredder and autoclaved in a tissue autoclave, provided that 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, and is shown to effectively inactivate a great number of biological agents, including those causing transmissible spongiform encephalitis (TSE).

Impact on public health

Since animal experiments are often an integral part of research activities, careful risk assessment and appropriate risk management in laboratory animal facilities are essential for ensuring maximum protection of public health and the environment.

Emergency plans

An emergency plan that takes both staff and animals into account should be put in place. Names, telephone numbers and emergency procedures should be clearly posted in animal facilities and training should be provided.

Biosecurity

The World Health Organisation (WHO) defines biosecurity as 'the protection,

control and accountability for valuable biological materials (VBM) within laboratories, in order to prevent their unauthorized access, loss, theft, misuse, diversion or intentional release'.

With regard to biosecurity in animal facilities, access control is of prime importance. Typically, electronic key cards and associated readers are used to control access and enable recording of the time, location and personal identification. ●

Reference

1. **Van Vaerenbergh B**, Van Droogenbroeck C, **Do Thi CD**, Verheust C, **Willemarck N**, **Brosius B**, **Leunda A**. Biosafety in laboratory animal facilities. A practical approach. Legal Depot 2011. D/2011/2505/47. http://www.biosafety.be/CU/PDF/LabAnimFacilities_SBB_2011_2505_47.pdf

FIGURE 1. Animal facility for rodents

