

Work instruction for working with genetically modified animals in animal laboratories

Introduction

This work instruction is intended as a base document for animal laboratories and should be supplemented according to the departmental situation. Indicate any supplementary regulations specified in the permit/notification (IenW).

Access

- Access is limited to those who are familiar with the risks to people and the environment of the work being carried out. Work may only be commenced following instruction by the responsible investigator, person responsible for the workspace, ABV, or BSO.
- Cleaning personnel have access to the workspace only as referred to in the cleaning procedure for GMO rooms. Maintenance personnel have access only in consultation with the ABV or person responsible for the room.
- Independent work with animals (transgenic or otherwise) by project employees is permitted only if those employees have authorization to carry out laboratory animal work from the Central Laboratory Animal Facility and if they are specified on the work protocol as employees.
- Other employees, including students, interns, and visiting employees, have access only if their supervisor (under whose supervision they work) is present.
- The access door will have a no-access light or an 'Experiment' sign, indicating that the door cannot be opened freely. This is part of the Regulation and intended to prevent animals from escaping. It indicates that an experiment involving animals is currently underway.

Clothing

- A closed lab coat (white) with long sleeves or a blue operating smock must be worn while working in the animal laboratories.
- A hairnet and overshoes must be worn in animal laboratories and when working openly, suitable gloves and an FFP-2 mouth/nose mask at a minimum.
- Shoes must be changed when in the Central Laboratory Animal Facility.
- In decentralized laboratories, overshoes must be worn in D-I laboratories.
- The lab coat, hairnet, respiratory protection, gloves, and overshoes must remain in the animal area once work is completed.
- Personal belongings such as jackets, jumpers, and bags must be stored outside the workspace.
- If wearing a blue operating smock while carrying out work, a white lab coat must also be worn when outside of the animal area.

Work techniques

1. General

- The doors and windows to the workspace must be kept closed while working.
- The enclosure must be locked if there are no employees inside. For large farm animals, the enclosure can remain open if they cannot escape the enclosure other than with human intervention or in the event of an emergency.
- The accommodation for genetically modified animals is clearly marked to distinguish the animals from non-genetically modified animals.
- Administration must not be carried out except for administration required to record experimental data.
- Hand and wrist jewellery is not permitted for D-I, DM-I and DM-II
- The workspace must be kept clean and tidy. There must be no excessive furniture or instruments in the laboratory.
- The presence of vermin is prohibited.
- Smoking, eating, drinking, the storage of food, applying of cosmetics, and putting in contact lenses are prohibited; hair must be tied back.
- Avoid hand-eye contact.

- Using a pipette with the mouth is prohibited – always use a bulb or pipetman.
- Filter tips may also be used to avoid contamination of a pipette's mechanical parts.
- Use only paper hand towels or tissues, if necessary.
- Before leaving the work area, wash hands with water and soap.

2. Preventing aerosol formation

- Prevent the formation of aerosols by waiting a few minutes before opening tubes to allow aerosols to precipitate, allowing a pipette to run out against the wall of a tube – do not force out under pressure, etc.

3. Using a biosafety cabinet

- See procedure [Safe use of a biosafety cabinet type II](#)

4. Syringes and needles

- To minimize the risk of puncture accidents, aerosol formation, and spillage, avoid using syringes with needles as much as possible.
- Use only disposable syringes with disposable needles; fill the syringe carefully and avoid the formation of air bubbles; hold the tip of the needle in the liquid when the content of the syringe is being pressed out.
- Deposit the needle straight into the designated sharps container.
- Never recap needles!!

5. Decontamination and disinfection

- An appropriate quantity of suitable disinfectants must be available before experiments are commenced see work instruction [Disinfection of laboratories](#).
- The work surfaces (table or biosafety cabinet) must be disinfected with a suitable disinfectant once work is complete and at the end of every working day.
- In the event of a spillage, the spillage must be cleaned up immediately by covering the liquid with tissues soaked in a suitable disinfectant. The disinfectant should be allowed to work for the stipulated time, after which it must be cleaned up; tissues used to clean spillages must be deposited in the blue/yellow WIVA container. The work surface should then be cleaned with water and disinfected again.

6. Biologically contaminated waste and biologically contaminated material

- With the exception of water used for hand washing, no contaminated waste water from the laboratory may be discharged into the sewer, unless it has been disinfected beforehand.
- Liquid waste can be autoclaved or decontaminated with active chlorine for at least 30 minutes. The end concentration of the chlorine needs to be at least 2000 ppm, e.g. 1 tablet in 750 GMO solution). This chlorine must be approved by the Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb). For more information regarding disinfectants and their concentration and minimal contact times see Table 1 of the work instruction [Disinfection of laboratories](#).
- In D-I labs, biological waste is collected in the blue WIVA containers. A deceased animal is not regarded as a GMO.
- In DM-I and DM-II, GMO waste is collected in the GMO waste container (yellow WIVA containers). Deceased animals and bedding material combined with GMOs must be treated as GMO waste.
- A full waste container is sealed with a lid. The exterior of the container must be disinfected with a suitable disinfectant.
- Contaminated glass that has come into contact with GMOs must be disinfected with a suitable disinfectant (bearing in mind the principle of minimal contact time), rinsed, and washed.

7. Centrifuging

- Ensure that the tubes are undamaged and seal them with care.
- Use sealed centrifuge tubes (ideally with a screw cap) and/or sealed buckets.
- Fill the centrifuge tubes to a maximum of 4/5 of the volume. If fixed angle centrifuge rotors are used, the centrifuge tube can only be filled to a maximum of 2/3 of the volume. The liquid inside the centrifuge tube must not come into contact with the cap on the tube while being centrifuged.

- After centrifuging, wait 30 minutes before buckets/tubes are opened in order to allow aerosols to precipitate. Alternatively, the buckets/tubes can be opened in a biosafety cabinet.
- Rotors that have been contaminated as a result of leaks or breaks must be decontaminated promptly once centrifuging is complete, see work instruction [Disinfection of laboratories](#).

8. Use of refrigerators, freezer drawers, and nitrogen tanks

- If GMO material is stored outside the animal laboratories, this should be stored in a ODG. A refrigerator, freezer or front porch of laboratories can be classified as ODG. The location of the ODG should be noted in the logbook. The ODG must be provided with an ODG label. Label all material that is stored outside the animal laboratories, so that is recognizable as GMO material. Level II and higher must be provided with a biohazard sign. All storage must be documented.

9. Water baths

- Change the water at regular intervals and add disinfectant (e.g. Prothermal or Aquaclean). Do not use sodium azide due to its explosive properties.

10. CO₂ incubators

- To prevent the formation of mould in the water pan, a copper water pan or piece of copper may be used. Change the water in the water pan in the CO₂ incubator at regular intervals and add disinfectant if necessary (e.g. Prothermal or Aquaclean).
- Clean CO₂ incubators at regular intervals to prevent the growth of bacteria and mould.

11. Ultrasonic and mixing equipment, magnetic stirrers, etc.

- This equipment should only be operated in a biosafety cabinet unless it contains sealed containers. If sealed containers are used, the containers must be opened in the biosafety cabinet. Wait a few minutes before opening the containers so that the aerosols can precipitate.

12. Shipment of transgenic animals

- Transgenic animals are always shipped via the Central Laboratory Animal Facility.

13. Transporting GMO material within the building

- Transgenic animals being transported to another location (including within the building) must be placed in a filter-top cage, which must be covered.

Abbreviations

ABV	Departmental Expert for Biosafety
BSO	Biosafety Officer
CPV	Central Laboratory Animal Facility
D-I	Animal enclosure class I
DM-I/II	Animal enclosure where GMOs are used, class I/II
FFP	Filtering Facepiece Particle
GMO	Genetically Modified Organism
IenW	Ministry of Infrastructure and Water Management
ODG	Another part of the GMO area
Ppm	Parts per million

References

- Decree and Regulation on Genetically Modified Organisms, Environmental Management 2013, IenW April 2014.
- [Website HSBM Maastricht](#)

Further information

For further information, please contact the [BSO](#).