Work instruction for ML-I containment level

Introduction

This work instruction is intended as a base document for ML-I laboratories and should be supplemented according to the departmental situation. Indicate any supplementary regulations specified in the permit/notification (IenW). Please also refer to the work instruction Working safely in laboratory. All employees working in an ML-I laboratory must be familiar with this work instruction and with the content of the permit/notification on which they perform work. In addition to this work instruction, a procedure has been compiled that sets out the organizational aspects relating to GMO work.

Access

- Project employees are permitted to carry out independent work with GMOs only after registering with the BSO and with authorization, see form Approval as GMO worker.
- Work may only be commenced following instruction by the responsible investigator, research leader, or the ABV.
- Cleaning personnel have access only for the work as referred to in the cleaning procedure for GMO areas.
- IDEE maintenance personnel are instructed by the BSO but have access only in consultation with the ABV.
- All employees and students who need access to a ML-I laboratory must register with the BSO as GMO employees.
- The ML-I laboratory must be locked at the end of the day.

Clothing

- A closed lab coat (white jacket with green collar or green strip on the collar or chest pocket and long sleeves) must be worn while carrying out work in the ML-I laboratory.
- The lab coat and gloves must remain in the ML-I area after completing work.
- In the event that the lab coat is contaminated by spillage or accidents with GMOs, the coat must be autoclaved immediately. All coats are placed in a plastic bag for autoclaving and sent to the CGSA.
- Wear disposable gloves if the skin is not in optimum condition or if required by the experiment.
- Personal belongings such as jackets, jumpers, and bags must be stored outside the lab.
- Avoid use of personal laptops, tablets, and mobile phones in the ML-I room. If their use is absolutely necessary, they should only be used with clean hands (not gloves). In the event of potential contamination of a laptop, tablet, or mobile phone, disinfection should be carried out with a suitable disinfectant (bearing in mind the principle of minimal contact time).

Work techniques

1. General information

- The doors and windows to the workspace must be kept closed while working.
- Administration must not be carried out in the laboratory except for administration required to record experimental data.
- The workspace must be kept clean and tidy. There must be no excessive furniture or instruments in the laboratory.
- Wearing jewellery (rings, bracelets, watches, etc.) while working is prohibited.
- 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.

- If there is no water or soap in the ML-I laboratory, a legally permitted hand disinfectant must be used to disinfect the hands before leaving.

2. Preventing aerosol formation

- Prevent the formation of aerosols by waiting a few minutes before opening tubes to allow aerosols to precipitate, allowing inoculation needles to dry out before flaming them, 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. If the sharps container is full, send an e-mail to afval@mumc.nl (with the subject 'GMO sharps container' and the room number) asking for it to be collected.
- Never recap a needle!

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 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.
- Liquid waste intended for destruction by the CGSA must be transported to the CGSA in a shatterproof, disinfectable container bearing GMO lettering.
- Solid GMO and biological waste must be collected in the yellow WIVA containers. If a container is full, it should be sealed with a red lid. The outside of the container must be disinfected with a suitable disinfectant and the lid fitted with a sticker bearing the GMO no./name/room no. Full containers must be collected in and removed from an agreed location.
- Liquid waste may be collected using the Serres gel system. With this system, the liquid is converted to a gel in a bag. The bag containing the gel can be deposited in the GMO waste container (yellow WIVA container).
- 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, freezers, and nitrogen tanks

- If GMO material is stored outside of the ML-I laboratory, it must be stored in another part of the GMO area (ODG). A refrigerator, freezer, or vestibule to a laboratory can be classified as an ODG. The location of the ODG must be stated in the logbook. The ODG must also bear an ODG label. All storage of GMOs 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. CO_2 incubators

- To prevent the formation of mould in the water pan, a copper water pan or piece of copper (e.g. a five Euro cent coin) may be used. Change the water in the water pan in the CO_2 incubator at regular intervals and add disinfectant if necessary (e.g. Prothermal or Aquaclean).
- Clean CO_2 incubators at regular intervals to prevent the growth of bacteria and mould.

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

- When used in combination with GMO material, 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. Shipping GMO material

- Please see work instruction Transporting GMOs and biological material for shipment of GMO material.

13. Transporting GMO material

- When transporting GMO material to another location (including within the building), a sealed, shatterproof, leak tight container bearing GMO lettering must be used.

14. Registration

- All GMO work is registered in a lab journal. The GMO material must be specified (host/vector/insert/origin of insert) along with the activities that were performed as well as the location of storage, if applicable, outside the ML-I laboratory. In the front of the lab journal it is stated at which GMO permit/notification number(s) activities are licensed/notified.
- It is mandatory to fill in the registration form in Labagenda or the logbook of the ML-I lab. Register date/name/department/tel no/activity/IG-no).
- Employees with an authorization to work in the ML-I laboratory, as well as any visiting employees, must be registered in the (digital) logbook.

Abbreviations

ABV	Departmental Expert for Biosafety
BSO	Biosafety Officer
CGSA	Central Glass Sterilization Department
Ctgb	Board for the Authorization of Plant Protection Products and Biocides
GMO	Genetically Modified Organism
IDEE	Instrument Development, Engineering, and Evaluation (UM)
IenW	Ministry of Infrastructure and Water Management
ML-I	Microbiological Laboratory Class I
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.