

**ML-I level Work protocol**  
**GMO Genetically Modified Organisms**  
**VMT Veilig Microbiologische Technieken**  
**BSO Bio-hazard Safety Officer**

**Access**

- During the activities, doors of the GMO laboratory must be closed.
- Only individuals who have a declaration signed by the BSO, stating that they are sufficiently familiar with safe microbiological technique, are allowed to work in the GMO laboratory. Exception: students and other persons working less than one year. The VMT officer adds those people to a list. This list contains name of the person working less than one year, the person who is responsible for him/her, GMO-permit number and the working period.

**Logbook**

- A logbook must be present in the GMO laboratory in which the data described in the GMO Manual (KWINT) are entered. This information, along with a laboratory journal which describes the individual experiments in greater detail, should provide insight into the way in which the activities are taking place in accordance with the GMO constructions and activities stipulated in the permit.

**Purity monitoring**

- At the beginning of the activities and afterwards, the material (micro-organisms and/or nucleic acid preparations) with which the activities will be/have been conducted must be checked for purity/identity every three months. If this is not demonstrated by the experiments that are being carried out (e.g. when the restriction pattern is viewed, one can see whether the initial material was correct), separate tests must be carried out. To prevent undesired organisms from being cultured, pure starting material must be used as much as possible (e.g. first make a pure culture with a glycerol stock and use an individual colony – which is checked for purity – to continue with).
- The results of this monitoring must be reported and dated in the logbook

**Hygiene procedures**

- The bench on which the activities are being carried out must be disinfected after the activities are completed. In any case, disinfection must take place on a daily basis. Disinfection must be carried out immediately following any spills.
- The GMO laboratory must be kept tidy and clean at all times.
- Before the activities begin, a sufficient quantity of disinfectant must be readily available. The disinfectant must be periodically checked for effectiveness and regularly replaced.

**Personal hygiene**

- When working in the GMO laboratory, do not place anything in your mouth, therefore do not eat, drink or smoke. Food, coffee cups, and other eating and drinking implements are not allowed in the workspace.
- Plants and animals (other than those being used for the experiments) may not be brought into the GMO laboratory.
- Personal belongings should be kept out of the GMO laboratory whenever possible.

- Avoid touching your face and hair with your hands; avoid manipulating contact lenses.
- For activities such as blowing your nose, use disposable tissues instead of cloth handkerchiefs.
- While working in the GMO lab, do not wear rings, bracelets or wristwatches.
- Wash your hands frequently, wash your hands always after handling potentially contaminated material and before leaving the laboratory. Washing with warm water and soap is recommended.
- Cross flow cabinets may not be used because these do not offer any personal protection.

### **Clothing**

- Wearing a lab coat or other laboratory clothing is mandatory. It is not allowed to wear these clothes outside the GMO laboratory.
- It is recommended that GMO laboratory clothing is labeled to differentiate from clothing for other types of laboratories.
- The lab coat must be buttoned when worn. The sleeves must entirely cover street clothes.
- The laboratory clothing must be changed frequently. The frequency of changing/washing/repairing clothing can be in accordance with the normal building procedures, except when clothing is contaminated by spills. In that case, the clothing must be first decontaminated by autoclaving.
- Wearing gloves, safety glasses etc is not essential at this level for microbiological reasons, but can be necessary for chemical reasons (disinfectants, solvents).

### **Work procedures**

- During all procedures the occurrence of aerosols should be prevented as much as possible.

### **Glassware use**

- It is recommended that screw top bottles are used for any potentially contaminated material.
- Glass containers and Erlenmeyer flasks that contain potentially contaminated material must be kept and transported in unbreakable, leak-proof containers. Add disinfectant to the water (for example, 0.1% chlorohexidin, added twice a week, change the water in the bath once a week). Sodium azide (explosion hazard) may not be used in this situation

### **Centrifuging**

- Whenever possible, use a centrifuge with a closed rotor or closed buckets.
- Centrifuge tubes must be made of unbreakable material and must be closed in a leak-proof fashion during centrifuging.
- Inspect the tubes for cracks and other imperfections before use and make sure the centrifuge still closes properly.
- The centrifuge tubes should never be filled so that the liquid can contact the lid during centrifuging. Therefore, they may only be filled to 4/5 of their volume; if angle rotors are used, they may only be filled to 2/3 of the volume.

- If leakage occurs while centrifuging, the tubes and the rotor must be disinfected (both internally and externally).
- The centrifuge must be regularly cleaned (e.g. inside of lid).

### **Pipette technique**

- Using the mouth for drawing liquids into the pipette is forbidden.
- To prevent the formation of aerosols, it is also forbidden to blow out pipettes.
- The outflow of the pipette must be directed along a surface to prevent dripping.
- Pipettes must be plugged with cotton wool.
- Glass pipettes must be disinfected in a pipette container after use and washed again with warm water and soap before they are reused.
- Disposable pipettes and/or pipette tips must be decontaminated after use, preferably by autoclaving.
- The use of water jet pumps is forbidden.

### **Injection needles and syringes**

- The use of injection needles and syringes is forbidden unless this is absolutely essential for the experiment.
- After use, the needles and syringes must be autoclaved or disinfected. Autoclaving is preferable.
- The mechanical pipette with disposable tips is a good alternative for certain activities with needles and syringes.
- When emptying the syringe, the point of the needle must be held in the liquid.
- The syringe must be filled carefully so that no air bubbles occur.
- Excessive liquid must be pressed out in a bottle with sterile cotton wool.
- Before autoclaving, the needle must be placed in a container with rigid walls, for example, one made of stainless steel.
- Do not replace the needle in the plastic protective sheath.

### **Ultrasonic disrupters/mixing equipment/stirrers/mortars**

- All such apparatus must be self-contained as much as possible.
- After use, you should wait several minutes before opening the apparatus to allow any aerosols to settle.

### **Freeze-drying**

- Ampoules for use in freeze-drying must be made of strong, heat resistant glass.
- When filling ampoules, allow the liquid to flow against the bottom of the ampoule.

### **Refrigerators/ freezers/dewar flasks**

- Periodically inspect the contents of refrigerators and freezers; if necessary clean them out.
- Periodically defrost and clean the refrigerators. Gloves must be worn during cleaning.
- All stored material must be provided with a label stating contents, name of the researcher and date.

## **Biological waste**

- All biological waste (intact organisms, cells or viruses), including any contaminated glassware or instruments, and all potentially contaminated liquid and solid waste, must be decontaminated before washing, reuse or disposal; autoclaving is preferable.
- The potentially contaminated material must be stored in a closed and adequately labeled container.
- Waste must be decontaminated at least once per week.
- Non-heat resistant material (such as dry Galsky spatel) should be decontaminated with ethanol.
- The transport of waste to the autoclave in room number 02.4444 must take place in leak-proof, closed containers.
- Following autoclaving or decontamination in another fashion, observe the normal rules that apply to household, chemical, radioactive and hospital waste.

## **Decontamination following spills of potentially contaminated material**

### *Slight contamination*

- First of all, wash your hands (and face if necessary) and change into clean laboratory clothing.
- Put on gloves.
- Remove as many obstacles as possible from the contaminated area.
- Cover the contaminated area with tissues soaked in disinfectant. Wait at least 10 minutes. Then remove the tissues.
- After this, wipe up the material with tissues.
- All the waste material will be decontaminated again by autoclaving.
- Clean the surface with soap and water.
- After these activities, wash your hands thoroughly.

### *For large spills (e.g. breakage of liter flask)*

- Remove as many obstacles as possible from the contaminated area and evacuate the room if possible.
- If possible, wait 15 minutes to allow any aerosols to settle.
- First of all, wash your hands (and face if necessary) and change into clean laboratory clothing if the clothing has been contaminated.
- Put on gloves.
- Be cautious if there are any glass shards or other sharp materials. Do not touch these materials.
- Make sure that the contamination does not spread any further by pouring a ring of disinfectant around the spill area.
- Cover the spill area with towels soaked in disinfectant and allow the disinfectant to remain for 10 minutes.
- Use a pair of tweezers to place the contaminated towels into a waste container.
- If necessary, cover the materials and surfaces with tissues or filter paper to absorb the liquid and repeat this measure until all the liquid has been absorbed.
- Deposit the waste (also the glassware etc) in the waste container.
- Wipe the spilled area with towels drenched in disinfectant, wiping from outside to inside.

- Clean the spill area with warm water and soap.
- If laboratory clothing has been contaminated, disinfect immediately by autoclaving.
- All waste materials (including towels, mops and mop water) must be decontaminated, preferably by autoclaving.
- Disinfect contaminated areas of the skin and wash with soap.
- Notify the VMT officer (if this has not yet been done) and record the information about the spill in the administration records in case of accidents and calamities.
- If there is an accident or calamity, the procedure as described in the GMO Manual (KWINT) must be followed.

### **Cleaning workspace**

- The GMO laboratory must be cleaned on a weekly basis. During this process, the floor and the work surfaces are mopped or wiped with warm water and soap.
- During cleaning activities, a laboratory coat and gloves must be worn.

### **Equipment repair**

#### *Repairs outside workspace*

- Apparatus that is to be repaired or disposed of must first be disinfected before it can be removed from the GMO laboratory.
- The VM makes arrangements with the individuals who will carry out the repair activities.
- The VM is responsible for making sure that the apparatus is cleaned and disinfected. The VM verifies this and fills in the release form (GMO Manual KWINT).
- The apparatus is then removed from the ML-I lab.
- The apparatus is taken to repair.
- Repairs made on apparatus and/or systems inside the GMO laboratory can take place only after the laboratory is thoroughly cleaned.

#### *Repairs in workspace*

- Before repairs or maintenance can take place, the workspace must be disinfected.
- An appointment is made to carry out these activities. The VMT responsible is notified about this appointment.
- The VMT officer is responsible for making sure that the apparatus and the space in which the activities must be conducted have been cleaned and disinfected. The VMT officer verifies this and signs a release form.
- The repair person reports at the appointed time to the VMT responsible, who then accompanies him/her to the location. The VMT responsible makes sure that the repair person follows the rules (puts on a lab coat, washes hands after completing activities etc.)
- During the activities of the repair person, no other activities can be carried out in the room unless the VMT officer gives permission.

## Transport of material

### *Internal*

- The material must be packed in a hermetically sealed, leak-proof, unbreakable outer container that is externally decontaminated before transport.
- The packaging must be provided with a label that lists the contents.

### *External*

- The transport of GMOs is regulated by stipulations from the Carriage of Dangerous Goods Act. This means that for transport by road, railway and inland waterways, packaging, documentation and labeling must satisfy the stipulations in the Provisions Concerning the Carriage of Dangerous Substances by Land (VLG, Stcrt. 235/1996), the Provisions Concerning the Carriage of Dangerous Substances by Railway (VSG, Stcrt. 237/1996) and the Provisions Concerning the Carriage of Dangerous Substances by Inland Waterways (VBG, Stcrt. 236/1996).
- In accordance with these provisions, any substances that can be infectious for humans or animals (micro-organisms, including viruses, endo-parasites and cells of pathogenicity class 2) are classified as class 6.2 and other non-infectious but environmentally hazardous biological agents (pathogenicity class 1) as class 9. These regulations can be summarized as follows:
  - *Pathogenicity class 1:* The biological material must be packaged in a hermetically sealed, unbreakable, leak-proof, inner container (Eppendorf tube, bottle, ampoule etc; all internal containers must have screw tops). This inner container must be packed in a hermetically sealed, unbreakable, leak-proof outer container with sufficient absorbent material to absorb the entire contents of the container. The content and the required containment level must be labeled on the inner container. The outer container must have a label containing the internationally recognized biohazard sign and the text, "BIOHAZARD" or "Infectious substances affecting humans". In addition, the attached letter on the outside container must report the contents, the sender and addressee, who must be notified in case of damage or leakage (including telephone numbers), under which conditions the package can be opened and the storage temperature. If dry ice is used, the outer container must be provided with a valve to release the carbon dioxide gas that forms inside (in this case the outer container may be placed in a non-hermetically sealed package). A transport label (class 9) only has to be used if: volume > 500 ml per inner container, total volume > 2 liter, mass of solid materials > 1 kg per inner container and the total mass > 4kg.
  - *Pathogenicity class 2;* follow regulations applying to class 1, but including the following additional stipulations. Packaging material must be officially certified. Multiple inner containers can be placed in a single outer container only if the materials are not packed together with substances from another class (such as class 9). The packaging must be labeled with hazard label 6.2.
  - *Pathogenicity class 3:* for transporting GMOs with a pathogenicity class 3, very extensive supplementary rules apply (see VLG, VSG or VBG: leak-proof inner container, filling material, leak-proof outer container, outer packaging, everything tested according to specific standards).

- All biological material (intact organisms, cells or viruses) that must be shipped by airplane abroad should be packaged in accordance with the IATA (“Dangerous Goods Regulations”) and provided with the label, “Etiological agent”.

### **Receipt of GMOs**

- When opening ampoules with potentially contaminated material, these must be wrapped in a towel soaked in disinfectant that is folded several times.
- As soon as possible after receipt, the project leader must check whether the received material is in accordance with the accompanying description.
- Information about receipt and inspection must be recorded in the logbook (purity monitoring).

### **Other remarks**

- Everyone who works in a GMO laboratory is required to obey supplementary regulations, measures or instructions given by the VMT officer and the BSO (Bio-hazard safety Officer) .
- In all cases where these safety regulations are not applicable, the VMT officer or the BSO must be consulted.
- Every member of staff is required to report irregularities to the VMT officer or the BSO.

### **Appeal procedure**

- Every regulation, measure and instruction drawn up or given by the BSO and/or VM can be appealed to the Executive Board.
- While an appeal is pending, the relevant regulation, measure or instruction remains in force.