BIOSAFETY PROTOCOLS
for molecular and microbiological laboratories

These guidelines describe safe working practices in molecular and microbiological laboratories, laboratory design and use of equipment.

Purpose of these guidelines is to realize a safe working environment, and to protect laboratory workers and the environment against exposure to potentially harmful biological agents. Another purpose is compliance with the law.

Please read (and follow) these instructions carefully!

Biosafety Contact Persons *(name, room, tel.)*

Responsible scientist (OL GMO):

Assistant OL:

Biosafety Officer: Fraukje Bitter (tst 7817)

Notification number(s):
BSL-I: IG ... ...
BSL-II-k: IG ... ...
BSL-II-k: IG ... ...

(in case of gmo work)

General remarks

- Everyone working in the molecular or microbiological labs, transgenic plant- or animal facilities should know and apply these rules.
- Standard microbiological practices and techniques ("VMT": Veilige Microbiologische Technieken) apply and should be strictly adhered to! Additional instructions apply to the Fytotron (check the Biosafety Fytotron Protocols).
- It is prohibited in the Netherlands to carry out contained use of genetically modified organisms (gmos) without a prior risk-assessment and approval by the BSO. Please report all new gmos and new gmo activities to your OL and/or BSO for risk-assessment and approval.
- A summary report of the risk assessment, listing the properties of the gmo (host, vector, donorsequence and donor-organism), the activities performed and the result of the risk assessment, should be kept available for the Human Environment and Transport Inspectorate (Ministry of Infrastructure and the Environment).
- For level I gmo activities, approval by the BSO and uptake in the summary report usually is sufficient. New level II-k gmos and activities should always be reported to the Min. of I&M. Activities with level II-v and level III gmos are prohibited unless a permit in response to an application for a license for these activities has been obtained.
- Personal health status may influence susceptibility to infections. Inform your supervisor or OL/VM if you have (or suspect) an increased vulnerability to infections with specific agents (e.g. due to immune-deficiencies, pregnancy, use of immune-suppressive medication, underlying illnesses like cystic fibrosis).

- Entry doors to laboratories should be closed during work to maintain a negative pressure relative to adjacent non-lab areas and to ensure sufficient ventilation of the lab.
- Laboratories should be kept clean and tidy.
- Materials that cannot be decontaminated easily (like books, reports) should be kept away from biohazardous materials. Keep paperwork separated from laboratory work benches.
- Use of laboratory coats is mandatory (see Personal Protective Equipment), the labcoats should be left behind in the gmo laboratory. It is not allowed to wear ML-I or ML-II labcoats in adjacent corridors that are not ML-I or ML-II.
- Wearing watches or jewelry on arms/hands is not allowed.
- In every lab an appropriate disinfectant[1] and Personal Protective Equipment (PPE) must be available (lab coats, gloves, and optionally face shield, shoe covers, and masks). Selection of PPE must be based on risk assessment.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics and storing food/drinks are not permitted.
- Mouth pipetting is prohibited (mechanical pipetting devices must be used).
- Creation of aerosols should be minimized.
- Open activities with sporulating yeast or fungal cultures should be performed in a Biosafety Cabinet Class II.
- Manipulation of (potentially) infectious materials must be conducted in a Class II BSC (check "Additional instructions for a level II facility".
- Work areas must be disinfected with an appropriate disinfectant before and after completion of work, and after any spill of viable material.
- Wash hands prior to leaving the laboratory, or disinfect hands (using a validated method).
- All exposure incidents, accidents or emergencies with respect to biological agents must be reported to the laboratory supervisor (OL) or BSO.
- If something is unclear: don't hesitate to contact one of the responsible persons.

Access to gmo area

- Registration at the OL/VM or BSO is required before one is allowed access to the gmo areas. Also guests, students or people who do not work with gmos need to be registered. The OL/VM will ask you to complete the worker registration form (Dutch or English). Check SOP A.07 for further information.

Laboratory design

- The laboratory must be designed so that it can be easily cleaned and decontaminated, bench tops must be impervious to water and resistant to heat, disinfectants, acids, alkalis and other chemicals.
- Laboratories must have a sink and soap dispenser for hand washing (hands free operation recommended for ML-I, obligatory for ML-II). Alternatively a (validated[1] and CTGB approved) hand disinfection unit may be used. At ML-II sink and/or disinfection unit should be positioned near the exits.
- Only non-adsorbent materials (including furniture!) are allowed in the labs.

[1] an *appropriate disinfectant* is effective against the micro-organisms present in the lab

- A sign indicating the biosafety level of the lab must be posted at the entrance of the lab, including the name and telephone numbers of the responsible person's (OL and BSO), as well as a biohazard sign for level II and higher.
- Check SOP A.03 for further and specific requirements.

Additional instructions for a level II facility (e.g. ML-II)

- Authorization to work in level II laboratories should only be given when the laboratory worker has proven that he has sufficient knowledge of safe working practices, that he has learnt all the specific safety procedures for this particular laboratory and knows how to apply them.
- The door must be locked when no one is present in the lab. For ML-II labs that are located within a gmo area, the door must be locked outside working hours.
- Wear gloves to protect hands from exposure to hazardous materials. Do not wash or reuse disposable gloves. Change gloves when contaminated or damaged and dispose of with other contaminated laboratory waste.
- All *aerosol-producing* activities must be conducted in a class II biosafety cabinet. *All open activities* with airborne infectious agents or with agents that are infectious via direct contact (wounds) should be performed in a BSC class II as well. The BSC should be located away from doors and other potential airflow disruptions (ideally at the rear of the lab). The cabinet should be included in an annual maintenance program and be tested and certified annually.
- FACS operating procedures should include aerosol containment and waste management. Awareness of the origin of samples is required; all unfixed materials (blood samples, body fluids, cultures cells and environmental samples) should be considered as biohazardous. Please check and implement the latest ISAC Biosafety Standards for cytometry and cell sorting.
- When potentially infectious material (if allowed) is handled outside the BSC, use eye and face protection when splashes or sprays might occur. Eye and face protection must be decontaminated before reuse.
- Instruction videos on class II BSC are available on the website, please get yourself acquainted with these!
- For working in a BSC: Replace glass pipettes (loops, cell spreaders etc.) with **disposables** whenever possible. The use of Bunsen burners or other flames in a BSC should be avoided: the turbulence created by the flame disrupts the sterile air flow and may cause aerosols to escape into the lab. In case it is absolutely necessary and there is no alternative: use touch-plate microburners. Small electric sterilization systems are available for annealing bacteriological loops and needles, these are to be preferred over using an open flame inside the BSC!
- ML-II lab coats must be left behind in the ML-II lab, and must be sterilized by autoclaving before being laundered. Coats should be put in an autoclave bag and must be sterilized immediately (storage in autoclave room is not allowed).

Working with cell lines or specimens of human or primate origin

- Human and other primate cell lines can harbor infectious viruses, like Hepatitis B, HIV, Epstein Barr or HPV. Therefore, they should be considered potentially infectious and handled under biosafety level II conditions. Only when a specific cell line has been well characterized and has been found negative for these viruses, working at BSL I is allowed.
- Level 2 (ML-II or BSL-II) practices and containment are recommended for working with all *potentially infectious* human and primate cell lines (like for working with human blood, body fluids, unfixed tissue etc.)!
- *Hepatitis B vaccination* is offered (and strongly recommended) to all workers who can be exposed to (human/primate) blood products. Please contact Health Services at your institute, your HR department or the BSO. Please note that not using available vaccination is at your own risk, but may lead to exclusion from working with certain biological agents.

Log book

- A log book (SOP A.08) is present in all gmo areas. Please get yourself acquainted with the location of the log book and its contents. In the logbook you'll find:
  - General and specific biosafety protocols and procedures (including these!), e.g. how to handle in case of incidents, accidents or spills.
  - Copy of gmo licenses (including the IG licenses of guests): make sure that the material you work with is officially permitted by the Government**.** *If not, contact the OL/VM or BSO: working with non-permitted strains, plasmids and / or donor sequences is a violation of the law.*
  - Floorplan of the gmo area, including location of laboratories and storage facilities.
  - A record of incidents, accidents and other occurrences (moves, temporary removal of the gmo restrictions).
  - Separate lists for gmo workers and non-gmo workers (alternatively, copies of the registrations forms can be included).

Personal Protective Equipment

- Clean, protective laboratory coats are available from ……
  - Lab coats for ML-I area are marked as follows:…….
  - Lab coats for ML-II labs are marked as follows:……
- All *lab coat* buttons should be fastened and sleeves rolled down so that street clothing and arms are completely covered. Coats should be changed and laundered at least every 2 weeks, earlier if necessary. In case of spill, change lab coat, disinfect spill or put it in an autoclave bag for sterilization.
- *Gloves* must be worn to protect hands from exposure to hazardous materials. Wear double gloves when extra protection is needed. Change contaminated gloves, or gloves that have been damaged or punctured. Used gloves should be disposed of as contaminated waste. Remove gloves and wash hands thoroughly before leaving the lab.
- Use *eye or face protection* when splashes of infectious or otherwise hazardous materials (e.g. liquid nitrogen) might occur.

Long term storage and registration of biological agents
(including gmos and seeds)

- Storage places can be located within the gmo area (e.g. ML-I or ML-II laboratory). Storage places require central registration; report location to the BSO.
- It is not allowed to move storage facilities (-80 freezer, N_{2lq} etc.) without permission of the OL/VM. New locations have to be reported to the BSO.
- The essential data of all biological agents/gmos should be kept in a (gmo) register. Essential data include the name of the host strain, host species, name of the plasmid, parental plasmid, donor sequences, donor-organism and biosafety level. Reference should be made to the applicable IG number. Reference can also be made to the construction method, plasmid maps or sequence data (SOP A.09).

Sharps

- Contaminated sharps are disposed of in a "sharps disposal container" that must be available on or near the workbench.
- The sharps disposal container should be leak-proof, puncture resistant, and closable (e.g. with a lid). It should be kept upright in order to avoid spilling of fluid or sharps.
- Containers should be marked with a sign indicating the hazard (e.g. biohazard).
- Containers must not be overfilled and never be emptied or handled in any other way that might cause exposure.
- Do not recap, bend or touch needles unless this is absolutely required and there is no appropriate alternative. If this is the case, use a safe technique: e.g. by holding the cap with a pair of tongs and placing it over the needle.
- Before the containers are removed, close the lid so that spilling is prevented. It can be placed in a secondary waste container (yellow or black) for transport to an external incineration facility (see below).
- Never pick up broken glass or sharps by hand, but use e.g. a pair of tongs, or brush/dust pan etc.

Biological waste
There are two possibilities for the appropriate handling of contaminated waste (Please complete and indicate method of choice).

❑ In case waste is decontaminated in an autoclave located in the same building, but outside the immediate laboratory area:
  - Collect contaminated waste in reusable containers that contain an autoclave bag. The autoclave bag must be securely tied (tie-strap, tape, or by using adhesive strips), and the container must be disinfected (outside) before transport to the autoclave.
  - The autoclave process must be validated and monitored, the autoclave should be tested and certified annually.
  - Location of autoclave:…… (*indicate building and room number)*

❑ In case of transport to an external incineration facility:
  - *Please check SOP F02 for additional information.*
  - Collect waste in black containers (level I facilities) or yellow containers (level II facilities) with red lid (in case of gmo)

- Hermetically seal full containers and complete the printed label on the lid. Before transport to the storage (and pick-up) location, disinfect the outside of the container.
- Don't let full containers accumulate in the laboratory area, they should be brought weekly to the pick-up location in your building, together with a transfer form for microbiological waste (keep a copy for your own records).

Disinfection

- Chemical substances for disinfection must be permitted in the Netherlands by the "College van toelating gewasbestrijdingsmiddelen en biociden" (www.ctgb.nl). Permitted disinfectants have been checked for effectiveness and safety for people and the environment. *Check whether the disinfectant you want to use is on the ctgb list* (if not, choose an alternative).
- Disinfection is a procedure that reduces the level of microbial contamination. It is not as lethal as sterilization. The effectiveness depends on a number of factors, e.g. nature and number of micro-organisms (e.g. the presence of spores), the incubation time, the amount of organic material present, the type of device that needs to be disinfected and the temperature. *Choose a disinfectant appropriate for your type of work and microorganism, and validate the procedure! Consult the disinfectants database on: http://www.bvfplatform.nl/*
- ***Surfaces*** can be routinely disinfected with 70% ethanol, larger surfaces (>0,5m^2) with 0,1% (1000 mg/l) chlorine, e.g. Stafilex, Actisan-D, Medicarine, Halamid. Prior cleaning (water and soap) is an important step in the disinfection procedure. It is necessary to remove proteinaceous material that might inhibit the effectiveness of the disinfectant.
  - Follow the manufacturer's instructions, otherwise validate the procedure.
  - *Ethanol*: is effective against a wide spectrum of bacteria, fungi and enveloped viruses. It is not effective against spores, non-enveloped viruses (like Hepatitis A or adenovirus) and prions. For lipid-enveloped viruses higher concentrations/mixtures might be used (e.g. 80% ethanol + 5% isopropanol).
    Wipe the surface using a siphon and tissues, or use commercially available pre-soaked tissues. The surface should remain visibly wet for at least 30 seconds and allowed to air-dry. Do not use ethanol *sprays*: spraying does not guarantee sufficient contact time, and poses a health risk (and is therefore legally forbidden).
  - *Chlorine* is a generic disinfectant with bactericidal, virucidal and sporicidal properties. Concentrations between 500 and 1000 mg/l are appropriate for most uses. In higher concentrations it is corrosive to metals and not recommended for aluminium surfaces (rinse with water after disinfection). Fresh solutions must be made, preferably daily. Note that organic compounds rapidly inactivate the chlorine activity: higher concentrations might be required when there is an excessive amount of organic material (e.g. spills of cultures). The surface should remain visibly wet for at least 5 minutes, and can be rinsed with water afterwards (to remove chlorine residues).
- ***Water baths****:* regularly clean to minimize microbial contamination: water baths should be heated regularly for 2 hr at 60°C or 30 min at 90°C. Old

and disinfected water can be discarded and bath refilled with fresh water. Alternatively, chemical solutions can be applied (e.g. Aquaclean, Biocidal).

- ***Contaminated reusable pipets*** should be completely submerged in an appropriate disinfectant.

Spills

*In case of a spill of biological agents **prompt action** must be taken to contain and clean up the spill in order to prevent exposure of laboratory workers and spreading of harmful agents into the environment.*

A spill of infectious agents outside the BSC:

- Prevent spreading of the material. Avoid inhaling infectious airborne material (hold your breath), leave the room quickly and notify others to leave as well. Close the door(s). Make sure no one enters the lab, post warning signs if necessary.
- Remove contaminated laboratory coat/clothes (put it in a bag for autoclaving), wash and disinfect hands and other exposed areas.
- Allow aerosols to settle for at least 30 minutes.
- Notify the laboratory supervisor (or OL/VM).
- Before re-entering the room: wear appropriate PPE's: laboratory coat, gloves and possibly shoe covers and respiratory protection.
- Start disinfection procedure (see below).
- Complete the incidents report form and send it to the BSO.

A spill inside a BSC

- Keep the cabinet running, and start disinfection procedure (see below) immediately. Wipe walls, work surface and equipment with an appropriate disinfectant solution. In case of a (larger) spill in the drip tray beneath the work surface, pour sufficient disinfectant onto the surface and through the grilles. Allow for sufficient contact time (20-30 minutes). Soak up solution with paper towels (the catch basin can be drained off into a disinfectant filled container). Lift the front exhaust grill/tray and wipe, disinfect and clean all surfaces and drip tray. Dispose of exposed materials in biohazard waste (check *disinfection procedure,* below).

A spill inside a centrifuge

- Turn centrifuge off and do not open it for 20 minutes, allowing for aerosols to settle. Post a warning sign on the centrifuge. After 20 minutes: put on appropriate PPE's (laboratory coat, gloves and possibly shoe covers and respiratory protection). Disinfect chamber and rotor (or buckets, on the outside), follow the disinfection procedure (see below). Remove rotor and bring to the nearest BSC, open, disinfect and clean. Contaminated equipment in the centrifuge can be put in a leak-proof bag and be moved to a BSC for decontamination.

Disinfection procedure:

- Put on appropriate PPE's: laboratory coat, gloves and possibly shoe covers and respiratory protection (FFP-2 or FFP-3 mask). Disinfect by pouring an appropriate disinfectant around the spill, allow solution to flow into the spill. Do not pour disinfectant directly onto the spill (this might produce aerosols). Use tissues (soaked in disinfectant) to carefully cover the spill area. Allow at least 20

minutes contact time before removing tissues, glass, liquid etc., and treat all waste as infectious waste (put it in the appropriate waste container). *Never* pick up broken glass or sharps by hand, but use e.g. a pair of tongs, or brush/dust pan etc. Then carefully wipe area with disinfectant-soaked tissues from the outside to the inside until it is dry and clean. Clean and rinse with water and soap.
  - Note: Disinfection procedures are much more effective when the area is dry and clean. Organic compounds decrease the effectiveness of many disinfectants; disinfection of solutions requires higher concentrations of the disinfectant. (Standard disinfections procedures recommend a cleaning step with water and soap first).

Incidents and accidents

- **In case you need first aid assistance, BHV, the fire department or an ambulance: dial (030-253) 4444!** *Report who you are, what happened and where, and whether there are any casualties.*
- Never hesitate to warn and/or ask your colleagues for help!
- In case of **spills**: follow the appropriate spill instructions
- In case of **exposure**:
  - **Exposure to infectious aerosols**: Avoid inhalation of infectious airborne material as much as possible (hold your breath), leave the room quickly and notify others to leave as well. Continue following the steps of the spill procedure for infectious airborne material outside the BSC.
  - **Wounds** (e.g. needle-stick incidents, cuts, splashes on damaged skin, animal bites etc.): allow small wounds to bleed freely, rinse extensively with warm water and soap and then disinfect (preferably with 70% ethanol, Sterilon or Betadine (available in first aid emergency kit). In case of severe bleeding: use sterile dressings for direct compression of the wound. In case of animal bites or wounds acquired outside (e.g. thorns), contact Health Services for getting a tetanus vaccination.
  - **Splashes onto intact skin**: remove contaminated clothing and rinse extensively with warm water and soap, then disinfect.
  - **Splashes into the eye, nose, mouth** etc.: immediately flush with clean, lukewarm water for 15 minutes (do not use disinfectants). For splashes into the eye: use an eyewash station if available, ask a colleague for assistance.

  Follow-up
  - Determine the infectivity status of the source material (identify the source individual or animal). Keep source material for further examination.
  - A blood sample can be drawn and kept at -70°C, for (retrospective) determination of baseline infectivity status. Contact Health Services for this.

- Report the incident/accident to your supervisor (in case of a gmo incident to the OL/VM), and discuss whether further action, e.g. consultation of a physician for prophylactic treatment, is advisable. If it is, contact the BSO (030-253 7817) or Health Services (035-688 0300) for further assistance.
- All incidents/accidents that pose a *serious risk for people or the environment* must be reported to the BSO immediately.

- Complete the incidents report form and send it to the BSO.

Sending and receiving biological agents

Receiving biological agents

- All strains, vectors and donor sequences that enter the lab need registration at the OL/VM. In case of gmos, the gmo license (or gmo risk assessment report) must be checked and extended where necessary. Please check SOP A.01 and SOP A.02 for further details, or contact the VM/BSO.
- For wild type strains: species that have not been registered at the BSO yet, must be reported to the BSO. A risk assessment (at species and strain level) must be performed to determine the appropriate biosafety level and practices.
- All strains, vectors and donor sequences require registration in the logbook or long term preservation records (SOP A.09).
- After check for authenticity: enter results in logbook.

Receiving animal by-products or animal pathogens

- An import license might be required, depending on the country of origin and nature of the material; check SOPs E.01 – E.04 on the website.

Sending biological agents or gmos

- Follow the steps of SOP F.01, or ask your OL/VM/BSO.
- Please note that using inappropriate packages, labels or forms, or choosing an inappropriate modality for sending can not only lead to delay or destruction of your package, but might also lead to fines/punishment for non-compliance of the law!

Transport of gmo to other gmo labs or areas (within the same building)

- Gmos can be transported in closed, plastic tubes (no glass), marked “gmo”. Tubes must be placed in a secondary, closed, leak proof container.
- Tubes and container must be decontaminated (outside) with 70% ethanol before being transported out of the gmo area.
- For transport over public roads, follow the instructions of SOP F.01.

Moving equipment

- It is not allowed to move equipment from ML-II to ML-I labs or vice-versa without permission of the OL/VM.
- It is not allowed to move equipment outside the gmo area /lab without permission of OL/VM (e.g. for maintenance).
- Equipment should be properly decontaminated or disinfected before being transported out of the gmo area, and/or before maintenance or repair. Check and follow the manufacturer’s instructions!

Leaving the GMO lab or area

- Work surfaces must be decontaminated routinely with an effective disinfectant, after work is finished, especially after any spills of viable material, and at least at the end of the day.
- Hands should be washed after finishing the work, removing gloves and before leaving the laboratory. Used gloves are disposed of as contaminated waste.
- Laboratory coats must be left behind in the gmo lab/area when leaving the area.

References and further reading:

- ARBO Informatie blad AI 9 (Biologische agentia) en AI 18 (Laboratoria).
- "Veilig werken met micro-organismen, parasieten en cellen in laboratoria en andere werkruimten, theorie en praktijk", Nederlandse Vereniging voor Microbiologie, derde druk, 2009.
- WIP richtlijnen, zie www.wip.nl, o.a. "Microbiologische veiligheid in diagnostische laboratoria 2013".
- Handreiking Bedrijfshulpverlening bij incidenten en ongevallen met gevaarlijke stoffen van de Universiteit Utrecht
- "Biosafety in Microbiological land Biomedical Laboratories", US Dept of Health services (http://www.cdc.gov/biosafety/publications/bmbl5/)
- NIH guidelines: http://oba.od.nih.gov/rdna/nih_guidelines_oba.html
- OSHA Fact Sheets: https://www.osha.gov/pls/publications/publication.AthruZ?pType=Types (on BSC's, on bloodborne pathogens, on sharps etc.)
- http://isac-net.org/Resources-for-Cytometrists/Biosafety.aspx