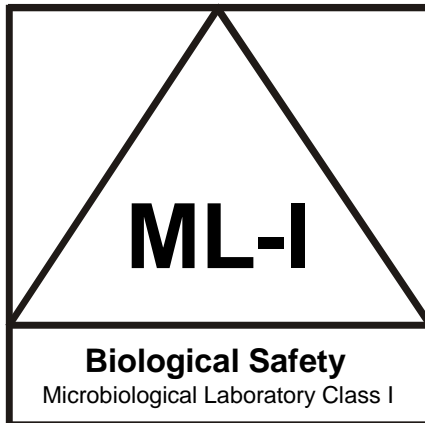


BVF:
tel.
Contactpersoon lab:
Naam
tel.



Short manual for the ML-I, ML-II laboratory (gmo-labs) and/or laboratory for working with biological agents / human materials

Based on the "Handboek Voorschriften voor het veilig werken met genetisch gemodificeerde organismen en biologische agentia" Universiteit Twente 2008 (kenmerk 374.341/PA&O)

2018

To work safely, to have success with the experiments and to have a workable laboratory, it is crucial to know how to work in the ML-I, ML-II laboratory and to be aware of the risks involved when working with genetically modified organisms (GMOs) or biological agents/human material. This manual will introduce people into the basics of the ML-I and ML-II laboratory, as well as into the basic principles of genetic modification. Together with instructions from the Principal Investigator (VM) or research leader (OL) and if necessary the Biological Safety Officer (BVF), one can start handling GMOs in the ML-I, ML-II laboratory at the University of Twente. If you work with animal tissue, see also the schedule safe working with animal tissue

This manual belongs to:

date:

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1. Biological Agents and Risk

Biological risk is a risk difficult to perceive and, similar to the risk from radiation or by genotoxic substances, that provokes damage over time which is difficult to associate to a particular exposure. Countless information exists regarding the danger of physical and chemical agents, but this cannot be said for biological agents.

Biological agents are infectious agents which include bacteria, rickettsia, viruses, yeasts, moulds, single and multicellular parasites and prions. Each species of infectious agent may have subtypes, strains and variants which differ in relation to pathogenic potential, host specificity, transmission, sensitivity to antimicrobial agents etc. Furthermore, biotechnology research laboratories produce a large variety of artificial vectors, with the aim of increasing the probability of genetic transfer between unrelated species. These new DNA fragments can self-recombine and give rise to dangerous products.

In conclusion, although biological agents are well defined by current regulations, it is difficult to perform a complete assessment of the risks associated with their manipulation. Numerous factors such as biological diversity, chemical complexity of the molecules, multiplicity of the diffusion pathways and host interaction specificity must be considered to evaluate the effect of these organisms on the health and the environment.

According to Article 4.84 of the Working Conditions Decree, there are four categories of biological agents (defined as cell cultures, human endoparasites and microorganisms, whether genetically modified or not):

- Category 1: an agent that is unlikely to cause disease in humans;
- Category 2: an agent that can cause a disease in humans and that can be hazardous to the health and safety of employees, but which is unlikely to spread among the population and for which an effective prophylaxis or treatment is available;
- Category 3: an agent that can cause a serious disease in humans and is very hazardous to the health and safety of employees, where the disease is likely to spread among the population, but for which an effective prophylaxis or treatment is available;
- Category 4: an agent that can cause a serious disease in humans and is very hazardous to the health and safety of employees, where the disease is very likely to spread among the population, but no effective prophylaxis or treatment is available.

Biological material originating from humans is potentially dangerous to people who work with it, because of the chance that the material is contaminated with microorganisms that are pathogenic to man, like the hepatitis virus, HIV-virus, etcetera.

The following are considered humane materials:

- blood, lymph, tissues (biopsies) and liquids excreted by the body like urine, saliva, sputum, faeces, sperm, etc.
- materials derived from humane tissues like cells that may or may not have been programmed into cell lines
- microorganisms that have been isolated from the materials listed above.

A genetically modified microorganism is "a microorganism (microbiological entity cellular or non-cellular, capable of replication or of transferring genetic material, including viruses, viroids, animal and plant cells in culture whose genetic material has been modified in an unnatural way, through multiplication or natural recombination."

2. Legislation for the lab and the project

Pathogenic micro-organisms and cells are classified under the definition of the Biological Agents in the Arbo law and Arbo decree. On the basis of information about the specific kind

and root of the micro-organism (commercially obtainable or primary cells, amount of samples per day, volume of tissue culture, used materials etc) a Risk Assessment should be established. For more information you can consult the [UT's working conditions and environmental coordinator \(VGM\) of the faculty.](#)

To start a GMO laboratory, the first thing that should be thought of is legislation from the local government. Without such legislation, no lab can be started. One should contact the BVF (biological safety officer) if a new room should be made ready to for working with biological agencies and /or gmo's.

For all activities with GMOs, the required containment level is stipulated. The containment level depends on the level of risk for people and the environment. Research involving GMOs takes place in specially equipped laboratories, animal facilities, plant growth chambers and greenhouses. A risk assessment (see chapter 3) is conducted to determine the necessary containment level of each room. The VM (principal investigator) can help you with the risk assessment.

Contained use has the aim of preventing the spread of GMOs to limit the hazard of genetic modification for the environment and staff as much as possible. Physical containment refers to containment that can be achieved by placing specific requirements on the laboratory and apparatus.

Physical containment therefore means closing off escape routes by means of special facilities in the workspaces and apparatus.

The following containment levels can be distinguished on the basis of EU Directive 98/81: Microorganisms Laboratory scale ML-I, ML-II and ML-III and IV-ML and (since 2015) new categories of physical containment: ODG and AP-I.

3. Risks

There are three hazards related to genetic modification:

1. An organism could become hazardous as a result of genetic modification. For example, due to the changes in the genetic material, the organism could become pathogenic or could begin to produce toxic substances.
2. A genetically modified organism could disturb the natural balance if the environment is exposed to it. For example, an ecological disturbance could be caused by the spread of genes that provide resistance to antibiotics.
3. It may be possible for the new genes of GMOs to transfer to other, non-modified organisms. As a result, these organisms could become hazardous or could disturb the ecological balance.

A distinction can be made between introducing GMOs into the environment and contained use. Contained use is where activities are conducted in special workspaces that meet specific requirements. All activities with GMOs at the University of Twente concern contained use.

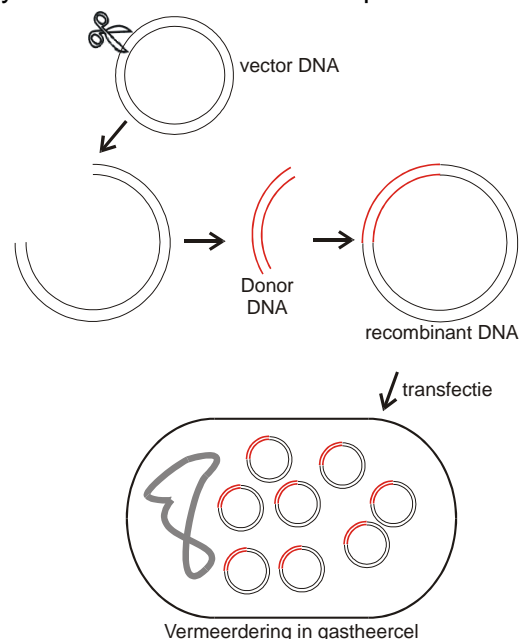
With contained use, measures are taken to prevent the spread of GMOs in order to limit the risk of genetic modification for the environment and for staff as much as possible. Contained use measures have the aim of preventing the spread of GMOs in order to limit the hazards of genetic modification for the environment and the staff as much as possible. There are three types of containment precautions:

1. **Biological containment:** using safe host organisms and vectors, i.e. host organisms and vectors with special properties that limit survival and spread in the environment.
2. **Physical containment:** sealing off any escape routes for the GMOs. The activities may only take place in laboratories, animal facilities, storage spaces, greenhouses etc, which are made suitable for such activities by installing special facilities and placing special demands on the apparatus. These rooms must be closed.
3. **Safe working procedure:** the application of Safe Microbiological Technique, i.e. special procedures and techniques that limit the spread of GMOs.

These safety precautions prevent GMOs from escaping from the workspace.

The risk of working with GMO's for employee and environment are dependent on the features of the inserted DNA. Risk can be relatively small because 'cripple' organisms like E.coli K12 are used. These organisms are not pathogenic anymore and won't survive long outside a laboratory. Besides, these organisms are only allowed to be handled in specific rooms. In these cases one speaks of contained use.

Theoretically a GMO is separable into: the host organism, in which the genetic information is inserted; the donor organism, from which the genetic information has been obtained; the vector which transfers the information between these organisms; the insert which contains one or more genes capable of displaying biological activity. Each of these parts, together with the final constructs must be taken into consideration in order to obtain accurate and proper risk assessment. Vectors are, by definition, plasmids, bacteriophages, viruses and other DNA elements



with a capacity for autonomous replication, in which a fragment of foreign DNA is inserted. A vector contains one or more replication sites and genes which confer a few phenotypic characters to the cells that contain it (e.g. antibiotic resistance). The latter makes sure that the modified cells can be separated from the unmodified cells. The vector may or may not integrate into the host DNA.

The classification of the biological safety level of activities involving GMOs where a microorganism is used as a host, a donor or vector is based partly on the degree of pathogenicity of this organism. There are four classes of microorganisms, the first class being non-pathogenic. The criteria used for classifying the microorganisms are listed in chapter 1. These criteria are also applied for the classification of pathogens which are not microorganisms, such as a number of parasites. In [Annex 4](#) of the regulation on GMO's the degree of pathogenicity is listed for many bacteria, fungi, yeasts, viruses and parasites.

- Donor sequence

In cloning, also when synthetically produced genetic material is involved, the donor of the genetic material is defined as the organism from which the relevant information originates. For example, a gene isolated from a human genome is still considered to be a human gene, even when it is isolated from a prokaryotic host in which the gene is present as a clone. The characterisation of the donor sequence and the hazard level of the genetic product which is coded by the donor sequence is also important for biological safety classification.

- Other properties important for classification

Other properties important for classification include the containment of the host/vector system the production of a toxin or the absence thereof, whether the virus being used is defective or biologically contained, whether animal cells are used in combination with a viral vector or not, and the use of plant cells.

Prior to the contained use of GMOs that have not been previously notified, a risk assessment should be made of the risks associated with the work of these GMOs. The risk assessment is done in accordance with Annex 5 of the Regulation on GMOs. Bureau GGO has developed a risk assessment tool for this. The tool is an aid in making a proper risk assessment and contributes to the proper applications for new activities or modifying existing activities.

The tool helps in determining the appropriate category of physical containment and thereby leads to the appropriate application form. Additionally, the tool helps in filling out the legally required risk assessment report which is part of the application / notification.

In the risk assessment tool the elements 'hosts', 'vectors', 'donor sequences' and 'activities' are described. In the activity table is determined which classification section of Annex 5, part I and consequently what category of physical containment is applicable to the work and which additional requirements for specific cases are applicable.

The risk assessment uses [Annex 2 \(List A1, list of non-pathogenic microorganisms, table A2, list of vectors, list A3, list of potentially harmful sequences\)](#) and [Annex 4 \(classification system for pathogenic micro-organisms and viruses\)](#) of the GMO regulation.

If [Annex 5](#) will not lead to an appropriate scaling or scaling down is desired, a request through the [2.8 form](#) is needed.

The risk of working with cell lines is determined by the micro organisms which could infect them. Even tumor cell lines seem not to be harmful for humans. Only cancer cell lines of personal cells might be dangerous. In the Arbo decree the pathogenic micro organisms (PMO's) are classified in risk category 1 to 4, in which 1 is the lowest risk category. The suppliers of the cell lines use the same classification, depending on the PMO in the cells. If the cell lines have been extracted from primary human or animal cells the risk classification can be gathered from the tissue of the cells, f.i human liver

cells can be contaminated with the hepatitis virus, human white blood cells with HIV.

The risk of working with blood is also determined by the possibility of contaminations. If not screened, blood should be considered hazardous/contaminated.

Within the UT human blood is used frequently. This blood may be contaminated by viruses such as the hepatitis B or hepatitis C virus and can thus pose a health hazard. Especially direct blood-to-blood contact is risky in this context.

When working with blood:

- there is a risk of pricking or cutting (pricking or cutting accident) with needles, razors or glass. This may cause (possibly contaminated) blood to end up directly in the bloodstream.
- possibly contaminated material may come into contact with damaged skin such as small wounds, splits, spots of eczema, etc.
- possibly contaminated material may come into contact with the mucous membranes, for example as a result of spattering in the eye or mouth.

Blood should at all times be considered as being possibly contaminated with infectious micro-organisms. To avoid contamination proper hygiene and avoiding the creation of aerosols by working calmly is important when working with blood. The work and arrangement regulations for working at ML-I level are therefore applicable (see chapter 5). Activities involving blood whereby aerosols may be created, are always to be carried out in a category II biological safety cabinet. In case of small wounds or chapped skin, gloves should be used for any handling of blood. All staff members who work with blood are given the opportunity to be vaccinated against hepatitis B.

What to do in case of pricking and cutting accidents:

- let the wound bleed out thoroughly;
- rinse with water;
- disinfection with betadine iodine or chloorhexidine 0,5% in 70% alcohol.

In case of spattering accidents:

- rinse with water;
- disinfection with betadine iodine or chloorhexidine 0,5% in 70% alcohol.

Follow-up in case of all accidents:

- if possible keep the source material and immediately contact the occupational health officer (088-2726312) to discuss the follow-up.

Fill in a [registrationform](#) for accidents, incident and unsafe situations.

4. Introduction of new staff members

In order to guarantee the expertise of staff members regarding working with GMOs, all staff members working with GMOs must be registered as project staff or if they do not meet the training requirements but do meet the conditions laid down in the Handbook GMO and BA University of Twente for other staff, they must be registered as temporary staff. Project staff may only carry out their activities after the VM/OL/BVF has labelled them to be competent for performing the relevant activities.

Before starting the activities the VM/OL will provide new staff members who will carry out GMO activities with information. The subjects to be covered will depend on the situation. Below you can find a list with subjects that are relevant and necessary in most cases.

Checklist for the introduction of new staff members

GENERAL DETAILS			
Name:			
Date of arrival:		Date of leaving:	
Name VM/OL:			
Number(s) of project(s) that will be worked on:			
WORK REGULATIONS AND PROCEDURES (GMO) LABORATORIES			
Has the general safety instruction been followed?	Yes / no / Irrelevant		
Has a VMT course been completed?	Yes / no / Irrelevant		
Have the internal rules been communicated?	Yes / no / Irrelevant		
Have the methods for disinfecting been explained?	Yes / no / Irrelevant		
Has it been explained how to act in case of calamities?	Yes / no / Irrelevant		
Has it been explained how contaminated material is to be removed?	Yes / no / Irrelevant		
Is it known how GMOs are to be transported?	Yes / no / Irrelevant		
Has it been explained in what way materials are to be stored?	Yes / no / Irrelevant		
Has the entrance regulation been explained?	Yes / no / Irrelevant		
Is it known which organisms are pathogenic?	Yes / no / Irrelevant		
.....	Yes / no / Irrelevant		
.....	Yes / no / Irrelevant		
TRAINING IN REQUIRED TECHNIQUES			
Does the staff member know how the steriliser works?	Yes / no / Irrelevant		
Is the staff member able to handle containment equipment safely?	Yes / no / Irrelevant		
.....	Yes / no / Irrelevant		
.....	Yes / no / Irrelevant		
COMPULSORY TRAINING PROGRAMME			
Reading through condensed manual and the GMO permits.	Yes / no / Irrelevant		
VROM video	Yes / no / Irrelevant		

Initials VM/OL for providing explanation to the above regulations and procedures:		Date:	
Initials staff member for receiving explanation to the above regulations and procedures:		Date:	

5. General handling regulations for handling biological agents and GMOs

5.1 ML-I (minimum containment level)

general

- a. The work space is to be kept clean and tidy;
- b. Eating, drinking and smoking, carrying food or drink, applying cosmetics or putting in contact lenses and storing food and drink in the work space is prohibited. Avoid hand-face contact.
- c. Using a pipette with the mouth is prohibited;
- d. Vermin is not allowed in the work space;
- e. Personal possessions, like coat, sweater, or bag must be put away outside the workspace;
- f. After contamination the contaminated surfaces are to be disinfected immediately;
- g. Clothing contaminated as a result of spilling or accidents involving genetically modified organisms or biological agents will need to be autoclaved immediately before washing;
- With the exception of students/students on work placement only persons who have a form signed by the VM/OL/BVF to the effect that in the opinion of the VM/OL/BVF they are sufficiently cognisant with safe microbiological techniques, may work with GMOs in the laboratory. Students/students on work placement may only work under professional supervision.
- Besides administration of the experimental data, no other administrative work is allowed.
- Before commencing the activities sufficient disinfectant must be ready for use. The disinfectant has to be renewed regularly and its effectiveness has to be checked regularly.
- Wearing personal protective gear including gloves, safety goggles etc. is not always necessary from a microbiological perspective. However, it is compulsory for chemical reasons (use of disinfectants, solvents). If ones skin is not in optimal condition or when the experiment does so require, disposable gloves should be worn.
- Wearing a headscarf for religious reasons, is only allowed when it meets the following rules:
 - Wear the headscarf so the material of the headscarf doesn't come into contact with (research) material or other objects in the lab.
 - If you wear a headscarf it should be short (no longer than until the collar of the lab coat) and clean. The headscarf should not hang over the protective clothing (lab coat).
 - Do not wear a headscarf of flammable material (think of work with a burner or open fire).
 - The headscarf should be replaced daily by a clean headscarf.
 - The headscarf should be washed daily at a temperature of 60 °C or higher.

during work

- h. During the work the doors and windows of the work space must be closed;
- i. During all activities the creation and circulation of aerosols must be avoided, for example by waiting several minutes with the opening of tubes until the aerosols have been settled down, leaving the graft needles to dry before flaming, and draining the pipette against the lining of a tube instead of using force by pressing the pipette;
- j. Fitting working clothes (laboratory coat) are to be worn. It is prohibited to wear these working clothes outside the work space. Each space has its own set of laboratory coats.

Lab coats belonging to a work space are not to be worn outside this space or taken to another space;

- k. Wearing wrist watches and jewellery on arms or hands is prohibited.
- l. The use of hypodermic needles and syringes is forbidden unless this is absolutely necessary for the experiment. Only when the use of hypodermic needles is necessary, keep the following rules in mind:
 - Only use disposable hypodermic needles and syringes
 - The syringe must be filled carefully so that no air bubbles occur
 - When emptying the syringe, the point of the needle must be held in the liquid.
 - After use, the needles and syringes must be placed directly into a sharps bin for disposal.
 - Do not replace the needle in the plastic protective sheath.

ending the activities

- m. The work surfaces are disinfected at the end of the work and at the end of each working day;
- n. Upon leaving of the work space, hands should be washed using soap;

waste and contaminated material

- o. All biological waste is collected in blue SZA (specific hospital waste) drums that can be locked and inactivated before being disposed of. This means storing followed by offering for combustion;
- p. Material that has been in touch with genetically modified organisms/biological agents is inactivated or disinfected before washing, reuse or removal as waste (see chapter 12);

other

- q. In case of simultaneous activities with non-genetically modified organisms the ML-I handling regulations are to be observed;

Logbook (only for gmo's)

Each ML-I, ML-II space is to have a logbook containing the following data:

- A list of names of persons having permission to work in the space (project staff, guests and students on work placement), whereby a distinction is made between GMO activities and non-GMO activities;
- A list of the numbers of the permits used in that particular space and a copy(ies) of the GMO permit(s);
- The dates and results of the checking of the cleanliness or the proper identity of the micro-organisms used;
- General workplace regulations and workplace regulations set by the VM/OL;
- An overview of the GMOs present per storage space;
- A list of hosts, vectors and sources of the DNA being used. These data are also to be kept during current projects and for a minimum of 5 years afterwards;
- A brief description of incidents in the GMO laboratory.

5.2 Additional handling regulations for ML-II laboratories

- A marked laboratory coat is to be used at all times. Before washing, the coat is to be sterilised.
- If no staff members are present, the work space is locked.
- Aerosol producing equipment is to be placed in the biological safety cabinet during use. If a pump or vacuum pipe is frequently used, it is recommended to protect it against aerosols released.

- Before materials, instruments or clothing such as lab coats are washed, reused or discarded, they need to be sterilised using an autoclave or if this is not possible, decontamination using a method the effectiveness of which has been proven.
- In working with GMOs or pathogenic micro-organisms a biological safety cabinet category II is to be used as much as possible. Activities that may involve aerosols being created, are always carried out in a category II biological safety cabinet.
- All biological waste, as well as waste that has been in contact with biological material, is to be sterilised or decontaminated before removal, or to be removed as specific hospital waste in breaking resistant, leaking resistant and airtight waste containers.

5.3 AP-I space

AP-I is an equipment room where small-scale operations (for example microscopy) with GMOs from level ML-I may be conducted in closed unit. In the ML-I lab the GMO's are brought into the closed unit and then transported to the AP-I space.

This instruction is intended as a general guidance for AP-I areas and, if necessary, has to be complemented by specific requirements in respect of the work to be performed.

- Access is limited to those who are aware of the risks of work;
- Personal possessions, like coat, sweater, or bag must be put away outside the workspace;
- The transport of GMOs between the ML-I lab and the AP-I space takes place in a sealed, unbreakable, leakproof container that is decontaminated on the outside before transport to the AP-I space;
- During the work the doors and windows of the work space must be closed;
- The work space is to be kept clean and tidy;
- Vermin is not allowed in the work space;
- Eating, drinking and smoking, carrying food or drink, applying cosmetics or putting in Contact lenses and storing food and drink in the work space is prohibited;
- While working with GMOs wear a lab coat;
- Avoid hand-face contact;
- After contamination, contaminated surfaces or materials are disinfected immediately;
- Biological waste is collected in blue SZA (specific hospital waste) drums. When the drum is full, it is sealed with a yellow lid. The outside of the drum is disinfected with an appropriate disinfectant (standard 70% ethanol).

5.4 ODG space

ODG (Other Part GMO area) is a space that has no physical containment, but where GMOs storage take place (storage of GMOs from level ML-I or ML-II of waste containing GMOs from level ML-I or ML-II).

- Access is limited to those who are aware of the risks of work;
- The work space is to be kept clean and tidy;
- After contamination, contaminated surfaces or materials are disinfected immediately;
- GMO waste is stored in blue SZA (specific hospital waste) drums with a sealed yellow lid.
- GMOs are packed in a closed unbreakable, leak-proof, double holder. The outer container in which the GMOs are packaged is labeled "GMO" (for ML-II also the biohazard sign). If in the same repository also non GMOs are stored, these are packaged separately.

6. Use of disinfectants

Materials that have been in contact with GMOs and/or biological agents are to be disinfected immediately after use, preferably in an autoclave. If this is not possible, disinfectants should be used. It should be ascertained whether a disinfectant or autoclave method is suitable for the organism in question. Below, guidelines are provided for this. Depending on the situation the regulations are to be specified in more detail. The disinfecting method used must have been validated. Table 1 (source: Maastricht University) shows an overview of frequently used disinfectants and their sphere of action.

Disinfecting of surfaces (such as table tops, handles, buttons, pipettes and floors)

- Only use disinfectants when necessary, for example after spilling or to clean a table top after an experiment. Surfaces not contaminated with pathogenics can best be cleaned with hot water and soap.
- Suitable disinfectants are:
 - 70% alcohol, drying by airing (not for traces)
 - Active chlorine solution (1000 ppm). The disinfecting activity of the chlorine solution strongly deteriorates after time.

Please note: chlorine is harmful to HEPA filters and erodes metals.

Disinfecting of skin (betadine soap may also be used)

When hands become contaminated during work, disinfecting is necessary. First the hands are to be washed during two minutes with water and soap, followed by disinfecting with disinfectants, e.g. 70% alcohol or better: 0.5% chlorohexidine in 70% alcohol.

Table 1: Cross tab report with disinfection methods for different types of GMO and PMO

Method	Working time (min)	Bacteria			Viruses		Yeasts/ moulds	Animal cells
		Vegetative, (e.g. <i>E. coli</i> K12)	Spores (e.g. clostridia)	Mycobacteria	Enveloped (lipophilic) e.g. lentivirus, vaccinia, influenza	Non-enveloped (hydrophilic) e.g. adenovirus bacteriophage M13		
Chlorine compounds (hypochlorite/dichloroisocyanuric acid)* (0.01-5%)	10-30	+	+(0.5%)	+(0.1%)	+	+(0.1%)	+	+
Ethanol (70%)	10-30	+	-	+	+	-	+	+
Isopropanol (70%)	10-30	+	-	-	-	-		
1-propanol (70%)	10	+	-	-	+	+		
Hydrogen peroxide	1-30	+	+	+	+	+	+	+
Quats** (0.1-2%)	10-30	+	-	-	-	-	+	
SDS (0.1-0.5%)	10				+	+(0.1% HIV; 0.5% adenovirus)		
Virkon S* (1%)	10	+	+	+	+	+	+	
Clidox ,Tristel or Twinoxide (sodium dioxide)) (acc. to manufacturer)*	10	+	+	+	+	+	+	
Terralin Protect (according to manufacturer)	5-15	+	-	+	+	+	+	

Method	Working time (min)	Bacteria			Viruses		Yeasts/ moulds	Animal cells
		Vegetative, (e.g. <i>E. coli</i> K12)	Spores (e.g. clostridia)	Mycobacteria	Enveloped (lipophilic) e.g. lentivirus, vaccinia, influenza	Non-enveloped (hydrophilic) e.g. adenovirus bacteriophage M13		
Caustic soda (1 M NaOH)	10	+			+?	+ (polio virus)	+?	+?
Water	10	-	-	-	-	-	-	+?
Thermal disinfection (autoclave)	15', 121°C	+	+(60 min)	+	+	+	+	+
	10', 134°C	+	+(30 min)	+	+	+	+	+
	30', 100 °C#	+	-	-	-	+(80°C)	+(60°C)	+
Thermal disinfection (dry heat)	160°C for 2 hours or 180°C for 1 hour	+	+	+	+	+	+	+
Membrane filtration		0.22 µm	0.22	0.22	0.22 µm (e.g. Planova 15N)	0.02 µm	0.45 µm	10 µm
Hand disinfectants with 70% ethanol like stokosept	30 sec	+	-	+	+	-	+	
Hand disinfectants with 80% ethanol such as Manugard Hand Rub	30 sec	+	-	+	+	+		

+ = good efficacy - = not effective Blank = unknown
+? Possibly good efficacy, but not published

* Aspecific reaction, therefore also for organisms that cannot be cultivated such as norovirus, etc.

**quats = quaternary ammonium compounds

7. Cleaning activities in ML-I and ML-II spaces

Cleaning staff is to be cognisant with the instructions relating to cleaning activities in ML-I and ML-II laboratories. For each laboratory it is established which cleaning activities are carried out by cleaning staff and what is cleaned by own staff.

The following rules are applicable to the cleaning:

- Instruction cleaning staff by VM/OL or BVF.
- Cleaning staff is to wear a white coat and disposable gloves during the cleaning activities.
- If a permanent employee is replaced this should be reported to the VM/OL.
- The floors are to be wet cleaned once a week (possibly during own duty chores), optionally alternating a (chlorite) disinfectant per week and water and soap the other week.
- Cleaning staff is not allowed to clean tables and equipment, only kitchen tops and sinks.
- It is required to use separate cleaning tools for contained spaces (separate trolleys). These are not to be used in other labs.
- The water used for cleaning activities is to be removed after use in the sink of the laboratory in question.
- Cleaning staff is not allowed to remove containers for biological waste from the laboratory.
- Only the waste packed in waste bags may be removed by cleaning staff. Removal is to take place twice a week.
- Any heaped waste, as well as gloves worn, are to be considered as contaminated waste and must therefore be put into the containers for biological waste in the laboratory.
- In case of doubt the VM/OL or BVF is to be consulted.

On the following page you will find the cleaning schedule for the (GMO) lab where you work.

8. Rules for cleaning, lab coats and maintenance

Per laboratory space further rules are set for cleaning and maintenance.
All this needs to be recorded as such in the logbook.

The ML-I space (space-no.....) is cleaned according to the schedule below (example).

Cleaning	Soap/desinfectant	Frequence	By whom?
Workspace/Bench (Biological safety cabinet)	70%-alcohol	Immediately after spilling	user
Workspace/Bench (Biological safety cabinet)	70%-alcohol	Immediately after end of activitiy	user
Floor	Water and soap	Weekly	Cleaning personel
Wall & doors	Water and soap	Monthly	
Water baths ¹⁾	Alconox / prothermal	Weekly	
Shaker	70%-alcohol	Weekly	
Other equipment	70%-alcohol	Immediately after use	user
37 °C stove ²⁾	70%-alcohol	Monthly	
Centrifuges	70%-alcohol	Monthly	
Furniture	70%-alcohol	Half yearly	
Fridge	70%-alcohol	Monthly	
Freezer	70%-alcohol	Monthly	
Waste		Frequence	By whom?
Removal of contaminated waste		Weekly	
Removal of normal waste		Weekly	Cleaning personel
Cleaning of labcoats		Twice a month	
Maintenance		Frequence	By whom?
Biological safety cabinet		Yearly	PMV

1) Refresh the water regularly and add disinfectants (f.i. Prothermal or Aquaclean).

2) To prevent fungus growth in the water pan a copper water pan can be used or use a piece of copper (5 eurocents). Refresh the water of the water pan in the CO2-stifle regularly and add disinfectants (f.i. Prothermal or Aquaclean).

9. Use of biological safety cabinet and centrifuge

Self-containment equipment and biological safety cabinets are used to prevent as much as possible the creation and circulation of aerosols. The creation of aerosols during centrifuging is often underestimated. For a proper functioning this equipment is to be properly maintained and periodically tested. Instructions for use and handling regulations of equipment are to be present in the workplace.

Biological safety cabinet

When working with GMOs or pathogenic micro-organisms a biological safety cabinet category 2 offers both product and personal protection. All aerosol producing activities are to be carried out in a biological safety cabinet. A cross-flow cupboard only offers product protection, and therefore cannot be used in ML-II laboratories and more contained laboratories. A biological safety cabinet category 2 gives a proper protection against releasing aerosols through a protective air curtain in the work opening and a proper protection of the experiment through a laminar down flow. The working rules are:

- Wear a tightly closed lab coat with cuffs or sleeve caps
- Let the Cabinet run at least 10 minutes before you start, so that the laminar air flow at all is set and only sterile air is blown in the Cabinet
- Don't wear rings, watches and bracelets and wear gloves if necessary
- Collect the materials required for the experiment in advance.
- Wipe the table top and air currents 10 minutes after switching on the biological safety cabinet using 70% alcohol.
- Bring the required materials into the work space of the biological safety cabinet after wiping this with 70% alcohol.
- Check the proper functioning of the biological safety cabinet. Disrupt the air current as little as possible when performing the activities (calm arm movements).
- Always keep the exhaust splits at the front and back of the work top free.
- Avoid the use of Bunsen burners.
- Avoid walking past the biological safety cabinet and open the laboratory doors as little as possible.
- Collect all disposable contaminated waste in a small box or bag in the safety cabinet. When the activities are finished, put the closed box/bag into the blue SZA drum.
- Disinfect the work surface of the cupboard after the work.
- After you have finished your activities keep the ventilator of the cabinet on for at least 5 minutes.
- Fill in the logbook of the biological safety cabinet.

Particulars:

- If the cabinet's ventilation falls out (e.g. in case of a power interruption), close immediately any opened tubes etc. and close the work opening.

Centrifuge

Centrifuges can be a cause of contamination due to the breaking of centrifuge tubes, or centrifuge tubes that have not been properly closed or leaking centrifuge tubes. In order to avoid this, the following rules must be observed:

- When possible, use centrifuges with lockable rotor or lockable buckets (self-containment centrifuges).
- If possible, use unbreakable tubes or holders that can be properly closed and check these before use on cracks or other irregularities.
- Check if the centrifuge still closed properly (valve rubbers).

- Never fill the centrifuge tubes up to the level that the liquid can reach the lid during centrifuging. With angle rotors the nominal volume is always smaller due to the slanting position. If necessary first test with water and mark the maximum filling level.
- After centrifuging you have to wait several minutes to let the aerosols subside. After this check the tubes for leaking.
- If necessary open centrifuge in the biological safety cabinet (e.g. in case of leakage).
- Clean and disinfect rotors and inside of centrifuge and lid after each use.

10. Reporting of accidents and incidents

- An **incident** is an unexpected, unplanned and unforeseen event, i.e.:
 - Every event where it is certain or probable that GMOs and/or biological agents have entered the environment.
 - Every event where GMOs and/or biological agents (may) have been released outside the contained work space/installation, even if this has not resulted in emission to the environment.
- An **accident** is an incident whereby personal "injury", contamination or wounds have occurred.
- **Calamities** are: incidents such as fire, explosions, leakage, escape of inflammable or toxic substances and technical failure that may cause a calamity.

General

- In each possible situation such measures need to be taken that an extension of the contamination, release or injury is avoided.
- In case of an accident, incident or calamity the VM/OL or BVF is to be warned. Accidents and/or incidents are to be reported to the BVF. [See registrationform for accidents, incidents and unsafe situations.](#) Reports are to be recorded in the logbook.

Task staff

Main tasks

- To take such measures that extension of the contamination, release or injury is avoided as much as possible.
- Reporting to the BVF and VM/OL (first contact as stated in the permit or if present the second contact)

Followed by

- **In case of incidents:**

Disinfect work space (see chapter 11).

- **In case of accidents:**

In case of slight injury, e.g. a pricking accident, let the wound be encouraged to bleed. Disinfect the skin and wounds with 70 % ethanol.

Use an eye shower in case of contamination of the eyes. Always contact the occupational health officer (tel. 088-2726312).

In case of serious injury: **call 2222**: state name, location of accident, type of injury.

- **In case of calamities:**

Fire: report fire: press fire alarm or **call 2222**: state name and faculty, location of the fire (space) and what is burning

Technical failure that may possibly result in an accident: bring persons into safety or ask others to do so and report failure.

11. Decontamination after spilling of potentially contaminated material.

Limited contamination

- First wash your hands (perhaps also your face) and change laboratory clothing.
- Put on gloves.
- Remove as many obstacles as possible from the contaminated place.
- Cover the contaminated place with tissues that have been moistened with disinfectant. Wait at least 10 minutes. Remove the tissues.
- Sweep up the material using tissues. Work from outside to inside. All the waste material should always be disinfected by autoclaving/sterilisation or put in the blue waste drums for specific hospital waste (SZA).
- Then clean the surface using water and soap. After these activities wash your hands thoroughly.

In case of spilling of large quantities (e.g. breaking 1-litre flask)

- Remove as many obstacles as possible from the contaminated place (and clear the space if possible).
- If possible wait 15 minutes to let the aerosols subside (depending on the organisms and pathogenic used) and ask a colleague for help.
- First wash your hands (perhaps also your face) and change laboratory clothing if this has become contaminated.
- Wear gloves.
- Be careful for fragments of glass and other sharp materials.
- Make sure that the contamination does not spread any further by pouring a ring of disinfectant around the place of spillage.
- Put cloths soaked in disinfectant on the place of spillage and let the disinfectant work into it for 10 minutes.
- Pick up the contaminated cloths using tweezers and put them in a waste container.
- Cover (if necessary) the materials and surfaces with tissue or filter paper to absorb the liquid and repeat this until all liquid has been absorbed.
- Put all the waste (including glasswork and such) in the blue waste drums for specific hospital waste (SZA).
- Sweep the place of spillage using cloths soaked in disinfectant, from the outside to the inside.
- Mop the place of spillage using warm water and soap.
- In case of contamination of laboratory clothing disinfect immediately by autoclaving/sterilisation.
- All the waste material (including cloths and mopping water) is to be decontaminated, preferably by autoclaving/sterilisation.
- Wash contaminated parts of the skin using soap and disinfectant.

12. Contaminated material and waste, storage and transport

All the biological waste (intact cells or microbes), including materials or (potentially) contaminated instruments are to be inactivated or decontaminated, before further handling, i.e. washing or reuse or before it is removed as waste. This will take place as quickly as possible and at least once a week, preferably by autoclaving/sterilisation. Prior to the inactivation/decontamination the waste and (potentially) contaminated material is at all times to be locked up and stored, labelled with a proper label. Any transport to the autoclave takes place in a closed, unbreakable and leak-tight container.

Contaminated material

- **Heat resistant and non-heat resistant, reusable material**

Depending on the type of organism that was present in the reusable material, the correct disinfectant is chosen from the table in chapter 6). 70% Ethanol and an active chlorine solution (1000 ppm) are the most common.

Waste

- **Solid waste (culture plastic, tissues, agar plates)**

is collected in SZA drums (blue waste drums for specific hospital waste).

- **Liquid culture waste**

is collected in a container with sodium hydroxide (50 gram for 2 litres) or household bleach (0,1% activated chlorine) (let stand for at least 20 minutes). If it is full, the contents are poured in a waste barrel (5-litre jerry can) and removed as hazardous waste. In case of a watery solution (pH between 6 and 9 and no addition of other chemical substances) the solution can be emptied via the sewer.

Liquid wastes may also be contaminated with 0.1% active chlorine in water. The disinfectant activity of chlorine is declining dramatically in a couple of days. Therefore replace the collection bottle preferably daily.

Storage of waste

The blue waste drums for specific hospital waste (SZA) are collected once a week by the UT's external waste collector. Storage takes place in the appropriate space (see § 5.4). The outside of the drums are to be disinfected (70% ethanol) and labelled. The drums must be reported to the waste contact person of the building via a "waste hazardous substances form". See the schedule removal of waste and hazardous waste of the University of Twente (361.831/PA&O).

Internal transport

For the internal transport of preparations a Bio-Transport carriage system is used. This is a locked system (using clips), break-resistant, transparent and can be autoclaved/sterilised. Before transport from the laboratory and after use it is to be disinfected in the laboratory.