



**Ciências
ULisboa**

Faculdade
de Ciências
da Universidade
de Lisboa

Approved by the Director

LABORATORY BIOLOGICAL SAFETY HANDBOOK



	INTERNAL SECURITY PLAN - PREVENTION PLAN	
	ACTION PLAN - CONFINED USE OF GMM/GMO	Date: oct 25
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1. ABBREVIATIONS

AAV - Adeno-associated viruses

ACT – Autoridade para as Condições do Trabalho

APA – Agência Portuguesa do Ambiente

LFC – Laminar Flow Cabinet

BSC – Biological Safety Cabinet

CSB.C – Biological Safety Commission of CIÊNCIAS ULisboa

DGAV – Direção-Geral de Alimentação e Veterinária

DGS – Direção-Geral da Saúde

DNA - Deoxyribonucleic acid

DOB – Date of Birth

PPE – Personal Protective Equipment

G3S – Safety, Health and Sustainability Office of CIÊNCIAS ULisboa

GM – Genetically Modified

HEPA – High Efficiency Particulate Air

IGAMAOT – Inspeção-Geral da Agricultura, do Mar, do Ambiente e do Ordenamento do Território

INSA – Instituto Nacional de Saúde Doutor Ricardo Jorge

LD - Lethal Dose

GMM – Genetically Modified Microorganism(s)

NIH – National Institutes of Health

BSLAF - Biological Safety Level of Animal Facilities

GMO – Genetically Modified Organism(s)

IBEP – Internal Biological Emergency Plan of CIÊNCIAS ULisboa

SOP – Standard Operation Procedure

ORBEA - Body Responsible for Animal Welfare

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2. GLOSSARY

Accident – Any incident involving a significant and unintentional release of GMM or GMO during their contained use, which may endanger, with immediate or delayed effect, human health or the environment.

Laminar Flow Cabinet (LFC) - Equipment designed to protect the **product** from contamination of particles present in the air. Provides a one-way flow of air filtered by HEPA filters (typically horizontal or vertical) over the work area, creating a sterile environment. Ensures that the product/handling is done in a clean environment. They are used in processes where the sterility of the product is essential, but where there is no risk of infectious agents.

Biological Safety Cabinet (BSC) - Equipment designed to protect the **operator, the environment and the product** from infectious and dangerous agents. They function as partial containment systems, based on directional air flow to ensure containment. The flow controls the directional movement of the air (through HEPA filters) to ensure that the operator and the environment are not exposed to aerosols or hazardous substances generated during handling. They are used in laboratories that handle potentially hazardous biological materials.

Incident – Any situation involving spillage, release or exposure to GMM or GMO during its contained use, without the occurrence of a significant release, which certainly does not endanger, with immediate or delayed effect, human health or the environment.

Lethal Dose 50 (LD50) – Amount of toxin that causes death to 50% of exposed animals.

Microorganism – Any microbiological entity, cellular or non-cellular, capable of replication or transfer of genetic material, including viruses and animal and plant cells in culture.

Laboratory Biological Safety Handbook of CIÊNCIAS ULisboa – Document in which the prevention measures to be adopted in association with the contained use of GMO/GMM in the CIÊNCIAS facilities are indicated, namely the organization, the human and material resources involved, the rules, the good practices and the procedures to be adopted.

GMM – Microorganism whose genetic material has been modified by a form of sexual reproduction and/or natural recombination that does not occur in nature.

GMO – Any organism, other than humans, whose genetic material has been modified in a way that does not occur naturally through interbreeding or natural recombination.

Internal Biological Emergency Plan of CIÊNCIAS ULisboa – Document in which the self-protection measures to be adopted in association with the contained use of GMOs/GMMs are indicated, to deal with a situation of spillage, release or exposure to agents that involve biological risk in the CIÊNCIAS facilities, namely the organization, the human and material resources to be involved and the procedures to be followed in this situation.

Contained use – any activity resulting in the genetic modification of GMM or GMO, in which they are cultivated, stored, transported, maintained, bred, destroyed, disposed of or otherwise used, using specific containment measures, with the aim of limiting their contact with the general population and the environment, ensuring a high level of safety.

User – Any person, or legal entity, responsible for the contained use of GMM or GMO.

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3. GENERAL PRINCIPLES

This handbook contains essential information on good laboratory practices in order to work with genetically modified organisms (GMOs) and genetically modified microorganisms (GMMs). The rules here presented are in line with Director's Resolution D/16/2022 regarding the *General safety rules in the laboratory* (Annex I - Order D/16/2022 General laboratory safety rules). Reading this handbook as well as completing the GMO/GMM Work Assessment Document (Annex II - GMO/GMM Work Assessment Document) should be carried out by all researchers and students working with biological samples or when starting to work with a new type of biological sample.

The first part of this handbook (Chapters 3 to 9) includes all the information, procedures and good practices to be considered in the different types of biohazard facilities existing in CIÊNCIAS ULisboa. The second part (Chapter 10) includes the procedure to be followed for requesting authorization for the contained use of GMO/GMM and the periodic actions to be taken while the authorisation remains in effect.

This handbook focuses on biosafety levels 1 and 2, as they are the levels currently being operated at CIÊNCIAS.

3.1. Classification/Definition of GMO/GMM

(According to Article 3, Decree-Law No. 55/2015, of 17 April)

A genetically modified organism (GMO) is any organism, other than humans, whose genetic material has been modified in a way that does not occur naturally through crossbreeding or natural recombination. A genetically modified microorganism (GMM) is a microorganism whose genetic material has been modified by a form of sexual reproduction and/or natural recombination that does not occur in nature.

Living organisms, when released into the environment, for experimental purposes or in the form of commercialized products, and/or as a result of their use/manipulation, are likely to reproduce in the environment, with effects that may be irreversible.

In order to ensure the sustainable progress and safe use of GMO, the European Union has established a regulatory framework to ensure the protection of human health, ecosystems and agriculture from the potential risks associated with the marketing and use of GMO.

In this context, and guided by the precautionary principle, obligations have been established to assess and control the risks resulting from the deliberate release into the environment and rules for marketing, cultivation and testing of GMO, as well as for the contained use of GMM/GMO.

3.2. Contained use (definition)

(According to the Agência Portuguesa do Ambiente, APA)

The contained use of genetically modified microorganisms (GMM) or genetically modified organisms (GMO) is any activity which results in the genetic modification of GMM or GMO or in which they are cultivated, stored, transported, maintained, created, destroyed, disposed of or otherwise used, using

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specific containment measures, with the aim of limiting their contact with the general population and the environment, ensuring a high level of safety.

In view of the potential risks to human health and the environment, the contained use of GMM and GMO is subject to strict national and Community standards for the assessment and management of these risks.

The contained use of GMM/GMO thus requires the risk assessment and risk management to human health and the environment, and requires specific containment measures, with the aim of limiting contact with the population and the environment, ensuring an adequate level of safety.

3.3. Classification of Confined Use Operations

(According to Article 7, Decree-Law No. 55/2015, of 17 April)

1 - Confined use operations shall be classified into classes, according to the risk inherent to the operation and in accordance with Annex III to Decree-Law No. 55/2015, which correspond to the levels of containment deemed necessary for the protection of human health and the environment, in accordance with Annex IV of the same Decree-Law:

- (a) 'Class 1' means operations of zero or negligible risk, where a level 1 containment is sufficient;
- (b) 'Class 2' means low-risk operations where a level 2 containment is required;
- (c) 'Class 3' means operations of moderate risk, where a level 3 containment is required;
- (d) 'Class 4' means high-risk operations, where a level 4 containment is required.

2 - In the case of doubt as to the class to be adopted, the classification corresponding to the next higher level shall be assigned, ensuring the protection of human health and the environment, unless there is information, accepted by the legally competent authority, that justifies the application of less stringent measures.

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3.4. Summary of requirements for Class 1 and Class 2 activities

(Class 1 and Class 2 – table I A, Decree-Law No. 55/2015, of 17 April)

Table 1 Containment measures and other protective measures applicable to level 1 and level 2 laboratory activities (extract from table IA of Decree-Law no. 15/2015, of 17 April)

Specifications	Containment Levels	
	1	2
Laboratory facilities: isolation - the laboratory is separated from other areas of the same building or is located in its own building	No	No
Laboratory: Can be sealed for fumigation	No	No
EQUIPMENT		
Surfaces resistant to water, acids, bases, solvents, disinfectants and decontamination agents, easy to clean.	Yes (bench)	Yes (bench)
Access to the laboratory through a vacuum chamber isolated from the laboratory. Its uncontaminated side should be separated from the restricted side by changing rooms or showers, preferably through doors with a coupling mechanism	No	No
Negative pressure relative to surrounding environment	No	No
The supply and extraction air from the laboratory must be HEPA filtered	No	No
Microbiological safety workstation	No	Optional
Autoclave	On the premises	In the building
Restricted access	No	Yes
Biohazard warning on the door	No	Yes
Specific measures to control the spread of aerosols	No	Yes. Reduce to a minimum
Shower	No	No
Protective clothing	Suitable protective clothing	Suitable protective clothing
Gloves	No	Optional
Effective control of vectors (e.g. rodents and insects)	Optional	Yes
WASTE		
Inactivation of GMM and/or GMO in effluents from sinks, drainage drains, showers and similar effluents.	No	No
Inactivation of GMM and/or GMO in contaminated material and waste	Optional	Yes
OTHER MEASURES		
Laboratories containing their own equipment	No	No
Observation window or equivalent that allows occupants to be seen	Optional	Optional



4. BIOLOGICAL SAFETY OF RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology involves combining genetic material from different sources, thus creating GMOs that could constitute an organism that did not previously exist in nature. Experiments involving the construction or use of GMO shall be carried out after a biosafety risk assessment has been carried out. The pathogenic properties and any potential hazards associated with these organisms may be new and not well characterised. The properties of the donor organism, the nature of the DNA molecules to be transferred, the properties of the recipient organism and the properties of the environment shall be assessed. These factors should help determine the level of biosafety required for the safe handling of the resulting GMO and to identify the biological and physical containment systems that should be used.

4.1. Biosafety considerations for biological expression systems

Biological expression systems consist of vectors and host cells. Several criteria must be met for its use to be effective and safe. An example of this biological expression system is the pUC18 plasmid. Often used as a molecular cloning vector in combination with *Escherichia coli* K12 cells, the pUC18 plasmid was fully sequenced. All genes needed for expression in other bacteria were eliminated from its precursor plasmid pBR322. *E. coli* K12 is a non-pathogenic strain unable to permanently colonise the gut of healthy humans or animals. Routine genetic engineering experiments can be safely performed in *E. coli* K12/pUC18 at BSL (*Biosafety Level*) 1, provided that the inserted foreign DNA expression products do not require higher levels of biosafety (details on BSL1 and BSL2 laboratories will be described in chapter 16 of this handbook). Higher levels of biosecurity may be required in situations where:

- The expression of DNA molecules derived from pathogenic organisms can increase the virulence of GMO.
- The inserted DNA molecules are not well characterized, for example: during the preparation of genomic DNA libraries of pathogenic microorganisms.
- Gene products have potential pharmacological activity.
- Genes encode toxins.
- Gene products have oncogenic potential.

4.2. Viral vectors for gene transfer

Viral vectors are used for the transfer of genes to other cells and include, among others, adenoviral, adeno-associated (AAV), lentiviral or retroviral vectors. Such vectors lack certain viral replication genes and are propagated in cell lines that complement the defect. Stocks of these vectors may be contaminated with viruses with replication capacity, generated by rare events of spontaneous recombination in propagating cell lines, or they may derive from insufficient purification. These vectors must be handled at the same level of biosafety as the original virus from which they are derived. Listed below are the characteristics of the main viral vectors according to the recommendations of the *National Institutes of Health* (NIH).

- Adenoviruses – can cause mild to severe respiratory illness in humans. Cleaning with 70% v/v ethanol does not inactivate this viral class, and there is a recommendation to use 10% v/v bleach

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(0.5% v/v sodium hypochlorite). The use of a replication-incompetent adenoviral system (BSL2) reduces the risk but requires several rounds of purification.

- b) Adeno-associated virus (AAV) – this viral class is replication-incompetent and is not known to cause disease in humans. If it is prepared using a helper plasmid instead of a helper virus, it can often be handled in BSL1. If prepared with a helper virus, it should be handled in BSL2. Cleaning with 70% v/v ethanol does not inactivate this viral class, and there is a recommendation to use 10% v/v bleach (0.5% v/v sodium hypochlorite).
- c) Lentiviruses – the best characterized lentiviral systems are derived from the human immunodeficiency virus (HIV), but their organization into multiple plasmids and the deletion of several HIV proteins reduces the likelihood of generating viruses with replication capacity. These systems are handled in BSL2/2+. The use of third-generation lentiviral vectors is recommended. By splitting the viral genome into distinct plasmids, third-generation lentiviral vectors increase safety, making the production of recombinant viruses even less likely.
- d) Retroviruses - are classified based on the types of cells they infect. For retroviruses that do not infect human cells, BSL1 may be appropriate (ecotropic vectors that are limited to a small group of species such as *Mus musculus* and *Rattus norvegicus*); if the infection is of human cells (amphotropic vectors, which infect mammalian cells, or pantropic, which infect mammalian and non-mammalian cells) the BSL2/2+ category is appropriate.

4.3. Transgenic animals and "knock-out"

Animals carrying foreign genetic material (transgenic animals) must be handled with containment levels appropriate to the characteristics of the introduced genes products. Animals with specific gene deletions (knock-out animals) generally do not present specific biological risks. Examples of transgenic animals include those that express receptors for viruses normally unable to infect that species. If these animals potentially escape from the lab and transmit the transgene to the wild animal population, they can theoretically create an animal reservoir for that specific virus. For each new line of transgenic animals, detailed studies should be carried out to determine the pathways by which the animals can be infected, the inoculum size required for infection and the extent of virus spread among infected animals. In addition, all measures must be taken to ensure strict containment of transgenic animals. The safety level for manipulation and/or experimentation with transgenic animals is related to the specific characteristics of the transgene. BSL1 transgenics are those whose transgene has the following characteristics:

- (a) synthetic nucleic acids (i) cannot replicate or generate nucleic acids that can replicate in a living cell (e.g. oligonucleotides or other synthetic nucleic acids that do not contain a replication origin or contain elements known to interact with DNA or RNA polymerase); ii) they are not designed to be integrated into genomic DNA; and iii) they do not produce a lethal toxin in vertebrates with an LD50 of less than 100 ng per kg of body weight.
- b) It is not present in organisms, cells, or viruses and has not been modified or manipulated (e.g., encapsulated in synthetic or natural vehicles) to make it capable of penetrating cell membranes.
- c) It consists only of the exact sequence of recombinant or synthetic nucleic acid from a single source that exists contemporaneously in nature.

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- (d) It consists entirely of nucleic acids from a prokaryotic host, including its indigenous plasmids or viruses when propagated only in that host (or in a closely related strain of the same species), or when transferred to another host by well-established physiological means.
- (e) It consists entirely of nucleic acids from a eukaryotic host, including its chloroplasts, mitochondria or plasmids (but excluding viruses) when propagated only in that host (or in a closely related strain of the same species).
- f) It consists entirely of DNA molecules from different species that exchange DNA by known physiological processes, although one or more of the segments may be a synthetic equivalent.
- (g) genomic DNA molecules that have acquired a transposable element, provided that the transposable element does not contain recombinant and/or synthetic DNA.
- (h) those which do not present a significant risk to health or the environment.

Situations not described above should be subject to a risk assessment.

4.4. Transgenic plants

A risk assessment should determine the appropriate level of biosafety to produce transgenic plants. The BSL1 level is recommended for all experiments with recombinant nucleic acids, recombinant synthetic nucleic acids, or plants containing synthetic nucleic acid molecules and plant-associated microorganisms. Examples of such experiments include those involving plants modified by recombinant or synthetic nucleic acid molecules that are not harmful invaders or unable to cross with harmful weeds in the immediate geographic area. Also included are experiments involving whole plants and recombinant nucleic acids or nucleic acids from modified molecules of non-exotic microorganisms, which do not have recognised potential for rapid and widespread dissemination or have a serious negative impact on ecosystems (e.g. *Agrobacterium* spp.).

The use of higher safety levels, namely BSL2, is recommended in the following cases:

- a) Plants modified by recombinant or synthetic nucleic acid molecules that are harmful weeds or can cross with harmful weeds in the immediate geographic area.
- b) Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent.
- c) Plants associated with recombinant or synthetic nucleic acid molecules modified from non-exotic microorganisms with a recognized potential for serious detrimental impact on ecosystems.
- d) Plants associated with recombinant or synthetic nucleic acid molecules modified from exotic microorganisms that have no recognized potential for a serious detrimental impact on ecosystems.
- e) Experiments with recombinant or synthetic nucleic acid molecules modified from arthropods or small plant-associated animals, or micro-organisms associated with them, if the microorganisms modified by synthetic nucleic acid molecules do not have recognised potential to cause negative impacts on ecosystems.

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4.5. Risk assessments of genetically modified organisms

The risk assessment of the contained use of GMO/GMM is carried out at first when applying for authorisation to be submitted to the Agência Portuguesa do Ambiente – APA, as described in chapter 10. *GUIDELINES FOR THE AUTHORISATION OF LABORATORY FACILITIES*. The form template is made available by APA on its website¹ and is available in Annex (Annex III – Class 1 Notification Form and Annex IV - Class 2 Notification Form).

Each facility/laboratory must carry out a risk re-assessment on an annual basis and may use the template available in the Annex for this purpose (Annex V – Form for Annual Risk Reassessment for Conducted Confined Use Activities).

Risk assessments for working with GMO should consider the characteristics of donor and recipient/host organisms. Examples of features to be considered include the following:

- a) Hazards directly arising from the inserted gene (donor organism) - Evaluation is necessary in situations where the product of the inserted gene has known biologically or pharmacologically active properties that could cause harm, for example:
 1. Toxins
 2. Cytokines
 3. Hormones
 4. Gene expression regulators
 5. Virulence factors or enhancers
 6. Oncogenic genetic sequences
 7. Antibiotic resistance
 8. Allergens.

Consideration of these cases should include an estimate of the level of expression required to achieve biological or pharmacological activity.
- b) Hazards associated with the recipient/host
 1. Host susceptibility
 2. Pathogenicity of the host strain, including virulence, infectivity, and toxin production
 3. Recipient immune status
 4. Consequences of exposure.
- c) Hazards arising from the alteration of existing pathogenic traits - Many modifications do not involve genes whose products are intrinsically harmful, but adverse effects may arise as a result of altering existing non-pathogenic or pathogenic traits. Modification of original genes can alter pathogenicity. To identify these potential hazards, at least the following points should be considered:
 1. Is there an increase in infectivity or pathogenicity?
 2. Can any disabling mutations within the host be overcome as a result of foreign gene insertion?
 3. Does the foreign gene encode a determinant of pathogenicity of another organism?
 4. If foreign DNA includes a pathogenicity determinant, is it foreseeable that this gene could contribute to the pathogenicity of the GMO?
 5. Is there treatment available?

¹ <https://apambiente.pt/prevencao-e-gestao-de-riscos/procedimento-autorizacao-de-uso-confinado>

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6. Will the GMO's susceptibility to antibiotics or other forms of therapy be affected as a result of genetic modification?
7. Is it possible to eradicate the GMO?

Other considerations - The use of whole animals or plants for experimental purposes also requires careful analysis. Researchers must comply with the regulations, restrictions and requirements for carrying out work with GMO in accordance with the legislation in place and with the guidelines of the competent authority for work with GMO that allow the identification of the appropriate level of biosafety. In some cases, the classification may differ from country to country or countries may decide to lower or higher classify the work when new information on a particular host vector/system becomes available. Risk assessment is a dynamic process that considers new developments and advances in science. Conducting proper risk assessments will ensure that the benefits of recombinant DNA technology remain available to humanity for years to come.

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5. LABORATORIES

5.1. Basic safety rules in Class 1 and Class 2 laboratories

a) Classification of laboratories:

- **Class 1 laboratory (BSL1)** – A laboratory where class 1 biological material is used, which presents no or negligible risk to healthy users and the environment. Examples of class 1 biological material are *Bacillus subtilis*, non-pathogenic strains of *Escherichia coli*, adeno-associated viral vectors (AAV), laboratory mammalian samples, and human or mouse cell lines (*Mus musculus*) when they do not contain or are contaminated with human or animal pathogens, and when not altered with vectors of a higher safety level (e.g. use of lentiviral vectors). Investigation with class 1 biological material is usually carried out on laboratory benches, without the use of special containment equipment. BSL1 laboratories are usually not isolated from the building where they are located but include a door that separates them from the rest of the building. New users must receive training on specific procedures from the responsible staff to ensure that the proper assessment of contained use of GMO/GMM assessment is carried out and the corresponding evidence obtained.
- **Class 2 laboratory (BSL2)** - A laboratory where class 2 biological material is used, which presents a low risk to healthy users and the environment. Class 2 biological material includes samples and some human cell lines, third-generation lentiviral vectors, adenoviral vectors, *Aspergillus fumigatus*, *Toxoplasma gondii*, *Salmonella typhimurium* and *Influenza A*. Research with class 2 biological material is usually carried out in designated locations, namely using a **laminar flow cabinet (LFC)/ biological safety cabinet (BSC)** with Class 2 certificate. BSL2 laboratories are generally not isolated from the building in which they are located but include a door that separates them from the rest of the building, and the placement of biohazard signs is mandatory. Only authorized users with prior training on specific procedures are allowed to operate in BSL2 laboratories.

b) Personal protection - all users must adopt the following preventive measures to reduce the risks associated with exposure to biological agents (in accordance with Director's Resolution D/16/2022, available at Annex I - Order D/16/2022 General laboratory safety rules):

- Lab coats or uniforms, preferably disposable, should always be worn when working in the laboratory.
- The hair must be tied up – or a protective cap must be worn.
- Appropriate gloves (in microbiologically approved and disposable latex or nitrile) should be used for all procedures that may involve direct or accidental contact with blood, body fluids and other potentially infectious materials or infected animals.
- After use, gloves should be removed aseptically and hands washed.
- Users should wash their hands after handling chemical, biological and animal materials as well as before leaving the work areas of the laboratory. Hand washing should be carried out for at least 20 seconds with soap and water, and these should be dried with paper towels.
- Safety glasses, face shields (visors), or other protective devices should be worn when it is necessary to protect the eyes from aerosols.
- The use of laboratory protective clothing outside the laboratory, such as canteens, coffee rooms, offices, libraries, personnel rooms and sanitary facilities, is prohibited.
- Street clothing must be appropriate for the work performed, and it is recommended to wear clothes that do not leave exposed skin below knee level.

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- Open footwear should not be worn in laboratories.
- Eating, drinking, smoking, applying cosmetics and handling contact lenses are prohibited in the work areas of the laboratory.
- Putting objects such as pens, pencils or chewing gum in the mouth while in the laboratory is not permitted.
- The use of electronic devices and headphones in the laboratory must not interfere with the clear hearing of alarms or verbal instructions.
- It is forbidden to store food or beverages for personal consumption in any part of the work areas of the laboratory.
- Laboratory protective clothing used in the laboratory should not be stored in the same lockers as street clothing, and should be checked and cleaned regularly, or replaced if damaged.
- People with cuts, scrapes or skin injuries should not work with any type of biological material without taking appropriate prevention/protection measures.

Note: When work is carried out using flame, laboratory protective clothing may not include disposable gloves, disposable masks, disposable lab coats or any other type of highly flammable material.

c) Procedures:

- Places and equipment must be correctly labelled (e.g. biological hazard signs; restriction of access to authorized persons, etc.).
- Appropriate working procedures must be established to avoid or minimise the release of biological agents. Depending on the type of facility concerned, the specific work procedures of each laboratory/facility should include:
 - good practices and care to be taken in the handling of biohazard materials, before, during and after the completion of the work, depending on the type of installation.
 - the appropriate personal protective materials for the installation and work to be carried out.
 - the rules for access to the laboratory/facility.
 - waste disposal rules.
 - the operating instructions of equipment (where applicable).
 - contact to be made in the event of a spill, **accident** or contamination.
- Depending on the laboratory/facility, a specific written procedure must be developed and followed for the clean-up of all spills and all users must have quick access to a spill kit, as provided for in the [Internal Biological Emergency Plan of CIÊNCIAS ULisboa](#) (IBEP). In case there is no need to apply specific procedures, the [OPERATIONAL INSTRUCTION – CONFINED USE OF MGM/GMO, IO-7: CLEANING PROCEDURES](#), belonging to the SAFETY PLAN – PREVENTION PLAN of CIÊNCIAS ULisboa, should be considered.
- All technical procedures must be carried out in such a way as to minimise the formation of aerosols and droplets, in particular through careful handling and without sudden movements. In order to contain the area with aerosols, work must be done on benches that are easy to clean and contain only the material strictly necessary for the work.
- Pipetting with the mouth is prohibited in laboratories.
- The use of hypodermic needles and syringes should be limited. They should not be used as a substitute for pipetting devices or for any purpose other than parenteral injection or aspiration of fluids from laboratory animals.

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- All spills, accidents and obvious or potential exposures to infectious materials should be reported to the laboratory supervisor. A written record of such accidents and **incidents** shall be kept as described in the [IBEP](#).
 - Written documents that are expected to be removed from the laboratory must be protected from contamination while in the laboratory.
 - Special attention should be paid to the use of contaminated equipment and sharp objects, such as needles, which should not be placed in normal waste, but in suitable containers.
 - Cleaning and disinfection must be always ensured, so all laboratories/facilities must have appropriate cleaning materials and agents available at all times (list available in Annex, Annex VI – List of disinfectants and contact times). In the absence of specific procedures, the [OPERATIONAL INSTRUCTION – CONFINED USE OF MGM/GMO, IO-7: CLEANING PROCEDURES](#) should be followed.
 - The destruction/inactivation of waste contaminated with biological agents must be ensured in accordance with the measures set out below.
- d) Laboratory equipment – Along with good procedures and practices, using equipment that allows proper safety handling will help reduce risks when it comes to biosecurity hazards. The equipment must be selected to take into account certain general principles, such as:
- Designed to prevent or limit contact between the operator and infectious material.
 - Built with liquid-impermeable, corrosion-resistant materials that meet structural requirements.
 - Manufactured so that it does not contain sharp edges and unattended moving parts.
 - Designed, built, and installed to facilitate simple operation and facilitate maintenance, cleaning, decontamination, and certification testing; Glass objects, and other breakable materials should be avoided whenever possible.
- e) Training – all users of Class 1 and Class 2 laboratories must be trained in specific procedures and safety measures. Users must be informed of the code of conduct and local guidelines, including the CIÊNCIAS ULisboa Biological Safety Handbook and other laboratory-specific manuals/documents. Measures must be taken to ensure that users have read and understood the guidelines, including completing the assessment regarding the appropriate contained use of GMO/GMM (with a minimum score of 75%), as well as the existence of a user registration system. The registration document must contain the user's name, category, date, contents and signature, and information about the evaluation of appropriate GMO/GMM confined use (example of record available in annex, Annex VII – Training Record). The training of the staff must always include information on safe methods for procedures applicable to the laboratory/facility concerned and involving:
- Inhalation risks (i.e. aerosol production) when using metal inoculation loops to inoculate agar plates, pipette, smears, open cultures, take blood/serum samples, centrifuge, etc.
 - Ingestion risks when handling samples, smears, and cultures.
 - Risks of percutaneous exposures when using syringes and needles.
 - Bites and scratches when handling animals.
 - Handling blood and other potentially hazardous pathological materials.
 - Decontamination and elimination of infectious material.
- f) Registration – all Class 1 and Class 2 laboratories must have a user's record sheet, with information regarding users, date of use, cell lines, culture media and, if applicable, other reagents to be used

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(e.g. Annex VIII – Records of use of use). They must also have a cleaning record (e.g. Annex IX – Cleaning Record) which must include the date, the equipment concerned and the user. Test/certification reports for equipment subject to periodic maintenance must be filed. In these cases, the date of the test/certification must be available on the equipment itself, alternatively, a maintenance record must be kept (e.g. Annex X – Maintenance Log), which must include the date, equipment and serial number. Records can also be made in digital format, namely through equipment management/use platforms, as long as the same evidence can be consulted.

5.2. Waste management

Everything that must be discarded is considered Waste. In laboratories, the decontamination of waste and its final disposal are interconnected. Most glassware, instruments and laboratory clothing are reused or recycled. The fundamental principle is that all infectious materials must be decontaminated or autoclaved in the laboratory or sent for treatment by a licensed operator in hazardous waste management, according to procedures defined by the G3S for the management of hazardous waste produced in CIÊNCIAS laboratories.

Procedures for disposing of hazardous waste:

- a) Decontamination - Steam autoclaving is the preferred method for all decontamination processes. Materials for decontamination and disposal should be placed in containers and/or autoclavable plastic bags.
- b) Procedures for handling and disposal of contaminated materials and waste - A system for identifying and separating infectious materials and their containers must be adopted. Categories should include:
 - Uncontaminated (non-infectious) waste that can be reused or recycled or disposed of as "household or unsorted" waste. Container with black bag.
 - Contaminated (infectious) "sharps" – hypodermic needles, scalpels and lamellae; these should always be collected in puncture-proof containers, fitted with lids and treated as infectious – cut-perforating container (provided by licensed operator).
 - Contaminated material for autoclave decontamination and subsequently washing, reuse or recycling.
- c) Sharps – after use, hypodermic needles should not be cut or removed, or the cap placed on disposable syringes. The complete set must be placed in a sharps disposal container. Disposable syringes, whether used alone or with needles, should be placed in sharps containers and incinerated. Sharps disposal containers shall be puncture-proof/puncture-resistant and shall not be filled to full capacity. When they are filled to three-quarters of their capacity, they must be placed in Group IV containers.
- d) Contaminated (potentially infectious) materials for autoclaving and reuse – There should be no pre-washing of any contaminated (potentially infectious) materials. Any necessary cleaning or repair should be done only after autoclaving or disinfection.

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e) Waste disposal – all liquid or solid waste (Group III and Group IV) must be placed in appropriate containers for scheduled delivery to a licensed operator. In case of doubt, waste should be disposed of in Group IV containers.

- Contaminated material for autoclaving and disposal (all material in contact with biological waste, e.g. gloves, media, vials/plates, disposable serological pipettes, tips) - container identified as Group III with white bag, supplied by licensed operator.
- Contaminated material for direct incineration (all material in contact with hazardous chemical waste, e.g. gloves, media, vials/plates, disposable serological pipettes, tips) - container identified as Group IV, with red bag, supplied by a licensed operator.

5.3. Biosafety requirements and procedures for Class 2 agents

Any laboratory that works with Class 2 agents must have standard operating procedures (SOPs):

- a) Physical Containment – In general, all work with Class 2 agents (e.g. lentiviral vectors) must be carried out in a **BSL2 laboratory**. This includes, but is not limited to, a room suitable for cell culture and a restricted-access door, and equipped with a Class 2 Laminar Flow Cabinet (LFC)/ Biological Safety Cabinet (BSC) and, where possible, a dedicated Class 2 cell and tissue culture incubator or a designated shelf. The vacuum system for aspiration of biological waste must be used, and the container must contain disinfectant solution (e.g. 10% v/v bleach), for waste inactivation. When working with **BSL2/BSL2+ virus** (e.g. lentivirus or adenovirus), in addition to the biohazard sign, an additional warning sign must be posted on the door alerting users to the presence of Class 2 agents. **The vacuum system cannot be used** for vacuuming. If concentration of media or solutions containing Class 2 agents requires the use of a centrifuge, the rotors shall be equipped with suitable features (e.g. sealing *o-rings*) to minimise the risk of aerosol production. Low-speed centrifugation buckets in swinging-bucket rotors shall be equipped with aerosol-tight safety caps. Microcentrifuges must have aerosol-tight rotors that can be removed and transported directly to the LFC/BSC.
- b) Personal Protective Equipment (PPE) - The following PPE must be worn when working with Class 2 agents: **nitrile gloves, lab coat**. A surgical mask and eye protection (goggles) are optional but recommended whenever there is a risk of aerosol production outside of LFC/BSC. In the case of work with **BSL2/BSL2+ viruses** (e.g. lentivirus or adenovirus), **double nitrile gloves should be worn**, with special attention to ensure that the skin of the wrists is covered. Alternatively, gloves with longer wrists than standard may be used and the cuffs of the sleeves should be attached to the lab coat. Potentially contaminated gloves should be removed and replaced with new gloves before touching any object or equipment outside of the LFC/BSC, such as the refrigerator, centrifuge, or incubator.
- c) Spill containment kit – The laboratory must have a spill containment kit or its components easily accessible in the event of a spill. This includes: instruction card; gloves, masks, goggles; disposable lab coats, paper towels to absorb contaminated liquids; disinfectant (e.g., 10% v/v bleach); tweezers to collect broken glass; biohazard collection bags (see detailed procedures in IBEP).

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d) Waste handling and disposal:

- Solid Waste:** Biohazard solid waste is collected into a white bag in a Group III container of 60 L or 30 L. Buckets or other bench containers, lined with white bags can be used as long as they are later placed in the Group III container after being closed. The bags are closed with plastic clamps before being placed in the containers that will then be sent for collection via a certified operator according to the waste disposal circuit in CIÊNCIAS.
Anything that comes into contact with solutions or containers **containing BSL2/BSL2 + viruses** must be decontaminated and contained before leaving the LFC/BSC. Solid waste must be collected in an autoclavable biohazard bag within the LFC/BSC. In these cases, only pipette tips with a filter may be used. The tips should be disposed of in a disposable container containing 10% v/v bleach. Disposable pipettes should be rinsed with 10% v/v bleach before being disposed of inside the autoclavable bag. At the end, the autoclavable biohazard bag must be closed, sprayed with 70% v/v ethanol and deposited in a Group III waste container.
- Liquid waste:** As a general rule, all liquid waste must be disposed of in a container containing 10% v/v bleach and subsequently placed for scheduled delivery by a licensed operator with a contract with CIÊNCIAS ULisboa. In order to minimize transfers of liquid waste or in the case of liquid waste **containing BSL2/BSL2+ virus**, these should be disposed of in a disposable container containing 10% v/v bleach, contained in an autoclavable bag and then placed in the Group III container for scheduled delivery by a licensed operator with a contract with CIÊNCIAS ULisboa.
- HEPA filters:** Periodic replacement of HEPA filters installed in LFC/BSC requires their subsequent disposal. For this purpose, facility/laboratory managers should contact G3S for collection and forwarding of HEPA filters for disposal via a certified operator. HEPA filters from CO₂ incubators can be disposed of via placement in Group III containers.

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6. LABORATORY FACILITIES FOR ANIMALS

Animal facilities (Vivarium), such as laboratories, can be classified as Level 1, 2, 3 and 4 biological safety of animal facilities (BSLAF), according to the risk assessment and risk group of microorganisms to be investigated.

Table 2. Containment levels of animal facilities: summary of safety practices and equipment (Adapted from Manual of biological safety in the laboratory, 3rd ED, World Health Organization, 2004)

LEVEL OF CONTAINMENT	LABORATORY PRACTICES AND SAFETY EQUIPMENT
BSLAF - 1	Limited access, protective clothing and gloves
BSLAF - 2	<u>NSBIA practices – 1 plus:</u> warning signs for dangers. LFC/BSC– Class 1 and 2 for aerosol-producing activities. Decontamination of waste and housing before washing.
BSLAF - 3	<u>NSBIA Practices – 2 plus:</u> Controlled Access. LFC/BSC and special protective clothing for all activities
BSLAF - 4	<u>NSBIA Practices – 3 plus:</u> Strictly Limited Access. Change clothes before entering. LFC/BSC – Class 3 or positive pressure suits. Shower at the exit. Decontamination of all waste prior to removal from the facility.

6.1. Animal Facility – Level 1 Biological Safety

This level is adequate for the retention of most animals after quarantine (except non-human primates, for which national authorities should be consulted) and for animals that have been deliberately inoculated with Risk Group 1 agents. Good microbiology practices are needed. The person responsible for managing the Vivarium must establish policies, procedures and protocols for all operations and access to it. An appropriate medical surveillance programme for personnel should be established. The number of entries in the Vivarium should be reduced to what is strictly necessary, not only for the protection of animals, but also to avoid wasting personal protective material.

At CIÊNCIAS ULisboa, the work carried out falls exclusively into Level 1, which means that the facilities are of limited access, and that personal protective equipment (PPE) is used: masks, lab coats, gloves, cap and shoe protectors.

The animal facilities at CIÊNCIAS ULisboa (Vivarium), located in building C2, floor 1, east wing, include the maintenance of laboratory animals (mice and fish) and wild animals such as fish, amphibians, reptiles and small mammals. The animal experimentation carried out in the Vivarium - CIÊNCIAS ULisboa is governed by Portuguese Law through Decree-Law No. 113/2013 of August 7, 2013 adjusted by Decree-Law No. 1/2019 of January 10, 2019, and by Community Directive 2010/63/EU of September 22, 2010. All authorized users of the Vivarium- CIÊNCIAS ULisboa must comply with current legislation as well as internal operating rules (see the *Vivarium Operating Rules, January 2023 edition*). The Vivarium has a scientific direction, executive management and a designated veterinarian, operating in coordination with the Animal Welfare Body (ORBEA).

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6.2. Access to the Vivarium

Access to the Vivarium is restricted to authorized users and previously authorized visitors. Authorization for use must be requested from the person responsible for the management of the Vivarium by filling out the user registration form. The visit authorization must also be requested from the person responsible for the management of the Vivarium, preferably 24 hours in advance, by filling out the visitor's form. Entry to the Vivarium is provided by handing over an electronic key to the main door. At the end of the period of use, it is mandatory to hand over the key to the person responsible for managing the Vivarium.

All users of the Vivarium must hold accreditation for the use of animals for scientific purposes authorized by the Direção-Geral da Alimentação e Veterinária (DGAV).

6.3. General Operating Rules

- The use of PPE is mandatory in all rooms of the Vivarium. Some rooms have specific rules for the use of personal protective equipment. In these cases, the rules of each of the rooms must be followed.
- Unauthorized visits are not allowed.
- It is not allowed to eat or drink inside the Vivarium facilities.
- It is not allowed to listen to music without headphones.
- The use of mobile phones is only allowed in "silent" mode.
- The storage and disinfection of all material and equipment in the Vivarium is the responsibility of the users.
- Personal protective equipment (lab coats) and cleaning accessories (towels, cloths) must be washed in the washing room on the 3rd floor of building C2, upon request from the laboratory support services.
- The cleaning services of CIÊNCIAS ULisboa can be requested by users as long as the person responsible for the management of the Vivarium is informed.
- Waste disposal should only be done in the containers provided inside each of the rooms.
- The use of hazardous waste containers (for example in the necropsy room) must be requested in advance to the person responsible for the management of the Vivarium.

6.4. Organization and Flow in the Vivarium

The Vivarium is divided into rooms for common use and rooms dedicated to different organisms (Figure 1). The rooms for common use include a toilet, a necropsy room, a washing room (consisting of a washing sink, washing machine, drying oven and an autoclave), a storage room with a cold storage area (freezers and refrigerators) and two quarantine rooms. Rooms dedicated to animal keeping include laboratory animal rooms (mice and fish) and wild animal rooms (small mammals, amphibians, freshwater fish, and marine fish).

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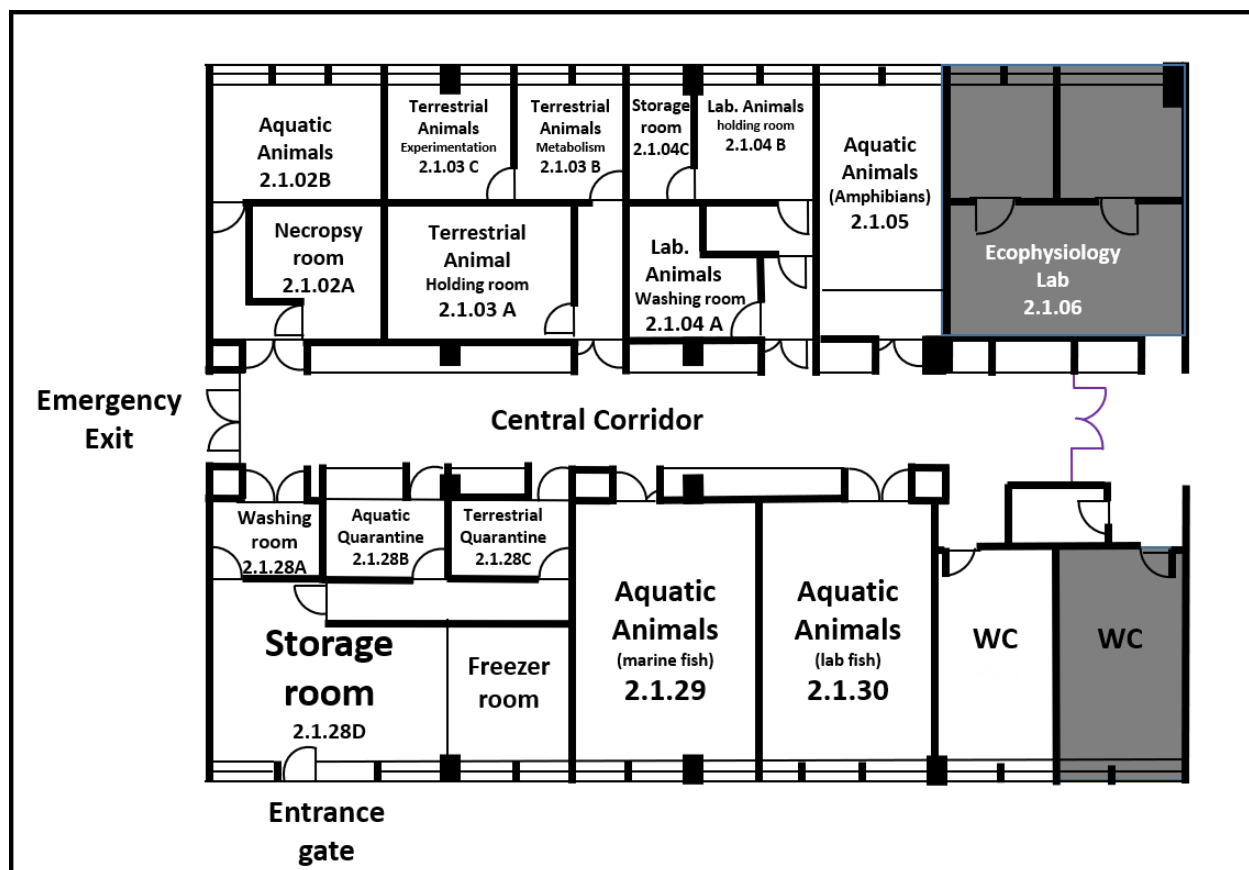


Figure 1 - Representative scheme of the Vivarium plant.

Laboratory animals purchased from duly licensed institutions for their breeding and marketing (e.g. The Jackson Laboratories) are transported to the Vivarium by licensed operators for the transport of animals in a controlled environment. They must be quarantined for a period of 5 to 10 days, during which they cannot be included in any procedure. In this case, the animals must enter the Vivarium through the emergency exit, being transported directly to the respective room. All animals must remain in these rooms until they are sacrificed or returned to the wild (in case they are wild animals). Necropsies must be carried out in the necropsy room and the carcasses must be stored in a freezer to be disposed of in a container for incineration.

6.5. Specific procedures for animals- Room 2.1.04

Room 2.1.04 is intended for specific procedures with small mammals, in particular mice (*Mus musculus*).

a) Facility Features:

This facility has five distinct areas: 1) antechamber with direct connection to the outside, 2) washing room, 3) food and washed material storage room, 4) animal room and 5) room with materials that are both washed and autoclaved (Figure 2). Inside the rooms there may be support objects, namely workbenches, sinks, air conditioning, ventilated cabinets, desk, shelves and cabinets.

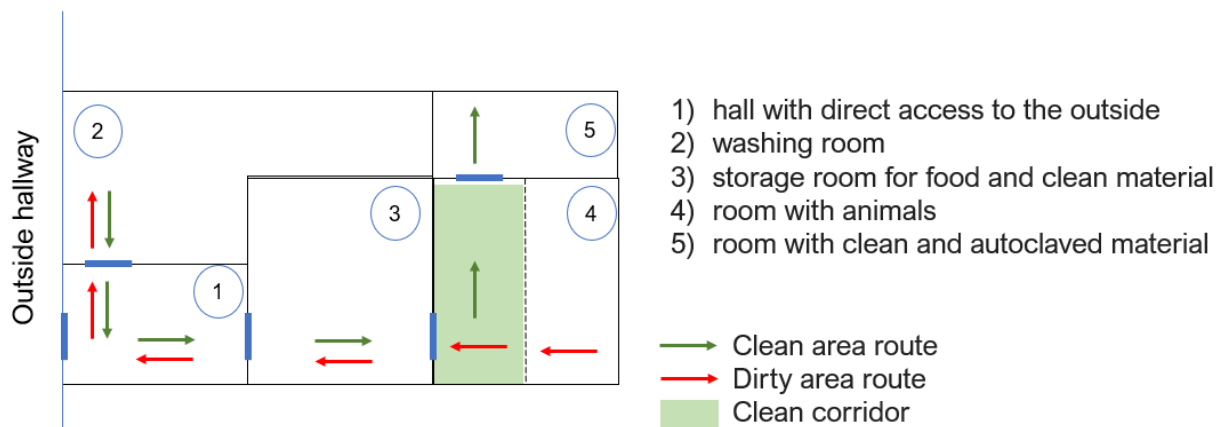


Figure 2 - Representative scheme of the organization and flow of the room 2.1.04

b) Procedures:

Before entering the Facility

- Put on personal protective equipment: labcoat (washed and autoclaved), shoe protection, cap, mask and gloves.
- Disinfect hands and all material that enters the Vivarium with 70% v/v ethanol.

In the Facility

- Disinfect workbenches with multipurpose spray disinfectant, followed by 70% v/v ethanol before and after each use.
- Record all the information about the mice in the animal registry book: number of cages, dates of birth (DOB), number of animals that were used and procedures performed.
- The number of animals per box must comply with the guidelines present in Annex V of Decree-Law No. 113/2013 of August 7, 2013.
- All cages must be numbered and well identified, using identification cards filling in all fields with information regarding the animals, according to the example below (Figure 3).

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STOCK CARD

LAB (<i>acronym or PI</i>)	USER (<i>name</i>)	CAGE (<i>nr.</i>)
STRAIN		
#M (<i>nr.</i>)	#F (<i>nr.</i>)	D.O.B. (<i>date of birth</i>)
ETHICS PROTOCOL NR: (<i>DGAV nr.</i>)		

NOTES:

(details on the genotype of the animals; if different D.O.B. register here)

Figure 3 - Example of identification cards for laboratory animals, divided into crossbreeding cards (record of crosses made), stock (stored animals) and experimental (animals used in an ongoing experiment).

c) Circulation in the Facility:

Since there is a single door for entry and exit and it is not possible to create a clean and a dirty corridor, the circulation in the Vivarium must meet some criteria (as illustrated in Figure 2):

- The circulation of clean and dirty material should not occur simultaneously.
- Entry of autoclaved material into the room (5) must be made through the corridor delimited on the floor (clean corridor).
- On the day of the change of cages, the clean cages must be placed in the animal room (4) before the change begins. The same applies to food and water.
- After staying in the animal room (4), re-entry into the autoclaved material room (5) must be avoided. If necessary, disinfect gloves and shoe protectors.

d) Cleaning the Facility and hygiene rules

- Personal protective material is for single use only - discard gloves, shoe covers, cap and mask. Place the lab coat in the designated container to be washed and autoclaved.
- The workbench must be cleaned after each use. The procedure involves first cleaning with a multipurpose disinfectant spray ensuring the removal of any potential biological material, followed by 70% v/v ethanol to ensure disinfection.
- Cleaning of the facility (benches and floor) should be done once a week. For benches, mouse racks and cabinets use the procedure with multipurpose disinfectant spray, followed by 70% v/v ethanol. For floors, sweep (if necessary) and wash with water and Virkon® (disinfectant with bactericidal, fungicidal and virucidal activity).
- The lab coats should be washed once a week after the cleaning procedure. The lab coats are taken to the washing room on the 3rd floor, where they are washed and dried and then

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autoclaved inside a proper bag. After sterilization in an autoclave, the closed bag is transported to the Vivarium. After disinfecting the outside of the bag with 70% v/v ethanol, it is opened in the Vivarium and the lab coats are left to dry in an appropriate box.

- The washing of the material (cages, grids and bottles) should be done once a week when the cages are changed. The bedding should be discarded directly into the trash, and the water poured. Food can be reused, but its reuse must be controlled. The material must be washed in the washing machine and then placed in bags and taken to autoclave. After autoclaving, the bag must be sealed and transported to the room designated for washed and autoclaved material (Figure 2, room 5). If the material is damp, place it inside the open cabinets or on the table in the autoclaved material room, but only temporarily. In the case of larger cages, which do not fit in the autoclave, after washing, they must be disinfected with 70% v/v ethanol.
- All waste produced must be collected once a week, after changing the cages. The trash container can only circulate between the washing room and the antechamber that gives access to the outside. Waste circulation is not allowed in the 3 other rooms (food stock room, animal room and autoclaved material room).

e) Material Preparation

- Autoclave tap water for the animal bottles into 2L bottles.
- Food and bedding bags should be stored in the designated room on shelves and never in direct contact with the floor and should always be autoclaved inside autoclave bags before use.
- Autoclave environmental enrichment materials (cotton, paper rolls, ...).
- Autoclaved material should only be handled in the autoclaved materials room.

f) Euthanasia of animals

The animals should be euthanised by cervical displacement after anesthesia with isoflurane. The euthanasia must take place in the necropsy room, outside the mice room. Animals can be transported in plastic bags from the Vivarium to the necropsy room. Carcasses must be frozen and then placed in a Group IV container only on the day of waste collection for immediate disposal by incineration.

6.6. Specific procedures for animals- Room 2.1.30

Room 2.1.30 is intended for specific procedures performed on model aquatic organisms such as zebrafish (*Danio rerio*) and *Danionella cerebrum*.

a) Facility Features:

Zebrafish should be housed in the room dedicated to model aquatic organisms (zebrafish *Danio rerio* and occasionally *Danionella cerebrum*). The room is therefore divided into two sections, with zebrafish housed on the right (east) side of the room and *Danionella cerebrum* on the left (west) side. Zebrafish should be housed in Tecniplast racks (Zeb Tec ZB2550SASXAB1) dedicated exclusively to the species. These racks allow the installation of up to 30 tanks of 3.5 L or 15 tanks of 8 L. This system allows for continuous monitoring

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of water salinity, conductivity, pH and temperature levels. On a weekly basis, or whenever needed, the parameters for ammonia, nitrites and nitrates should be assessed manually. *Danionella cerebrum* must be housed in the two metal racks, each of which consists of two shelves where up to 8 tanks of 20 L can be installed. *Danionella cerebrum* must be kept in individual tanks, each equipped with its own filtration system and water temperature regulation. All the above physicochemical parameters for zebrafish should also be checked manually for *Danionella cerebrum* at least once a week. The room is equipped with support benches dedicated to each of the species and a sink. There are also two dedicated water treatment systems. The water used in zebrafish must be supplied by a reverse osmosis equipment while the water to be used in the tanks of *Danionella cerebrum* is filtered by an ultraviolet light equipment. The room is also equipped with a ventax for air circulation and an air conditioning unit.

b) Procedures:

Before entering:

- Put on personal protective equipment: lab coat (washed and autoclaved), shoe covers, cap, mask and gloves.
- Disinfect hands and all material that enters the room with 70% v/v ethanol.

In the room:

- Ensure that the door to the room is closed before starting any type of procedure that involves handling animals.
- Disinfect workbenches with multipurpose spray disinfectant, followed by 70% v/v ethanol before and after each use.
- Record all information about the fish in the animal registry book: number of tanks, dates of birth (if applicable), number of animals that have been used and procedures performed.
- The number of animals per tank must comply with the guidelines present in Annex V of Decree-Law No. 113/2013 of August 7, 2013.
- All tanks must be numbered and well identified, with the following information: Identification of the mutant strain or line, date of crossing or transfer, number of animals.

c) Circulation:

Since there is only one access door, it is not possible to create a clean and a dirty corridor. Thus, the circulation in the room meets the following criteria:

- The circulation of clean and dirty material should not occur simultaneously.
- Entry into the washed material room must be through the corridor delimited on the floor.
- On the day the tanks are cleaned, the cleaned tanks must be placed in the animal room before starting the changeover.

d) Cleaning the Facility and hygiene rules:

- Personal protective material is for single use only - discard gloves, shoe covers, cap and mask. Place the lab coat in the appropriate container to be washed and autoclaved.

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- Cleaning of the facility (benches and floor) should be done once a week. For benches, fish racks and cabinets use the procedure with multipurpose disinfectant spray, followed by 70% v/v ethanol. On the floor, sweep (if necessary) and wash with water and Virkon® (disinfectant with bactericidal, fungicidal and virucidal activity).
- The lab coats should be washed once a week after the cleaning procedure. The lab coats are placed in a closed bag, taken to the washing room on the 3rd floor, where they are washed and dried and then autoclaved inside a proper bag. After sterilization in an autoclave, the closed bag is transported to the Vivarium. After disinfecting the outside of the bag with 70% v/v ethanol, it is opened in the room and the lab coats are left to dry and then placed in an appropriate box.
- The washing of the material (tanks and filters) should be done once a week when the tanks are changed. The material must be washed in the washing machine dedicated exclusively to the material used with zebrafish.
- All waste produced must be collected once a week, after changing the tanks.
- Carcasses of fish found dead or fish euthanised must be disposed of in designated containers.
- Fish resulting from crosses involving mutant lineages should always be considered mutants and therefore handled and discarded properly.

e) Prevention of animal release

To prevent the undue release of animals, it is essential to ensure that:

- Genetically modified zebrafish are only kept in equipment specifically designated for the species as it is properly prepared to prevent the undue release of animals.
- The tanks are kept with water close to their maximum capacity, which prevents egg laying, as zebrafish only spawn in shallow water.
- The filters used have a fine mesh that prevents the circulation of eggs, larvae and adults between tanks.
- The integrity of the filters is checked when feeding the fish.
- The racks have additional physical barriers (grids/screens), to prevent fish from swimming out of the tanks during water changes or filter maintenance.
- The tanks have lids that are kept tightly fitted and closed to prevent fish from jumping between tanks.
- The flow of water is controlled to prevent an excessive current that leads to fish being transported passively.

f) Euthanasia of animals

Adult fish should be immersed in a buffered solution (pH 7.0 – 7.4) of Tricaine MS-222 (250 mg/L) for at least 30 minutes until there is loss of opercular motion. The euthanasia of larvae between 5 and 14 days requires at least another 20 minutes of exposure to Tricaine MS-222, with verification of the absence of opercular movements. Embryos up to 7 days old should be immersed in sodium hypochlorite (6.15% v/v bleach). The euthanasia must be done in the animal maintenance room in an individualized tank. Carcasses must be frozen and then placed in a Group IV container only on the day of waste collection for immediate disposal by incineration.

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7. LABORATORY FACILITIES FOR PLANTS

In an infrastructure with adequate implementation of containment measures, the probability of accidental release of genetically modified plant material is very low. However, despite the general rules and good practices for risk prevention and safety in the CIÊNCIAS ULisboa laboratories being duly documented, the following recommendations, based on the guidelines from official Health and Environment authorities, have been compiled to support the work of researchers in handling genetically modified plants. Table I-B of Annex IV of Decree-Law No. 55/2015 of 17 April should also be consulted.

Table 3 Containment and other protective measures applicable to greenhouses and growing chambers.

Specifications	Containment Levels	
	1	2
The terms "greenhouse" and "growth chamber" refer to structures with walls, roof and floor, designed and used primarily for the growth of plants in a controlled and protected environment. All provisions of Table I-A of Decree-Law No. 55/2015 of 17 April apply in this handbook, as transposed and presented in Table 1, with the following additions or amendments:		
Building		
Greenhouse: permanent structure The greenhouse should consist of a structure with a continuous waterproof cover, located on a site with a slope such as to prevent runoff of surface water and have automatic-closing doors	No	Yes
Equipment		
Access via a separate compartment with two doors with locking mechanism	No	Optional
Control of contaminated runoff water	Optional	Reduce runoff to a minimum (if transmission through the ground is possible)
Working system		
Control measures for undesirable species such as insects, rodents and arthropods	Yes	Yes
The procedures for transferring living material between the greenhouse/growth enclosure, the protective structure and the laboratory must control the spread of GMM and/or GMO	Reducing the spread to a minimum	Reducing the spread to a minimum

In CIÊNCIAS ULisboa, the work carried out involving genetically modified plants falls exclusively under Level 1 of biological safety. The handling of GMM, donors or vectors, used in the genetic manipulation of plant samples, also classified in Level 1 of biological safety, must follow the good microbiological practices described in Chapter 8 of this handbook (8. *Good Microbiological Practices*).

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7.1. Plant Facilities – Level 1 Biological Safety

Facilities used for handling genetically modified plants include:

1. Laboratories with LFB for manipulation under aseptic conditions, located in building C2, floor 1 and floor 4.
2. Growth chambers, including phytoclimatic chambers and *walk-in* equipment, for growing genetically modified plants in pots under controlled conditions of light, temperature and/or humidity, located in building C2, floor 1.
3. Culture room for *in vitro* growth of genetically modified plants located in building C2, floor 1.

7.2. Access to the Plant Facilities

Access to the facilities located in building C2 (laboratories, phytoclimatic chambers, *walk-in*, culture room) where genetically modified plants are handled is restricted to people involved in the ongoing work, duly authorized by the research manager, and to previously authorized visitors. The entrance to the facilities is duly marked with "restricted access", "keep the doors closed", "contact of the person in charge" and risk class signage.

7.3. On-site activities

All genetically modified plant samples must be labelled, properly identified and registered in a log book for this purpose, or electronic system, which must contain information on the type of sample, ID, quantity (if applicable), activity carried out (e.g. storage in the refrigerator or freezer, for growth, to be analyzed in the lab, etc.) and name of the user. The person in charge should check all entries in the logbook, and keep both the logbooks and backup copies archived.

7.4. Procedures in all laboratories where activities with genetically modified plants take place

1. It is mandatory to wear a cotton-based lab coat (a non-flammable fabric), preferably with buttons or Velcro® to facilitate removal in the event of an accident. The sleeves should be adjustable.
2. Gloves should be changed whenever contaminated and should not be used to handle objects of common use (telephone, computer, door handles or drawers, etc).
3. Dispensers for liquid or powder soap should be provided in the laboratory. Dispensers should always be washed before refilling. Alternatively, products in disposable packaging can be used.
4. If hands are accidentally contaminated, a disinfectant agent (e.g. 70% v/v ethanol) should be used in addition to washing.

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a) Procedures in growth chambers for genetically modified plants in soil

Safe handling

- To minimise the risk of spillage, all material used during manipulations should be kept on trays during ongoing activities. All spills must be immediately cleaned up according to the procedures outlined in the Internal Biological Emergency Plan of CIÊNCIAS ULisboa.
- Solid waste that includes disposable laboratory material, soil, paper and protective material must be collected in bags for autoclaving. Solid biological waste must be placed in a separate bag and placed in Group III solid waste containers for collection by the waste management company.
- Liquid waste, including solutions and run-off water, must be transported in containers and decontaminated by autoclave before being disposed of. All equipment and accessories must be cleaned and decontaminated before being removed from the facilities for repair or maintenance.

b) Cleaning and disinfection of the premises

- Facilities (surfaces and permanent structures) should be easily washable and cleaned, and kept in good hygienic conditions. All cleaning operations must be carried out by authorised users after each cycle of use.
- Additional precautions should be taken to prevent the spread of pollen from the phytoclimatic chambers/*walk-in* located in building C2 (floor 1). If the plants are grown until they reach the reproductive stage, a bag of suitable material should be used to enclose the fully developed flower organs. When necessary, exposed surfaces should be sanitized, and the application of hydrogen peroxide vapor (does not generate toxic residues), or a suitable disinfectant, is recommended.
- All growing surfaces should be regularly cleaned with disinfectant solutions (e.g. bleach). Support benches should be decontaminated with bleach or 70% v/v ethanol at the end of the work. Experimental materials must be inactivated by chemical methods or autoclaving, including growth vessels and other accessories. Autoclaving should be carried out at 1.2 bar and 121°C for 15-180 min depending on the type and condition of the material. Cleaning accessories and lab coats should be sterilized before washing.

c) Transport of genetically modified plant material between facilities

Transport between the various facilities mentioned here, of any type of GMO/GMM biological material, whether in *Petri* dishes, tubes/microtubes or other containers, must be carried out, mandatorily, in covered boxes or trays suitable for transport. Cultures grown in liquid medium should be transported inside boxes, basins or buckets, using appropriate supports or laboratory carts. Biologically contaminated material should be transported to the washing rooms on laboratory carts in basins or trays. Pack the materials carefully inside the containers to avoid breakage, dropping and/or spillage during transport.

d) Use of the washing room or the services provided there

In building C2 there are two support rooms for washing material (floors 1 and 4) where the following tasks are carried out (i) washing laboratory material; (ii) decontaminating biological waste, culture media and laboratory material; (iii) sterilizing laboratory material, culture media and solutions. The use of these rooms

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or the sending of materials must follow the necessary rules in order to protect users and staff from exposure to chemical and biological hazards, namely:

- a) Glass containers with biological contamination or with contaminated media should be transported covered to the washing room, to avoid spills.
- b) Needles and blades with biological contamination must be placed in proper containers in accordance with the waste management implemented in CIÊNCIAS ULisboa.
- c) Appropriate gloves should be used for handling and washing contaminated materials with biological/chemical agents.
- d) Whenever it is necessary to decontaminate the materials with bleach or disinfectant solution.
- e) The normal conditions of sterilization by autoclaving are: 121°C, 20 min.
- f) Containers with liquid medium for autoclaving must not be filled to more than 80% of their nominal volume.
- g) All users of the washing rooms (washing, decontamination and sterilization) or the equipment that within them (e.g. autoclaves and drying ovens) must follow the rules implemented therein.

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8. GOOD MICROBIOLOGICAL PRACTICES

The preparation of microbial cultures or cell cultures involves a series of procedures in asepsis that must be performed under appropriate conditions.

Asepsis is the technique used to avoid contamination of materials and environments during microbiological procedures. It involves the proper use of personal protective equipment, decontamination of surfaces, use of flames to sterilize instruments, and the careful handling of microorganisms.

Throughout this chapter, good microbiological practices are addressed in contexts such as work area, personal hygiene, reagents and sterile materials, pipetting, etc.

8.1. Working Area

The simplest and most cost-effective way to reduce contamination from particles and aerosols, such as dust, spores and sneezes, is to use a Laminar or Biological Safety Flow Cabinet (LFC/BSC) for the handling of microorganisms and/or cell lines. The LFC/BSC must be correctly installed in an area restricted to cell culture and with reduced movement of people.

The work area should be clean and organized, containing only the items necessary to carry out a certain procedure, and should not be used as a storage area. Before and after each use, the work area as well as all equipment used must be cleaned with an appropriate disinfectant (see Annex VI – List of disinfectants and contact times). The room must be cleaned regularly with the use of appropriate disinfectant in accordance with the cleaning procedure provided in the [OPERATIONAL INSTRUCTION – CONFINED USE OF MGM/GMO, IO-7: CLEANING PROCEDURES](#).

8.2. Laminar Flow Cabinets / Biological Safety Cabinets

According to the recommendations of the World Health Organization, the limitations and procedure for the use of LFC/BSC should be explained to all potential users. The LFC/BSC should only be used if it is in full operation and the glass panel should not be opened when it is in use.

In the LFC/BSC, the presence of excessive equipment and materials must be avoided to ensure adequate air circulation in its rear space. The use of Bunsen burners within the LFC/BSC is prohibited.

All operations must be carried out in the center or at the rear of the working area, and must be visible through the glass panel.

The movement of people around the operator must be reduced to the minimum necessary.

The operator should not interrupt the airflow by repeatedly inserting or removing their arms from the cabinet or placing documents inside the LFC/BSC.

Air grids should not be obstructed with papers, pipettes or other materials, as this interrupts airflow and increases the risk of material contamination and operator exposure.

When starting/finishing the work and at the end of the day, the surface of the LFC/BSC should be cleaned with a suitable disinfectant. The cabinet fan should run for at least 5 minutes before and after work is performed to ensure the sterility of the work area.

In addition, LFC/BSC ultraviolet light can be used to sterilize both the air and the work area between uses. Preferably, the LFC/BSC should remain on throughout the workday and only be turned off when not in use for long periods, such as at night and weekends.

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8.3. Personal Hygiene

It is important to wash hands before and after working with microorganisms and cell culture. In addition, the use of proper PPE not only protects the operator from hazardous materials but also reduces the likelihood of contamination.

The application of cosmetics is prohibited in laboratories.

8.4. Reagents and Sterile Materials

All reagents and materials used in the manipulation of microorganisms and cell culture must be sterile, whether disposable or not.

8.5. Handling

Before starting, it is necessary to disinfect hands and the work area with a proper disinfectant. Culture flasks and plates should be disinfected before being placed inside the LFC/BSC.

Direct use of reagents from their bottles and vials should be avoided.

Reagent bottles and vials should be closed after use. No sterile flask, bottle, petri dish, etc., should be opened until the user is ready to use it. Reagent bottles and vials, as well as materials to be used for handling, should never be opened outside the LFC/BSC.

When removing a cover, if necessary, the lid should be with the opening facing down on the work surface. Avoid talking during procedures performed in a sterile environment.

Procedures should be carried out as quickly as possible to minimize contamination.

8.6. Pipetting

In the course of working with microorganisms or cell lines, when pipetting, it is mandatory to use a suitable pipetting device, such as a pipettor or pompette. It is forbidden to pipette with the mouth as well as to blow into the pipette to expel the liquids from the pipette.

All serological pipettes (disposable or glass) must be sterile and have a cotton filter to avoid contamination of the pipetting devices.

Preference should be given to graduated pipettes that do not require the expulsion of the last drops.

Pipetting using micropipettes should be done using appropriate tips for micropipettes. Whenever aerosol production is likely, filter tips should be used.

Pipetting should never be done using syringes with a hypodermic needle.

8.7. Aerosols

The particles and droplets (>5 µm in diameter), produced during microbiological handling, quickly settle in the work areas and in the operator's hands. The use of disposable gloves is recommended. Laboratory personnel should avoid touching their mouth, eyes, and face. During procedures that may generate projectiles of materials with a potential risk to the operator's health, it is necessary to protect the face, eyes and mouth.

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8.8. Use of centrifuges

The use of centrifuges in the culture of microorganisms and cell lines is a routine procedure that must be carried out in compliance with the rules of good use of this equipment. It is essential that laboratory centrifuges are in perfect mechanical operation to ensure biological safety during use, as well as to carefully follow all instructions provided by the manufacturer.

Centrifuge tubes/microtubes/cups suitable for centrifugation should be used. When placing the tubes in the centrifuge, they must be well covered, positioned and balanced in order to prevent accidents during centrifugation and potential contamination. The volume of liquid in the tube/microtube/cup must be respected following the manufacturer's guidelines, thus ensuring safe operation. This point is particularly important when using swinging-bucket rotors.

To balance empty tubes/microtubes/cups, it is recommended to use distilled water.

8.9. Maintenance and use of refrigerators and freezers

Refrigerators and freezers (-20°C and -80°C) are important equipment in the practice of culturing microorganisms or cell lines. They are used to storage not only samples but also reagents and, therefore, it is essential to ensure their proper functioning.

In this way, refrigerators and freezers should be defrosted and cleaned periodically. Appropriate PPE must be worn during cleaning.

All materials stored in refrigerators and freezers must be properly labelled and well covered.

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9. DISINFECTION AND STERILIZATION

9.1. Definitions

Several terms are used for disinfection and sterilization. The most common ones in biosafety are highlighted below:

- Antimicrobial – Agent that kills microorganisms or suppresses their growth and multiplication.
- Antiseptic – Substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to the surfaces of the body.
- Biocide – General term for any agent that kills organisms.
- Chemical germicide – A chemical or mixture of chemicals used to kill microorganisms.
- Decontamination – Any process of removal and/or elimination of microorganisms. The same term is also used to refer to the removal or neutralization of hazardous chemicals and radioactive materials.
- Disinfectant – A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.
- Disinfection – A physical or chemical means of killing microorganisms, but not necessarily spores.
- Microbicide – A chemical or mixture of chemicals that kills microorganisms. The term is often used instead of "biocide," "chemical germicide," or "antimicrobial."
- Sporicidal – A chemical or mixture of chemicals used to kill microorganisms and spores.
- Sterilization – A process that kills and/or removes all classes of microorganisms and spores.

9.2. Procedure

Disinfection and sterilization procedures should consider the GMO/GMM to which they relate, as well as the location where they are applied (e.g., Laminar or Biological Safety Flow Cabinet, LFC/BSC; centrifuge). The [OPERATIONAL INSTRUCTION – CONFINED USE OF MGM/GMO, IO-7: CLEANING PROCEDURES](#), belonging to the SAFETY PLAN – PREVENTION PLAN of CIÊNCIAS ULisboa, describes the procedure to be carried out. If there is a need to apply specific procedures associated with the facility in question, they must be properly documented and known to the users involved. Clean-up operations should be documented in the relevant register (Annex IX – Cleaning Record or equivalent).

Any accidents and spills should be reported to the researcher in charge or person designated for this purpose and if necessary, call the CIÊNCIAS ULisboa emergency number (ext. 20000; 217 500 600). The procedures associated with an occurrence of a spill involving GMOs/GMM are detailed in the [Internal Biological Emergency Plan of CIÊNCIAS ULisboa](#).

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10. GUIDELINES FOR THE AUTHORISATION OF LABORATORY FACILITIES

10.1. General principles

The **Agência Portuguesa do Ambiente (APA)** is the competent authority for the deliberate release into the environment and for the contained use of GMM/GMOs, and is responsible for the assessment of environmental risks as well as the authorisation for:

- placing on the market products containing or consisting of GMO (other than those intended for human and animal consumption);
- deliberate release of GMO into the environment for any purpose other than placing on the market, including for experimental purposes;
- GMM/GMO contained use operations.

The **Direção Geral de Alimentação e Veterinária (DGAV)** is the competent authority for:

- regulating the cultivation of genetically modified varieties in order to ensure their coexistence with conventional crops and organic production;
- authorising the placing on the market of products containing GMO intended for human and animal consumption.

According to Decree-Law No. 55/2015, of 17 April, which regulates the contained use of GMM/GMO, when it is intended to carry out contained use operations with GMM/GMOs, a notification must be submitted to the APA, according to the respective risk class:

- Class 1 - zero or negligible risk operations where a level 1 containment is required (Annex III – Class 1 Notification Form);
- Class 2 - low-risk operations, where a level 2 containment is required (Annex IV - Class 2 Notification Form);
- Class 3, moderate risk operations, where a level 3 containment is required;
- Class 4, high-risk operations, where a level 4 containment is required.

The user of GMM or GMO in confined environments is obliged to:

- a) assess the contained use in the light of possible risks to human health and the environment, including the disposal of waste and effluents;
- b) classify the confined use operation to one of the classes;

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- c) apply the general principles and containment and other appropriate protective measures to ensure that exposure in the environment and workplace is kept as low as possible and a high degree of safety is ensured;
- d) Carry out notification and authorization procedures;
- e) Annually review the risk assessment, containment measures and any other protective measures adopted;
- f) Immediately review, considering the provisions in the previous paragraph, the risk assessment, the containment measures applied and any other protection measures adopted;
- g) Submit to APA, I. P., the amended notification as a result of the revision specified in the previous paragraph;
- h) Maintain an annual record of the risk assessments of the confined use activities carried out, which must be made available to APA, I. P., and other competent entities, whenever requested;
- i) Develop procedures related to accident prevention, action in case of emergency, training of personnel and treatment of waste and effluents, and for this purpose:
 - i. Draw up an emergency plan that contemplates the safeguarding of human health and the environment, to be adopted in the event of failure of the planned containment measures;
 - ii. Inform the organizations and entities likely to be affected in the event of an accident, about the emergency plans and safety measures that must be applied, informing APA, I. P.;
 - iii. Inform APA, I. P., about issues related to security;
 - iv. In the event of an accident, carry out the measures outlined in paragraph 1 of article 15 and in the provisions of the [Internal Biological Emergency Plan of CIÊNCIAS ULisboa](#);
- j) Ensure, under the terms of the law, the protection of the safety and health of workers against risks resulting from exposure to biological agents during work, providing a high level of safety, without prejudice to the measures corresponding to the respective class of confined use, outlined in Decree-Law 55/2015 of 17 April;
- k) Ensure, under the terms of the law, the application of good practices in microbiology and safety and hygiene at work;
- l) Provide APA, I. P., with the following information:
 - i. Relevant information of which you are aware;
 - ii. Changes in the contained use of an GMM or GMO that may imply a change in the associated risk levels;
 - iii. Changes in the class of confined use;
 - iv. Annual presentation of the activities carried out in accordance with this Decree-Law, according to the model available on the APA, I. P. website, on the Internet, including the conclusions of the audit, if carried out;
 - v. Communication of any interruption of the contained use activity;
 - vi. Submission of documentation or additional information requested by APA, I. P.;

Before starting the contained use, APA, I. P., must ensure:

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- a) The preparation of an emergency plan for cases where the failure to contain measures poses a serious and immediate or late danger to persons outside the facilities and/or to the environment, unless the emergency plan has already been drawn up in accordance with other legislation;
- b) Communication of emergency plans to the authorities and organizations that may be affected by an accident, including the safety measures to be adopted.

10.2. Notification of the first contained use operation

- a) The person in charge of the facility or laboratory where GMOs/GMM will be used must notify the Commission on Biological Safety (CSB. C) via e-mail (csbciencias@ciencias.ulisboa.pt) the intention to start work with GMM/GMO by submitting the GMM/GMO form (Annex II - GMO/GMM Work Assessment Document) duly completed.

It is up to the CSB. C, evaluate the application by verifying whether the facility is able to meet the requirements necessary for the contained use of GMO/GMM. The assessment is carried out using the checklist corresponding to the required biological safety level, available in the Annex (Annex XI – Checklist of requirements for Class 1 contained use and

- b) Annex XII – Checklist of requirements for Class 2 confined use). After verification, in case of compliance with the requirements necessary for the contained use of GMO/GMM, CSB. C forwards the request to G3S for submission to APA.
- c) CIÊNCIAS ULisboa, through G3S, notifies APA, I. P., of the intention to proceed with the use of facilities to carry out the first confined use operation, in order to obtain authorisation and, regardless of the risk classification of the operation, submit the information contained in the Notification Form for Contained Use of GMM and/or GMO associated with the respective safety level (Annex III – Class 1 Notification Form or Annex IV - Class 2 Notification Form) and which are available on the APA website: <https://apambiente.pt/prevencao-e-gestao-de-ricos/procedimento-autorizacao-de-uso-confinado>. The completion of the form is the responsibility of the person in charge of the facility or laboratory where GMO/GMM will be used and must be subsequently reviewed by the members of the CSB. C before its submission to the APA.

The first class 2 confined operation may be initiated:

- a) Immediately, upon authorization of APA, I. P.;
- b) 45 days after the submission of the notification containing the elements provided for in paragraph a) of the previous paragraph, with the authorisation of APA, I. P.

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10.3. Subsequent Class 1 Contained Use Operations

After the submission of the notification as outlined in the preceding Article, subsequent Class 1 contained use operations may take place without further procedures.

10.4. Subsequent Class 2 Contained Use Operations

Subsequent Class 2 Contained Use Operations may take place immediately after the submission of the Notification Form for Contained Use of GMM and/or GMO (Annex IV - Class 2 Notification Form) and which are available on the website: <https://apambiente.pt/prevencao-e-gestao-de-riscos/procedimento-autorizacao-de-uso-confinado> and which have been subject to a prior notification procedure for the purpose of carrying out Class 2 or higher contained use operations as long as the requirements set out in Decree-Law No. 55/2015, of 17 April are fulfilled. The completion of the form is the responsibility of the person responsible for the facility or laboratory where GMO/GMM will be used and must be subsequently reviewed by the members of the Biological Safety Commission.

By the analysis of the notification by the APA in this context, the payment of a fee is due, under the terms of Portaria No. 295/2018.

The decision on the confined use of GMM/GMO is made based on the assessment of the risks of GMM/GMO to human health and the environment considering the characteristics of GMM/GMO, facility conditions, implemented containment measures, waste management among other aspects.

In the evaluation process and under the terms of the legislation, the APA makes a decision on the confined use of GMM/GMOs, being heard the Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA), the Direção-Geral da Saúde (DGS) and, in the case of plants, the Direção-Geral de Alimentação e Veterinária (DGAV), and taking into account the results of the public consultation when considered relevant.

It is incumbent upon APA, I. P., after hearing the entities consulted:

- a) To verify the compliance of notifications for conducting GMO and GMO contained use operations, including:
 - i. The submitted documentation and the reliability of the information provided;
 - ii. The assessment of potential risks to human health and the environment resulting from contained use, including the disposal of waste and effluents, and the class of the contained use;
 - iii. Containment measures, waste and effluent management, as well as emergency action and other protection and safety measures;
- b) To authorize the start of confined use;
- c) To carry out visits to the facilities of confined use or adopt other monitoring or control measures to ensure that the user complies.

According to Decree-Law No. 55/2015, of 17 April, which regulates the contained use of GMM/GMO, the notifier must prepare an annual report of the activity carried out of the operations of confined use with GMM/GMO by submitting a report to APA.

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The annual report must be submitted at the beginning of each year, referring to the activity carried out in the previous year (Annex XIII - Annual Reporting Form for Contained Use of GMM/GMOs).

In the event of an accident, the notifier must inform the APA. Detailed information in the [Internal Biological Emergency Plan of CIÊNCIAS ULisboa](#).

10.5. Periodic actions and responsibilities

Authorisations for contained use of GMO/GMM require the guarantee of procedures under the responsibility of the Biological Safety Commission, those responsible for the authorised facilities and their users.

a) Users of GMO/GMO Contained Use Facilities

It is the responsibility of users of GMO/GMM contained use facilities to:

- Comply with the rules and good practices provided for in this handbook;
- Comply with operation and cleaning procedures;
- Comply with waste disposal rules and procedures;
- Identify waste collection jerrycans with waste designation and respective ELW code;
- Comply with specific access rules and procedures implemented in the facility;
- Ensure the record of contained uses of GMO/GMM (see Basic safety rules in Class 1 and Class 2 laboratories and Annex VIII – Records of use);
- Ensure the record of cleaning and decontamination actions of equipment and spaces (Annex IX – Cleaning Record);
- Report spills or accidents involving GMO/GMM to the laboratory manager and those responsible for Laboratory Infrastructures, or for the management of facilities, if they exist (see Internal Biological Emergency Plan of CIÊNCIAS ULisboa);
- Communicate failures or difficulties in implementing the measures provided for in this handbook to the laboratory manager and/or those responsible for Laboratory Infrastructures, or for the management of facilities, if any.

b) Responsible for the laboratory/facility where GMO/GMM will be used

It is the responsibility of the person in charge of the laboratory/facility where GMO/GMO will be used:

- Ensuring the training of new users and corresponding record (see Basic safety rules in Class 1 and Class 2 laboratories and example of record available in the Annex VII – Training Record);
- Ensure the respective assessment of the contained use of GMO/GMM;
- Ensure the elaboration/clarification of specific procedures complementary to this handbook;
- Establish and implement access control rules for Class 2 facilities in conjunction with the G3S;
- Ensure the updating of procedures and rules;
- Ensure that the facility/laboratory has the information, means and resources necessary for the application of the procedures provided for in this manual, namely the availability of appropriate

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cleaning material and agents and a spill containment kit, to act in the event of a spill, release or exposure to people involving biological risk;

- Ensure the awareness of users for the good practices of contained use of GMO/GMM and compliance with all associated rules, namely the Biological Safety Handbook (BSH) and the Internal Biological Emergency Plan of CIÊNCIAS ULisboa (PIEB);
- Monitor inspection actions;
- Organize and keep updated the file of records and documentation related to the installation;
- Communicate to CSB.C any need to amend the contained use authorization for GMO/GMM.

b.1) Annual responsibilities

Annually, those responsible for the facility or laboratory where GMO/GMM will be used must:

- Prepare and send to the CSB.C, the annual reporting of contained use activities in the previous year up to January 31st of each year (Annex XIII - Annual Reporting Form for Contained Use of GMM/GMOs);
- Ensure the execution of Operational Instruction 8 (IO-8) DISINFECTION QUALITY CONTROL PROCEDURES, belonging to the SAFETY PLAN – PREVENTION PLAN of CIÊNCIAS ULisboa and respective registration;
- Ensure the maintenance and annual certification of existing Biological Safety Cabinets and incubators in class 2 installations and keep their records available in the installation file (Annex X – Maintenance Log);
- Prepare the annual risk assessment of the contained use activities carried out (Annex V – Form for Annual Risk Reassessment for Conducted Confined Use Activities) and communicate to the CSB.C necessary changes to the contained use authorization as a result of the evaluation (e.g. use of new GMO/GMM).

c) CIÊNCIAS Biological Safety Commission

It is the responsibility of the Biological Safety Commission to:

- Define the mechanisms for evaluating users, namely, the evaluation questionnaires;
- Communicate to G3S new applications for contained use of GMO/GMM or changes to contained uses of GMO/GMM authorised to submit to the APA;
- In case of the need to respond to a failure in the planned confinement measures, ensure the applicability of the Internal Biological Emergency Plan of CIÊNCIAS ULisboa;
- Ensure the revision of the Biological Safety Handbook and the Internal Biological Emergency Plan whenever an update of procedures and rules is needed;
- Monitor inspection actions;
- Respond to requests arising from inspections.

c.1) Annual responsibilities

Annually, CSB.C must:

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- Submit to the APA the annual report of the activities of confined use of the previous year by February 28th of each year;

Attached (Annex XIV - Model of the Annual Calendar of Responsibilities), a sample schedule is available that can be used as a calendar of procedures under the responsibility of the CSB.C and those responsible for the authorised facilities and their users.

10.6. INSPECTION ACTIONS

Without prejudice to the competences of other entities, the supervision of compliance with the rules contained in DL No. 55/2015 is the responsibility of the Inspeção-Geral da Agricultura, do Mar, do Ambiente e do Ordenamento do Território (IGAMAOT) and the Autoridade para as Condições do Trabalho (ACT), within the scope of their respective competences.

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Internal Biological Emergency Plan of CIÊNCIAS ULisboa,

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12. ANNEXES

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12.1. Annex I- Order D/16/2022 General laboratory safety rules

Despacho D/16/2022

Sumário: Regras gerais de segurança em laboratório.

Considerando o Regime Jurídico da Promoção da Segurança e Saúde no Trabalho estabelecido pela Lei n.º 102/2009, de 10 de setembro, na sua atual redação;

considerando que o referido Regime Jurídico estabelece no seu artigo 15.º que o empregador deve zelar, de forma continuada e permanente, pelo exercício da atividade em condições de segurança e de saúde para o trabalhador, tendo em conta princípios gerais de prevenção;

ao abrigo das competências que me são conferidas nos termos da alínea y) do artigo 55.º dos Estatutos da Faculdade de Ciências da Universidade de Lisboa, publicados em anexo ao Despacho n.º 11913/2021, de 2 de dezembro, e ouvido o Gabinete de Segurança, Saúde e Sustentabilidade, determino que a utilização de laboratórios por parte de trabalhadores, alunos e visitantes, obedeça ao cumprimento das seguintes regras gerais de segurança em laboratório:

- conheça previamente os riscos e as medidas de prevenção associados às atividades, aos equipamentos e aos agentes químicos, físicos e biológicos com que vai trabalhar;
- Conheça previamente as fichas de dados de segurança de todos os agentes químicos que vai manusear;
- não fume, coma, beba ou guarde alimentos no laboratório;
- use vestuário e calçado apropriados e equipamentos de proteção individual adequados. Prenda os cabelos compridos;
- não utilize telemóveis nem toque em botões de elevadores e puxadores de portas com luvas contaminadas;
- identifique inequivocamente todos os recipientes contendo materiais e produtos. Minimize a quantidade de reagentes armazenados no laboratório;
- cumpra as regras para eliminação de resíduos perigosos produzidos no laboratório, e nunca os elimine diretamente para o esgoto ou balde do lixo comum;
- mantenha a bancada de trabalho limpa e arrumada;
- conheça a localização e o modo de utilização dos equipamentos de emergência (extintores, lava olhos, chuveiros, caixas de primeiros socorros, manta ignífuga, etc.);
- mantenha desobstruídos os acessos a percursos de evacuação, saídas de emergência ou equipamentos de emergência;
- não permita que o som dos dispositivos eletrónicos e a utilização de auscultadores no laboratório interfiram com a audição clara de alarmes e instruções;
- não permaneça com a bata vestida em espaços comuns, nomeadamente em bares e bibliotecas;
- lave as mãos antes de sair do laboratório;

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- em caso de emergência: mantenha a calma, ligue ext. 20000 ou +351 217 500 600, identifique o tipo e local da ocorrência (edifício, piso e sala) e coloque-se em segurança até à chegada da equipa de emergência, prestando todas as informações sobre o sucedido.

Assinado por : **JORGE AUGUSTO MENDES DE**

MAIA ALVES

Num. de Identificação: BI050394304

Data: 2022.03.11 19:16:01 GMT Standard Time



Jorge Maia Alves

Subdiretor

(Em substituição do Diretor, nos termos do disposto no Despacho n.º 5364/2018, de 29 de maio)

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12.2. Annex II- GMO/GMM Work Assessment Document

(available at: <https://cirrus.ciencias.ulisboa.pt/owncloud/s/yARqcJeDxtxsc5k>)

GMO/GMM Work Assessment Document

- Biological Safety Commission of CIÊNCIAS ULisboa, csbciencias@ciencias.ulisboa.pt

1 – The work involves the use of (mark with X):

a) Animals		
b) Plants		
c) Microorganisms		

2 – Is there (at the time of this assessment) or is it expected that there will be (during the experimental procedures) alteration(s) of the genome of the organism/microorganisms compared to the wildtype, namely through the introduction of genetic material (e.g. plasmids, viral genome)? (mark with X)

a) Yes		
b) No		

3 – The organisms/microorganisms fall into which of the groups indicated below (mark with X):

	Tick (X)	Notes
Biosafety level 1 (BSL1) biomaterial	<i>Bacillus subtilis</i>	
	Non-pathogenic strains of <i>Escherichia coli</i>	
	Adeno-associated viral vectors (AAV)	
	Mouse cell lines (<i>Mus musculus</i>) when they do not contain or are contaminated with human or animal pathogens, and when not altered with vectors of a higher safety level (e.g. use of lentiviral vectors).	
	Human cell lines (<i>specify in the Notes field</i>)	
	Laboratory mammal samples	
	Transgenic organisms that contain nucleic acids that do not exist in the wild strain and that cannot replicate or generate nucleic acids that can replicate in a living cell (e.g. oligonucleotides or other synthetic nucleic acids that do not contain a replication origin or contain elements known to interact with DNA or RNA polymerase); ii) they are not designed to be integrated into DNA; and iii) they do not produce a lethal toxin in vertebrates with an LD50 of less than 100 ng per kg of body weight.	
	Plants modified by recombinant or synthetic nucleic acid molecules that	

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Biosafety level 1 (BSL1) biomaterial	are not harmful weeds or that cannot come across harmful weeds in the immediate geographic area		
	Whole plants and recombinant nucleic acids or nucleic acids from modified molecules of non-exotic microorganisms, which have no recognised potential for rapid and widespread spread or with a serious negative impact on ecosystems (e.g. <i>Agrobacterium</i> spp.)		
	Other similar to one of the above categories		
Biosafety level 2 (BSL2)	Human samples (specify in the Notes field)		
	Human cell lines (specify in the Notes field)		
	Third-generation lentiviral vectors		
	Adenoviral vectors		
	<i>Aspergillus fumigatus</i>		
	<i>Toxoplasma gondii</i>		
	<i>Salmonella</i> Typhimurium		
	Influenza A		
	Plants modified by recombinant or synthetic nucleic acid molecules that are harmful weeds or may interbreed with harmful weeds in the immediate geographic area.		
	Plants associated with modified recombinant or synthetic nucleic acid molecules of non-exotic microorganisms with a recognized potential for serious detrimental impact on ecosystem management.		
Biosafety level 2 (BSL2)	Plants associated with modified recombinant or synthetic nucleic acid molecules of exotic microorganisms that have no recognized potential for a serious detrimental impact on ecosystem management.		
	Experiments with modified recombinant or synthetic nucleic acid molecules from arthropods or small plant-associated animals, or from micro-organisms associated with plants, if the micro-organisms modified by synthetic nucleic acid molecules do not have a recognised		

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	potential for adverse impact on ecosystems.		
	Other similar to one of the above categories		

Users:

Name (function/quality in which you participate in the assessment)

Name (function/quality in which you participate in the assessment)

(...)

(Location), (Date)

(signature, name)

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12.3. Annex III – Class 1 Notification Form

This form is available in Portuguese only. The translation provided here is for guidance only.



Notification Form for Contained Use of GMM and/or GMO Class 1 (1st use)

To be submitted to the APA, for the purposes of complying with article 8 of Decree-Law no. 55/2015, of 17 April, in a format in accordance with article 16 of the same diploma, which must be accompanied by the respective risk assessment for human health and the environment (according to Annex II of the form in question) and include the potential harmful effects and consequent containment measures concerning: a) the donor organism/micro-organism; b) to the vector or vector/host system; c) the recipient organism/microorganism; d) the resulting GMM/GMO; e) to organisms that, although not directly involved in the process in question, are the starting point (give rise) to others that will be used. It should be noted that according to Article 9 of Decree-Law 55/2015, subsequent uses of Class 1 do not require notification.

I) Identification of the notifier

Name of the notifier

NIPC

Address

Telephone

Fax

Email

II) Description of Contained Use (1st use)

MGM

☐

GMO

☐

(Check the correct option)

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Confined use class

Description of the activities
developed

[see note of support in Annex I]

(Include the respective work plan)

III) Description of MGM/GMO

Identification
characteristics
GMM/GMO

and
of

[see note of support in Annex I]

IV) Installation Description

Facility Address

Installation Overview

[see note of support in Annex I]

(Include the respective plant(s) of the facility showing the location of the GMM/GMOs)

V) User description

Name of users and those
responsible for
surveillance and security

Training, qualifications
and experience (years) of
those responsible for
surveillance and security



Data on any committees or working groups set up (security, maintenance, emergency, risk assessment or others)

VI) Risk assessment

Summary of the risk assessment for human health and the environment

Rationale for the risk class according to the outcome of the risk assessment

(Include the respective assessment report prepared in accordance with Annex I to this form, as well as the map of the location that should be an integral part of it)

VII) Risk management measures implemented

Containment/protection measures implemented

Waste management measures

Measures to prevent incidents, accidents and action in case of emergency

(Include the contingency plan to be adopted in case of failure of the planned containment measures)



VIII) Conclusion on the risk

Conclusion on the acceptability of the risk (taking into account the risk class, the environment and the suitability of the measures implemented)

IX) Confidentiality request

Identification and substantiation of information considered confidential

X) Reporting Officer

Signature

Name

Date

Attachments:

- Installation plans
- Report of the Risk assessment for Human Health and the Environment carried out in accordance with Annex I to this form
- Location map
- Internal Emergency Plan



Annex I

Notes to support filling out the form

II. Description of Contained Use

Description of the activities developed

It is intended that the notifier briefly provide a general description of the activities carried out on the premises in connection with the contained use of GMM/GMOs (including activities involving manipulation as well as activities giving rise to GMM/GMOs).

[This information should be complemented with the work plan].

III. Description MGM/GMO

If GMM/GMOs are used as donors, vectors or recipients, the notifier will have to indicate which micro-organism(s) gave rise to them, whether the manipulation has taken place at the site of installation and will have to identify and describe this GMO/GMM.

Example:

In the case of the use of vectors without the possibility of autonomous recombination ("disarmed") that come from pathogenic microorganisms (e.g. HIV), it is important that the notifier refers to which microorganism (classified according to Decree-Law No. 84/97 and Ordinance 1036/98) originated the vector, and whether or not the microorganism will be handled at the installation site, as the containment measures will have to be evaluated according to the situation.

Identification and characteristics of GMM/GMO

Identification and description of the characteristics of all GMMs/GMOs involved in the procedures to be carried out, including not only the final GMO/GMM but also all GMOs/GMOs handled during the procedures carried out.

Example:

If it is intended to introduce genes responsible for the production of beta-carotene into the rice plant and if it is necessary to amplify a given plasmid in E. coli and use the modified bacterium Agrobacterium tumefaciens (without T-DNA and transformed with the plasmid amplified into E. coli) to insert the genes of interest into the rice plant, the notifier will have to identify as the GMM/GMO involved in the process, the rice plant transformed with the genes responsible for the synthesis of beta carotene, the strain of E. coli used to amplify the plasmid and the modified strain of Agrobacterium tumefaciens.

IV. Installation Description

Installation Overview

Briefly describe the facility (type of building, number of floors, how the various types of laboratory areas and the administrative area are divided, planned ventilation system and GMOs/MGMs handled or produced in these areas). Attach a map of the same showing the location of the GMM/GMOs.

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Annex II

Elements necessary for the assessment of risk to Human Health and the Environment

I. Identification of harmful effects

Identify the potentially harmful effects associated with the recipient micro-organism/organism, inserted genetic material, vector, donor micro-organism/organism, the resulting GMM/GMO and other effects considered relevant.

Diseases in humans, including allergenic or toxic effects, diseases in animals or plants, are considered potentially harmful effects; effects that have no treatment or for which effective prophylaxis is not available; effects resulting from fixation or dissemination in the environment; effects that result from the natural transfer to other organisms, of inserted genetic material.

a) Diseases in humans

Describe any significant effects of GMM on humans, including probable direct or indirect interactions and any hazardous characteristics that are likely to cause allergenic or toxic effects.

b) Diseases in animals or plants

Describe any significant effects of GMM on animals or plants including insects, symbionts, pathogens (e.g. viruses and bacteria), birds and mammals. Probable direct or indirect interactions and any characteristics that are hazardous and likely to cause allergenic or toxic effects shall be considered.

c) Effects that have no treatment or for which no effective prophylaxis is available

Refer to the effects of MGM for which no treatment is known.

d) Effects resulting from fixation or dissemination in the environment

Refer to the effects of GMM in the event of its spread into the environment.

e) Effects resulting from the natural transfer of inserted genetic material to other organisms

Refer to the effects resulting from the increase or decrease in the transfer potential of the genetic material of the GMM.

II. Severity of the effects considered to be potentially harmful

Describe the severity of the effects identified in point I.

III. Likelihood of occurrence of effects considered to be potentially harmful

Indicate the probability of occurrence of the and effects identified in point I.

IV. Classification of the Confined Use Operation

The classification of contained use operations shall be based on the relevant information available, and the bibliographic references supporting such classification shall be mentioned.

In case of doubt as to the class to be adopted, the classification corresponding to the next level must be assigned in order to safeguard the protection of human health and the environment.

*GMMs with the following characteristics are considered to be appropriately included in Class 1:
(a) the recipient or parental micro-organism is unlikely to cause disease in humans, animals or plants
(b) GMM is unlikely to cause disease in humans, animals or plants and to have adverse effects on the environment*

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(c) the nature of the vector and the inserted element does not give rise to an MGM with a phenotype that is likely to cause disease in humans, animals or the environment

V. Description of the surroundings

Identification and characterization of the environment that may be affected. Identify, specifically, environmentally sensitive and/or contamination-prone elements.

Include a map of the location of the installation, where the elements mentioned above are identifiable.

VI. Identification and selection of containment/protection measures

Identify and conclude on the adequacy of the containment/protection measures implemented, based on the risk class of the GMO/MGM, as well as the characteristics of the environment likely to be negatively affected, characteristics of the activity and unconventional operations (see applicable minimum requirements tables IA-C and II of Annex IV of Decree-Law No. 55/2015, of 17 April).

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12.4. Annex IV- Class 2 Notification Form

This form is available in Portuguese only. The translation provided here is for guidance only.



Notification Form for Contained Use of GMM and/or Class 2 GMOs

To be submitted to the APA, for the purposes of complying with articles 8 and 10 of Decree-Law no. 55/2015, of 17 April, in a format in accordance with article 16 of the same diploma, which must be accompanied by the respective risk assessment for human health and the environment (according to Annex II of the form in question) and include the potential harmful effects and consequent containment measures concerning: a) the donor organism/micro-organism; b) to the vector or vector/host system; c) the recipient organism/microorganism; d) the resulting GMM/GMO; e) to organisms that, although not directly involved in the process in question, are the starting point (give rise) to others that will be used.

XI) Identification of the notifier

Name of the notifier

NIPC

Address

Telephone

Fax

Email

XII) Framework for Contained Use

MGM

☐

GMO

☐

(Check the correct option)

1st Use

☐

Subsequent Use

☐

(Check the correct option)

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Confined use class

Description of the activities
developed

[see note of support in Annex I]

III) Identification of the previously authorized Contained Use *(fill in only for subsequent uses)*

Permitted use class

Date

Notification No.

Date

IV) Description of Contained Use

Description of the work to
be carried out, including its
planning, objective and
expected results

[see note of support in Annex I]

(Include the respective work plan)

Approximate
volume/quantity of culture/
to be used



V) Description of the GMM/GMO

Identification and characteristics of GMM/GMO

[see note of support in Annex I]

Origin and function of the genetic material involved in genetic modification (donor)

[see note of support in Annex I]

Vector/host system used

[see note of support in Annex I]

Identification of the micro-organism/recipient organism

[see note of support in Annex I]

VI) Description of the installation

Facility Address

Installation Overview

[see note of support in Annex I]

(Include the respective plant plant(s), showing the location of the GMM/GMOs)

VII) Description of users

Name of users and those responsible for surveillance and security



Training, qualifications and experience (years) of those responsible for surveillance and security

Data on any committees or working groups set up (security, maintenance, emergency, risk assessment or others)

VIII) Risk assessment

Rationale for the risk class according to the outcome of the risk assessment

(Include the respective assessment report prepared in accordance with Annex II to this form, as well as the map of the location that should be an integral part of it)

IX) Risk management measures implemented

Waste management measures

Measures to prevent incidents, accidents and action in case of emergency

(Include the contingency plan to be adopted in case of failure of the planned containment measures)



X) Conclusion on the risk

Conclusion on the acceptability of the risk (taking into account the risk class, the environment and the suitability of the containment and risk management measures implemented)

XI) Request for confidentiality

Identification and substantiation of information considered confidential

XII) Responsible for the notification

Signature

Name

Date

Attachments:

- Installation plans
- Report of the Risk assessment for Human Health and the Environment carried out in accordance with Annex II to this form
- Location map
- Internal Emergency Plan

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Annex I

Notes to support filling out the form

II. Framework for Contained Use

Description of the activities developed

It is intended that the notifier briefly provide a general description of the activities carried out on the premises in connection with the contained use of GMM/GMOs (including activities involving manipulation as well as activities giving rise to GMM/GMOs).

IV. Description of Contained Use

Description of the work to be carried out, including its planning, objective and expected results

The notifier is intended to provide a description of the work of contained use to be carried out, including a summary and explanation of all procedures, as well as the objective and expected results.

Example:

The aim of this work is to increase the expression/reduce the expression/silence/introduce, in cell lines/microorganisms/organisms, the x genes, using the y technique, which involves the use of the z vector. The procedures described aim to study the cellular mechanisms involved in the processes w and the role of the gene x in the mechanism k.

[This information should be complemented with the work plan].

V. Description MGM/GMO

If GMM/GMOs are used as donors, vectors or recipients, the notifier will have to indicate which micro-organism(s) gave rise to them, whether the manipulation has taken place at the site of installation and will have to identify and describe this GMO/GMM not only in the respective items ("Origin and function of the genetic material involved in the genetic modification (donor)", "Vector/host system used", "Identification of the micro-organism/recipient organism") but also in the item "identification/description of the GMM/GMO".

Example:

In the case of the use of vectors without the possibility of autonomous recombination ("disarmed") that come from pathogenic microorganisms (e.g. HIV), it is important that the notifier refers to which microorganism (classified according to Decree-Law No. 84/97 and Ordinance 1036/98) originated the vector, and whether or not the microorganism will be handled at the installation site, as the containment measures will have to be evaluated according to the situation.

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Identification and characteristics of GMM/GMO

Identification and description of the characteristics of all GMMs/GMOs involved in the procedures to be carried out, including not only the final GMO/GMM but also all GMOs/GMOs handled during the procedures carried out.

Example:

If it is intended to introduce genes responsible for the production of beta-carotene into the rice plant and if it is necessary to amplify a given plasmid in *E. coli* and use the modified bacterium *Agrobacterium tumefaciens* (without T-DNA and transformed with the plasmid amplified into *E. coli*) to insert the genes of interest into the rice plant, the notifier will have to identify as the GMM/GMO involved in the process, the rice plant transformed with the genes responsible for the synthesis of beta carotene, the strain of *E. coli* used to amplify the plasmid and the modified strain of *Agrobacterium tumefaciens*.

Origin and function of the genetic material involved in genetic modification (donor)

Identify where the genes to be introduced into the recipient organism come from, with clear identification of the donor organism (organism from which the genetic material of interest originates) and what is the intended function (see diagram in the annex). Explain whether or not donor organisms will be handled during the procedure, as containment measures will have to be appropriate to the classes of all microorganisms/organisms handled during the process. If the donor micro-organism/organism is pathogenic, it is important to know whether it is going to be handled in the facility or whether the notifier is just going to handle its nucleic acid.

Example:

In the introduction of genes responsible for the production of beta-carotene into the rice plant, the genes responsible for the synthesis of beta-carotene could, for example, come from the maize plant, and the intended function would be to increase the beta-carotene content in the rice grain.

Vector/host system used

Describe the vector/host system used (see attached schematic).

This system can simply be used to amplify the genetic material of interest, but it can also be used to effect the transfer of the recombinant vector to the recipient.

A vector is the nucleic acid molecule used to transport the genetic material of interest to a recipient cell. Examples of vectors are, namely, plasmids and viruses.

Example:

In the introduction of genes responsible for the production of beta-carotene into the rice plant, it would be necessary to describe the vector/host system *E. coli*/recombinant plasmid (to amplify the genetic material of interest) and *Agrobacterium tumefaciens*/recombinant plasmid (to effect the transfer of the recombinant vector to the receptor).

Identification of the recipient micro-organism/organism

Identification of the organism/microorganism that will receive the genetic material of interest in order to obtain the GMO/GMM (see attached diagram)

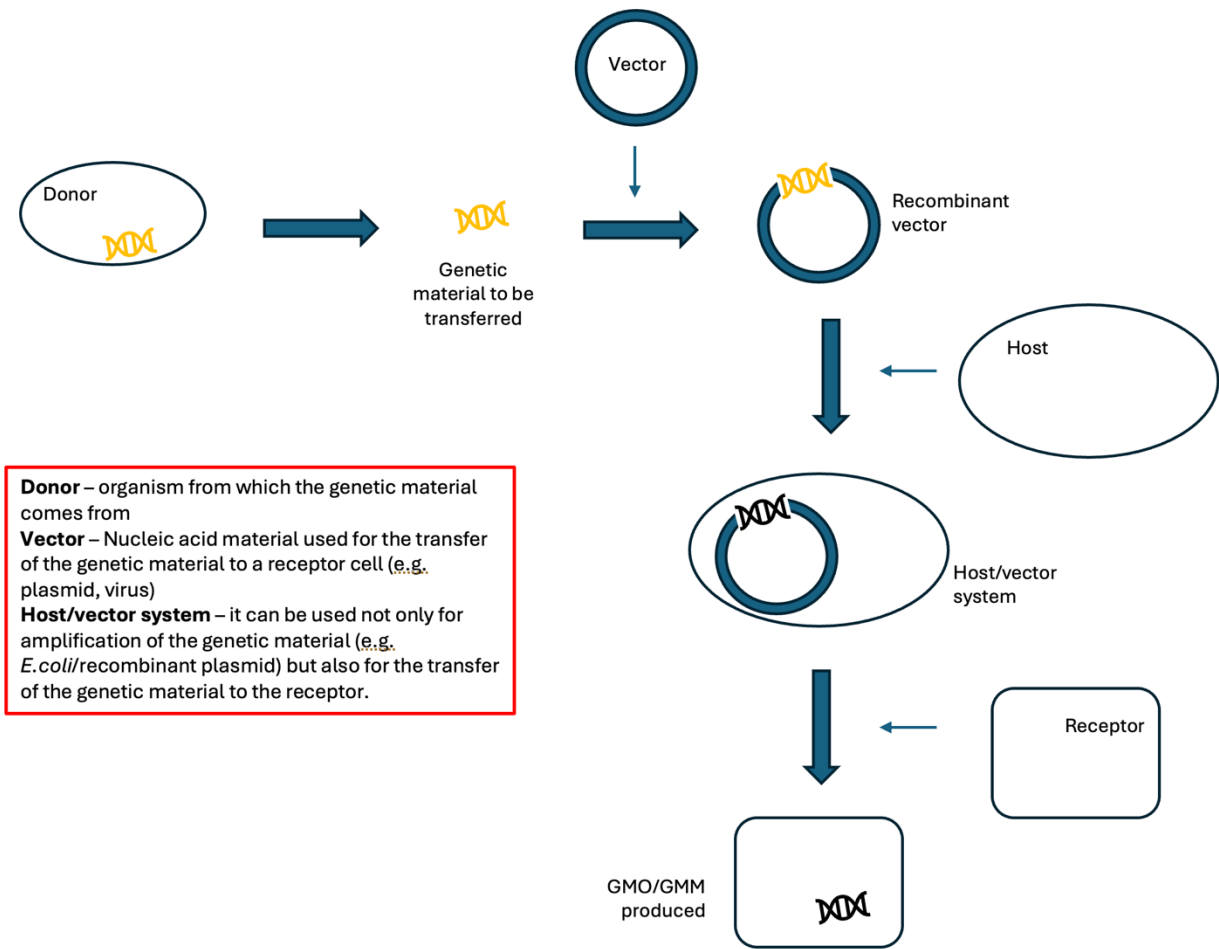
SAW. Installation Description

Installation Overview

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Briefly describe the facility (type of building, number of floors, how the various types of laboratory areas and the administrative area are divided, planned ventilation system, and GMOs/MGMs handled or produced in those areas). Attach a map of the same showing the location of the GMM/GMOs.

Figure 1: Illustrative scheme of genetic manipulation





Annex II

Elements necessary for the assessment of risk to Human Health and the Environment

VII. Identification of harmful effects

Identify potentially harmful effects associated with the donor micro-organism/organism, inserted genetic material, vector, receiving micro-organism/organism, the resulting GMM/GMO and other effects considered relevant.

Diseases in humans, including allergenic or toxic effects, diseases in animals or plants, are considered potentially harmful effects; effects for which there is no effective treatment or prophylaxis; effects resulting from fixation or dissemination in the environment; effects that result from the natural transfer of the inserted genetic material to other organisms.

f) Effects on humans

Describe any significant effects of GMM/GMO on humans, including probable, direct or indirect interactions and any hazardous characteristics that are likely to cause infection, allergenic or toxic effects.

g) Effects on animals or plants

Describe any significant effects of GMM/GMOs on animals or plants including insects, symbionts, pathogens (e.g., viruses and bacteria), birds, and mammals. Probable direct or indirect interactions and any characteristics that are hazardous and likely to cause allergenic or toxic effects shall be considered.

h) Effects for which no effective treatment or prophylaxis is available

Refer to the effects of GMM/GMO for which no treatment is known.

i) Adverse effects resulting from establishment or dissemination in the environment

Refer to the effects of GMM/GMOs in the event of their spread into the environment.

j) Effects resulting from the natural transfer of inserted genetic material to other organisms

Refer to the effects resulting from the increase or decrease in the transfer potential of the genetic material of the GMM/GMO.

VIII. Severity of the effects considered to be potentially harmful

Describe the severity of the effects identified in point I.

IX. Likelihood of occurrence of effects considered to be potentially harmful

Indicate the likelihood of the effects identified in point I.

X. Classification of the Confined Use Operation

The classification of contained use operations shall be based on the relevant information available, and the bibliographic references supporting such classification shall be mentioned.

In case of doubt as to the class to be adopted, the classification corresponding to the next level must be assigned in order to safeguard the protection of human health and the environment.

XI. Description of the surroundings

Identification and characterization of the environment that may be affected. Identify, specifically, environmentally sensitive and/or contamination-prone elements.

Include a map of the location of the installation, where the elements mentioned above are identifiable.

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XII. Identification and selection of containment/protection measures

Identify and conclude on the adequacy of the containment/protection measures implemented, based on the risk class of the GMO/MGM, as well as the characteristics of the environment likely to be negatively affected, characteristics of the activity and unconventional operations (see applicable minimum requirements tables IA-C and II of Annex IV of Decree-Law No. 55/2015, of 17 April).

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12.5. Annex V – Form for Annual Risk Reassessment for Conducted Confinement Activities

(available at: <https://cirrus.ciencias.ulisboa.pt/owncloud/s/yARqcJeDxtxsc5k>)

Confined use of MGM and GMO

Annual register of risk assessments of the confined activities carried out

Date:		Laboratory:	
Authorization:		Installation:	
Responsible person/people:			

1. Description of confined use:

- a. Description of the work to be carried out
(Describe the changes or indicate "No alterations to report")
- b. Approximate volume/amount of culture to be used
(Describe the changes or indicate "No alterations to report")

2. Description of GMM/GMO:

- a. Identification and characteristics of GMM/GMO
(Describe the changes or indicate "No alterations to report")
- b. Origin and function of the genetic material involved in genetic modification (donor)
(Describe the changes or indicate "No alterations to report")
- c. Vector/host system used
(Describe the changes or indicate "No alterations to report")
- d. Identification of the micro-organism/recipient organism
(Describe the changes or indicate "No alterations to report")

3. Facility description:

- a. Facility address
(Describe the changes or indicate "No alterations to report")
- b. Facility overview
(Describe the changes or indicate "No alterations to report")

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4. Description of users:

- a. **Name of users and those responsible for surveillance and security**
(Describe the changes or indicate "No alterations to report")
- b. **Training, qualifications and experience (years) of those responsible for surveillance and security**
(Describe the changes or indicate "No alterations to report")
- c. **Data on any committees or working groups set up (security, maintenance, emergency, risk assessment or others)**
(Describe the changes or indicate "No alterations to report")

5. Risk assessment

- a. **Rationale for the risk class according to the outcome of the risk assessment**
(Describe the changes or indicate "No alterations to report")

6. Risk management measures implemented

- a. **Waste management measures**
(Describe the changes or indicate "No alterations to report")
- b. **Measures to prevent incidents, accidents and action in case of emergency**
(Describe the changes or indicate "No alterations to report")

7. Conclusion on the risk

- a. **Conclusion on the acceptability of the risk (taking into account the risk class, the environment and the suitability of the containment and risk management measures implemented)**
(Describe the changes or indicate "No alterations to report")

8. Confidentiality request

- a. **Identification and substantiation of information considered confidential**
(Describe the changes or indicate "No alterations to report")

Risk Assessment for Human Health and Environment

1. Identification of harmful effects

- a. **Effects on humans**
No alterations to report (Describe the changes or indicate "No alterations to report")
- b. **Effects on animals or plants**
(Describe the changes or indicate "No alterations to report")

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- c. **Effects for which no effective treatment or prophylaxis is available**
No alterations to report (Describe the changes or indicate "No alterations to report")
- d. **Adverse effects resulting from establishment or dissemination in the environment**
(Describe the changes or indicate "No alterations to report")
- e. **Effects resulting from the natural transfer of inserted genetic material to other organisms**
(Describe the changes or indicate "No alterations to report")
- 2. **Severity of the effects considered to be potentially harmful**
(Describe the changes or indicate "No alterations to report")
- 3. **Likelihood of occurrence of effects considered to be potentially harmful**
(Describe the changes or indicate "No alterations to report")
- 4. **Classification of the confined use operation**
(Describe the changes or indicate "No alterations to report")
- 5. **Description of the surroundings**
(Describe the changes or indicate "No alterations to report")
- 6. **Identification and selection of containment/protection measures**
(Describe the changes or indicate "No alterations to report")

Participants in the review:

Name (function/capacity in which you participate in the risk assessment)

Name (function/capacity in which you participate in the risk assessment)

List of attachments:

(delete if not applicable)

Annex 1: (...)

Annex 2: (...)

(place), (date)

(signature, name)

(role/capacity in which you participate in the risk assessment)

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12.6. Annex VI – List of disinfectants and contact times

DISINFECTANT AGENTS AND CONTACT TIMES				
Product	Active Agent	Concentration	Contact Time	Observations
Bleach	Sodium Hypochlorite	Solutions of 5000 ppm, 0.5% corresponding to a dilution of 1:10 v/v of commercial bleach	Surface disinfection - 1 min Disinfection of liquid waste – 20 min	Corrosive to metal surfaces so it should be cleaned with water after its contact time. Diluted solutions should be prepared daily and kept in an opaque container. Often used to disinfect surfaces and equipment.
		10,000 ppm, 1% solutions corresponding to a dilution of 1:5 v/v commercial bleach for biological waste containing a high organic load (e.g. blood, proteins or lipids)	Surface disinfection - 1 min Disinfection of liquid waste - 20 min	
Alcohol	Ethanol	70% v/v	10 min	Flammable. Often used to disinfect surfaces and equipment.
Oxidants	Hydrogen peroxide	As per product indication	1 min	It can damage some metals (e.g. iron, copper, brass, zinc, steel). Non-toxic and decomposes into water and oxygen. Used to disinfect surfaces, equipment and waste. Caution should be applied in the presence of wounds.
ZF™ Biocidal	Quaternary Ammonium Compounds	As indicated of the product	10 min or as per product indication	Some are irritating to the skin, eyes and respiratory tract. Used to disinfect surfaces and equipment. Reduced effectiveness in the presence of organic matter.

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12.7. Annex VII – Training Record

(available at: <https://cirrus.ciencias.ulisboa.pt/owncloud/s/yARgcJeDxtxsc5k>)

Training: "Rules of use of the Culture Room (*to be specified*)"

ACTION DATA

Name of the
training
action:

Research
centre or
training
department:

Place:

Duration:

Training
program/sum
mary

Training
scheme *Classroom*

DATA OF THE TRAINEES AND TRAINER

User Name	Date of Training	Category	Signature	Trainer and signature	Date of Evaluation

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12.8. Annex VIII – Records of use

(available at: <https://cirrus.ciencias.ulisboa.pt/owncloud/s/yARqcJeDxtxsc5k>)

Observations								
Plates/ Bottles								
Mycoplasma test								
GMM/GMO								
Incubator								
LFC/BSC								
End Time								
Start Time								
User								
Date								

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12.9. Annex IX – Cleaning Record

(available at: <https://cirrus.ciencias.ulisboa.pt/owncloud/s/yARqcJeDxtxsc5k>)

CLEANING LOG

Facility/Lab: _____

Sheet No. _____

[illegible]



12.11. Annex XI – Checklist of requirements for Class 1 contained use

Check-list – Level 1 of biological safety

Date:		Laboratory:	
Responsible:			

	Yes	No	N/A	Comments
Laboratory/Facility				
Benches resistant to water, acids, bases, solvents, disinfectants and decontamination agents, easy to clean?				
Effective control of vectors (e.g. rodents and insects)?				
Are the doors kept closed?				
Notice to keep windows closed posted?				
Cleaning and disinfecting agents available?				
Spill kit available at the facility?				
Procedures				
Appropriate protective clothing/equipment?				
Procedure and rules of good practice communicated in the training of new users?				
Clean-up procedures in case of spillage?				
Equipment utilization procedures?				
Prohibition of applying cosmetics, storing food for human consumption, eating, drinking, smoking in the laboratory/facility?				
Prohibition of pipetting with the mouth?				
Equipment				
Autoclave on site?				
Waste				
Procedures for the disposal of biohazard waste (solid and liquid)?				
Inactivation of GMM and/or GMO in contaminated material and waste?				optional
Properly labelled and closed containers? (ELW code placed on the jerricans with the biological waste)				
Waste disposal standards posted in the laboratory/facility?				
Records				
Record of spills and accidents involving GMO/GMM?				
Annual record of risk assessments of contained use activities carried out?				

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12.12. Annex XII – Checklist of requirements for Class 2 confined use

Check-list – Level 2 of biological safety

Date:		Laboratory:	
Responsible:			

	Yes	No	N/A	Comments
Laboratory/Facility				
Benches resistant to water, acids, bases, solvents, disinfectants and decontamination agents, easy to clean?				
Biohazard warning on the door?				
Restricted access?				
Effective control of vectors (e.g. rodents and insects)?				
Are the doors kept closed?				
Notice to keep windows closed posted?				
Cleaning and disinfecting agents available?				
Spill kit available at the facility?				
Procedures				
Decontaminated work surfaces before and after each procedure, daily and after spills?				
Control measures at source				
Appropriate protective clothing/equipment?				
Implemented control measures for reusable personal protective clothing (e.g. washing labcoats)?				
Procedure for training new users?				
Clean-up procedures in case of spillage?				
Reporting procedure in the event of a spill?				
All GMO/GMM spills and accidents reported to the laboratory supervisor?				
Equipment utilization procedures?				
Specific measures to control the spread of aerosols?				
Tests carried out <u>biannually</u> for the presence of viable manipulated organisms outside the primary area of physical containment?				
Prohibition of applying cosmetics, storing food for human consumption, eating, drinking, smoking in the laboratory/facility?				
Prohibition of pipetting with the mouth?				
Equipment				
Autoclave in the building				
Biohazard warning posted on equipment?				
Laminar flow cabinet with annual maintenance performed?				
CO2 incubators with annual certification carried out?				
Live flames used inside the cabinet?				Forbidden!

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	Yes	No	N/A	Comments
Waste				
Procedures for the disposal of biohazard waste (solid and liquid)?				
Inactivation of GMM and/or GMO in contaminated material and waste?				
Properly labelled and closed containers? (ELW code placed on the jerricans with the biological waste)				
Waste disposal standards posted in the laboratory/facility?				
Proof of disposal of HEPA filters by an accredited operator?				
Records				
New user training registration?				
Record of use of the room/equipment with user indication/date/cell line used?				
Record of periodic cleaning carried out on equipment and installation?				
Record of spills and accidents involving GMOs/GMMs?				
Annual record of risk assessments of contained use activities carried out?				
Disinfection quality control record in the BSC and outside the primary containment zone (benchtop) (IO-8)?				



12.13. Annex XIII - Annual Reporting Form for Contained Use of GMM/GMOs

This form is available in Portuguese only. The translation provided here is for guidance only.

Annual activity reporting form

Contained Use of GMM/GMOs

To be submitted to the APA, for the purposes of complying with article 6 of Decree-Law no. 55/2015, of 17 April, in a format in accordance with article 16 of the same diploma.

XIII) General Information

Reporting period

Type of Authorized Contained Use

Notification number^(s)

Class(es) of Contained Use

XIV) Identification of the notifier

Name of the notifier

Address

Telephone

Email

XV) Description of the Contained Use activities developed

MGM/GMO used

Confined Usage Class

Origin

Quantity

Prepared by:

Commission on Biological Safety

Version:

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Revision:

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Description of the activity
carried out

--

Specify any changes to the
notification(s)

--

specify, inter alia, changes in the level of the GMM(s) used; of confined use operations; Facilities (including any changes in the confinement mediates; work programme(s); users and those responsible for confined use, surveillance and safety; emergency procedures and waste management)

Indicate if any accident or
anomalous situation has
occurred

--

XVI) Responsible for the report

Signature

--

Name of the person
responsible for preparing the
report

--

Date

--

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12.14. Annex XIV- Model of the Annual Calendar of Responsibilities

Confined use of GMM and GMO – FACILITY MANAGERS

Laboratory:		YEAR:	
Authorization:		Installation:	
Responsible:			

ANNUAL CALENDAR OF RESPONSIBILITIES

- ☐ UNTIL 31 JAN: Preparation of the annual report of the activities of confined use
- ☐ UP TO _____: Prepare the annual risk assessment of the contained use activities carried out and communicate to the Biological Safety Commission any necessary changes
- ☐ UP TO _____: Ensure the maintenance and annual certification of existing Laminar Flow Cabinets and incubators in class 2 facilities
- ☐ UP TO _____: Execute IO-8 – PROC. DISINFECTION QUALITY CONTROL
- ☐ UP TO _____: Execute IO-8 – PROC. DISINFECTION QUALITY CONTROL

DON'T FORGET:

- ☐ Ensure the update of procedures and rules
- ☐ Ensure that appropriate cleaning material and agents and a spill containment kit are available

NEW USERS:

- ☐ Ensure training and ensure the evaluation of new users and their registration
- ☐ Ensure user awareness of good practices for the contained use of GMO/GMM and compliance with all associated rules, namely compliance with the Biological Safety Handbook and the Internal Biological Emergency Plan

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Confined use of GMM and GMO – BIOSAFETY COMMISSION

ANNUAL CALENDAR OF RESPONSIBILITIES

- ☐ UNTIL FEB 28: Submission of the annual report of the activities of confined use
- ☐ UP TO _____: Ensure the revision of the Biological Safety Handbook
- ☐ UP TO _____: Ensure the revision of the Internal Biological Emergency Plan
- ☐ BY _____: Communicate to the APA new requests for contained use of GMO/GMM or changes to contained uses
- ☐ UP TO _____: respond to requests arising from Inspection actions

DON'T FORGET:

- ☐ Communicate to the APA new requests for contained use of GMO/GMM or changes to contained uses;
- ☐ Ensure the revision of the Biological Safety Handbook and the Internal Biological Emergency Plan whenever needs to update procedures and rules are identified
- ☐ To monitor and respond to requests arising from inspection actions

NOTES / DON'T FORGET: