

Biosafety Level 2 regulations

Only the risk group 2 microorganisms can be currently manipulated at the University of the Balearic Islands (UIB). Such tasks shall be developed at the laboratories authorized by the Biosafety Committee and carried out only by personnel with specific Biosafety Level 2 training.

General regulations for the manipulation of microorganisms, samples potentially infected with biological agents and genetically modified organisms (GMOs)

For the proper manipulation of microorganisms and materials potentially infected with biological agents (human or animal specimens, wastewater, GMOs, etc.) the following biosafety regulations must be followed:

1. Hygiene measures

- Wash your hands with soap and water before and after manipulating potentially infectious materials. Dry them using paper, not a cloth towel. For more information on how to wash your hands properly, please follow this [link](#).
- Cover your hand wounds with a waterproof dressing.
- Do not wear rings or jewellery while working. Do not wear nail polish either.
- Do not use contact lenses in the laboratory.
- Eating, drinking, smoking and even chewing gum are not permitted in the laboratory. Do not use your mobile phone or any other device while wearing gloves.
- Food and drink cannot be stored in the laboratory refrigerators.
- Keep clean and tidy your working area. Store your personal items and garments in safe wardrobes or storage areas.
- Cover your work surface with absorbent paper.
- Mouth pipetting is strictly forbidden. Manual or automatic pipettors, propipettes, etc. should be used instead. Pipettes should have cotton plugs to prevent contamination.
- Laboratory windows and doors must be closed.

2. Personal protective equipment (PPE)

Inside the laboratory, at all times, you should wear **closed shoes** with non-skid soles and a long-sleeved laboratory coat. Leave the laboratory coat at the university (there is a laundering service). You should also wear **waterproof gloves** when manipulating cultures, blood and other potentially infectious materials or objects, patients, etc. Do not touch any other object (doorknobs, telephones, chairs, etc.) while wearing gloves. Do not touch your mouth, eyes or face either.

Safety glasses and cellulose masks (type P3) must be worn when splashes or aerosols may be generated and when working within a biological safety cabinet is not possible. For instance, in the event of spillages involving infectious materials.



3. Biological safety cabinets (BSCs)

It is required to work within a class II biological safety cabinet in the following cases:

- When aerosols may be generated (aspiration, agitation, centrifugation, sonication, homogenization, opening of a pressure tube or vial, etc.).
- When air-transmitted biological agents or samples infected with those agents are manipulated (wastewater, for example).

Do not confuse a BSC with a laminar flow cabinet, which only protects the sample that is being manipulated but does not offer personnel protection. Do not manipulate infectious samples within a laminar flow cabinet.

Working protocol

- Before starting to work, turn on the BSC for 5 minutes in order to obtain a stable airflow. If the BSC has ultraviolet light, turn it on. After completion of work, keep the BSC on for at least 5 more minutes.
- Before starting to work, place all the required equipment inside the cabinet, to avoid a continuous in-and-out movement. For instance, the pipette tip disposal container should be placed inside the BSC.
- Decontaminate any packaging before entering and removing materials.
- Work at least 10 cm away from the front edge of the BSC.
- Do not block the front and rear air grilles with any material.
- Doors, windows, air conditioners and personnel may cause undesired airflows, which may alter the vertical flow. Move your arms and hands slowly when working inside the cabinet.

- Bunsen burners should not be used inside the cabinet. The flame can distort the airflow and may damage the HEPA filters. Instead, use sterile disposable loops or sterilize the loops with a microincinerator.
- Clean up immediately any accidental spillage of biological material inside the BSC and decontaminate the surface and all the equipment placed inside the cabinet.
- HEPA filters must be regularly replaced, according to the manufacturer's instructions.

4. Use of sharps

- Avoid the use of syringes. If it is not possible, use safety syringes.
- Glassware (tubes, pipettes, bottles, etc.) should be replaced with plastic ware whenever possible.
- Be extremely cautious when manipulating sharp-edged or cutting objects.
- Needles and sharp-edged or cutting objects should never be recapped. Discard them in yellow rigid biosafety containers.



5. Laboratory waste management

- Laboratory wastes will be collected once a month by an authorized company and finally incinerated. There are two types of containers: **yellow containers** (for contaminated sharps, such as needles, box cutters, contaminated broken glass, slides, etc.) and **black containers** (for contaminated solids).
- In the laboratory there must be a specific container for clean broken glass (free of microorganisms). Do not discard contaminated broken glass in the container for clean broken glass.
- Containers must not be filled excessively. If necessary, the person responsible for the laboratory should provide new containers.
- Do not dispose of contaminated materials through the drain or with general rubbish.
- Solid wastes (culture plates, tubes with cultures, pipette tips, contaminated gloves or papers, etc.) shall be collected in autoclavable bags. Once the materials are sterilized, they shall be discarded in black biosafety containers and finally removed.
- Put glass tubes with cultures in tub racks and autoclave them. After that, throw the liquid through the drain and clean the tubes.



For more information on waste management, please read this [protocol](#).

6. Disinfection and sterilization

After completion of work, **disinfect** all the surfaces (working table, biological safety cabinet, centrifuge, pipettes, etc.) applying one of these products:

- Sodium hypochlorite (bleach) diluted with water: at 1% of active chlorine if the surface is highly contaminated (for instance, with blood) or at 0.5% of active chlorine if it is slightly contaminated. The solution must be fresh and should be kept on in contact with the materials for at least 20 minutes.
- Fresh solution of ethanol at 70%: let it stand for at least 5 minutes.
- Broad-spectrum commercial disinfectant (follow the manufacturer's instructions).

Disinfectant effectiveness is limited by the presence of organic matter. Therefore, instruments should be washed with soap and water before applying the disinfectant.

All the microorganisms are destructed by **sterilization**, including bacterial spores. In laboratories, moist heat sterilization (autoclaving) is the most common way to sterilize (for 20 minutes at 121 °C and 1.1 atm of pressure). It is important to regularly check that the autoclave is working properly (there are microbiological tests on the market).

7. Generation of aerosols and dispersion of infectious materials

- It is safer to use disposable loops or an electric microincinerator to sterilize inoculating loops, instead of a Bunsen burner.
- Any technique that may generate aerosols (aspiration, sonication, homogenization, opening of a pressure tube, etc.) shall be developed inside a BSC.
- To avoid splashes, use screw-capped tubes instead of those with a press-on cap.

8. Vaccination (active immunization)

All the personnel working in direct or indirect contact with infectious blood or other body fluids, wastewater or other sources of biological contamination should be vaccinated.

For instance, personnel manipulating human blood should vaccinate against hepatitis B and personnel working with wastewater, against hepatitis A. Furthermore, they shall regularly undergo specific medical examinations. In the event of doubt, please contact the medical unit of the UIB Risk Prevention Service.

9. Transport and storage of microorganisms and samples

To transport microorganisms or materials potentially infected with biological agents use secondary airtight containers to avoid accidental spillages. Containers must be labelled with the biohazard warning sign.



Only shipping companies complying with the regulations in force can transport microorganisms or infectious samples to other laboratories. They must provide the biosafety containers and labels. Personnel sending or receiving those samples should be aware of the potential risks involved and adopt the general biosafety precautions, especially when manipulating broken containers.

Biological samples should be stored in restricted access areas, inside clearly labelled refrigerators or freezers, which should be connected to the emergency power system of the building.

10. Emergency procedures

Biological accidents include spillages of infectious samples and puncture wounds, cuts or splashes on the skin or mucous areas with blood or other body fluids, cultures, wastewater, etc., including animal bites and scratches.

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Immediate action

- **Spillages:** in the event of contaminated broken glass, put on your gloves, remove the glass safely (preferably with tongs) and discard it in a yellow waste collection container. Use paper to absorb the spillage and decontaminate the surface with a solution at 1% of active chlorine. Finally, autoclave all the residues.
- **Puncture wounds and cuts:** force the wound to bleed, clean it with soap and water and disinfect it with povidone-iodine.
- **Splashes** in the eyes or mouth: clean the affected body part with physiologic serum or use running water from the emergency eyewash fountain for at least 5 minutes.
- **Accidental ingestion** of infectious materials: go to the Medical Unit of the UIB Risk Prevention Service.

After the initial action, go to the medical unit (Cas Jai building, from 8:00 to 17:30, telephone extension 34 61). If the accident occurs when the medical unit is closed, head to Son Espases (i.e. the nearest public hospital to campus), or to another medical centre indicated by your insurance company.

Follow this [link](#) to fully read the biological accident protocol.

Specific regulations for the manipulation of animals

In addition to the previous general regulations, the following instructions should be considered:

1. Working protocol

- Animals can only be manipulated inside the animal facilities of the UIB.
- Animal cages and confinement boxes should be labelled with the identification of the animals they contain. This is especially important in the case of deliberately infected animals.
- Access to the animal facilities must be restricted to authorized personnel.
- Observe strict hygiene, focusing on hand washing, which should be done after manipulating dead or alive animals, after removing your gloves and always before leaving the animal unit.
- Wounds caused during the manipulation of animals shall be treated immediately: stimulate the bleeding, wash it with soap and water, cover it with a dressing and, if necessary, start a drug treatment.
- All working surfaces should be decontaminated with an effective disinfectant after completion of work and in the event of splashes or spillages of infectious materials. After working with infectious materials, all the equipment should be decontaminated as well. Equipment should be decontaminated too in the following cases: before starting maintenance or repair work, before removing it from the animal facilities or in the event of procedural changes to avoid cross-contamination. These operations should be done with the proper personal protective equipment.
- It is forbidden to leave the animal facilities with your working clothes or with the personal protective equipment.
- Working procedures should be designed to avoid or minimize the generation of aerosols. If this is not possible, work within a class II biological safety cabinet. The same care shall apply when tasks involve the manipulation of infectious materials, including animals.
- Equipment, materials and wastes to be cleaned, disinfected or discarded must be transported in appropriate containers, perfectly labelled and closed.
- Animal carcasses to be eliminated must be transported in leak-proof containers to avoid spillages. Carcasses shall be stored in specific freezers and finally incinerated.
- All accidents, including bites and scratches, should be reported to the person responsible for the animal facilities.
- Any unexpected disease or death among animals shall be immediately notified. Animals with an unexpected disease shall not be touched as long as no specific instructions from the person responsible for the animal unit are received.

- Personnel working with animals should be immunized against tetanus and all the other diseases deemed necessary by the medical unit of the UIB Risk Prevention Service, according to the biological materials they manipulate.
- Established flows of people, materials and animals should be respected (always from the clean to the dirty area, never the other way round).

2. Personal protective equipment (PPE)

- Personal protective equipment should be adequate to the risks involved. Protective clothing, gloves, footwear, etc. should be bite and scratch resistant.
- Masks and safety glasses are recommended to avoid sensitization to the existing allergens in the animal facilities. Its use will be mandatory when developing any technique that may generate aerosols, splashes or projections, such as necropsies, collection of tissues or intranasal inoculation in infectious animals.
- There should be safe devices to immobilize animals if necessary, for instance when taking samples or administering substances.
- Before leaving the animal unit or the animal facilities, clean and, if necessary, disinfect the non-disposable protective equipment that has been used.

Specific regulations for the manipulation of cell cultures

When manipulating cell cultures, the previous general regulations should apply. Moreover, when working with cell cultures at the UIB, the following specific regulations shall be complied with:

1. Facilities and access

- Tasks that involve working with cell cultures should only be developed at the facilities and laboratories authorized by the UIB Biosafety Committee.
- Access to the facilities and laboratories is restricted to the personnel that have received appropriate training in cell culture manipulation.

2. Working protocol

- It is compulsory to wear appropriate personal protective equipment (PPE) according to the manipulation that is being conducted. A specific laboratory coat should be worn when working with cell cultures. It has to be left at the cell cultures facilities.

- Except for the well-characterized cell lines and the primary cultures obtained of animals certified as specific pathogen free (SPF), the manipulation of cell cultures will be developed inside a class II biological safety cabinet. In the event of doubt, consult the manual of the Biosafety course or contact the UIB Biosafety Committee.
- Infectious cell cultures shall be manipulated according to the biosafety level of their infectious agents and with the approval of the other users of the facilities.
- Follow the BSC usage regulations (see section “Working protocol”, page 2). In the case of cell cultures, it is recommended to turn on the ultraviolet light, even if the BSC is switched off, in order to minimize accidental contamination.
- Work with sterile materials (opened inside a BSC) maintaining optimal cleaning and hygiene conditions.
- Clean and disinfect the materials before and after working and, specially, in the event of spillages of culture medium.
- In the case of long-term cultures, verify periodically their properties and develop controls to detect any contamination with Mycoplasma.

3. Wastes

- Liquid wastes of cell cultures should be collected by aspiration in a container with bleach. After work routine, a disinfectant shall be aspirated (for instance, ethanol at 70%) in order to clean the aspiration system.
- Solid wastes of cell cultures should be autoclaved in autoclavable bags and discarded in black biosafety containers. Sharps will be discarded in yellow biosafety containers.
- Papers, cardboards and plastics that have not been in contact with biological materials can be discarded as general rubbish.

Specific regulations for the manipulation of genetically modified plants (GM plants)

The manual of the Biosafety course details the forms and documents that should be filled in by the researcher responsible for the experiment.

1. Specific working protocol

- As a general rule, GM plants shall be grown separately in time with other non-genetically modified plants. Exceptions shall be made in duly justified cases, according to experimental criteria.
- GM plants must be properly identified. Identification shall be done by labelling the plant pots with the Entry Register code. In this way, the same code will be used to identify stored seeds and plants that are being grown.
- To minimize the risks of gene flow, and as a general rule, experiments should finish before anthesis (flower opening). Alternatively, flowers shall also be cut before anthesis. Exceptions shall be made in duly justified cases, according to experimental criteria.
- As a general rule, the door of the confinement facilities shall be closed.
- The person responsible for the surveillance and control of the confinement facilities must periodically verify the fulfilment of the confinement and protection measures.

2. General working protocol

- Personnel manipulating GM plants shall attend and pass the UIB Biosafety course and, specifically, the module on contained use of GM plants.
- Personnel manipulating GM plants shall comply with the general biosafety regulations stated at the beginning of this document.
- Personnel manipulating GM plants shall wear a laboratory coat and comply with the personal protective additional measures required in every single experiment. In the event of using gloves, they shall be discarded in double autoclavable bags. Laboratory coats should be left in the confinement facilities, using the clothes hangers located at the exit.

3. Inactivation of cultures and materials in contact with GM plants and cleaning of the facilities

- After completion of every experiment with GM plants, the researcher responsible for the experiment shall verify that the facilities are left clean and tidy. The Culture Register includes information on cleaning measures and inactivation of GM plants and the resulting wastes.

- Plants should be discarded, along with substrates, in autoclavable bags, which should be inactivated in the autoclave located in the same Area of Plant Physiology.
- Non-disposable containers and instruments that have been in contact with GM plants (including plant pots) should be inactivated with sodium hypochlorite at a final concentration superior to 1% before starting a regular cleaning. Disposable containers will be discarded, with their caps on, in autoclavable bags.
- The cleaning service shall regularly disinfect the confinement facilities (at least, twice a year).

4. Accidental spillages

- There is only one possible situation of accidental spillage of GM plants in our facilities. Due to the expected workload, spillages will be, in all cases, small. In the case of a plant culture spillage, plants that cannot be reused should be individually collected and discarded in autoclavable bags. All other solid wastes will be cleaned up with paper and discarded in the same way. Floors and affected surfaces shall be cleaned with paper soaked in sodium hypochlorite at 20% or in ethanol at 70%. Paper shall be disposed of in autoclavable bags. At the end, bags containing wastes should be inactivated in the autoclave located in the same Area of Plant Physiology.
- In the event of an accident, the researcher responsible for the experiment shall include all the relevant information in the Culture Register and inform the person responsible for the surveillance and control of the confinement facilities as soon as possible.

5. Storage and transport of GM plants and seeds

- Storage, cultivation, including germination, and manipulation of GM plants should be done in a phytotron, a type of confinement facility.
- Transport, by any means, of GM plants, its seeds or parts requires an assessment of health and environmental hazards. Specific regulations on industrial hygiene and safety (article 13.2 of the Royal Decree 178/2004) should be complied with. Transport of GM plants, its seeds or parts to and from the European Union shall include the required documentation stipulated in the Cartagena Protocol on Biosafety. For more information, consult the manual of the Biosafety course or contact the person responsible for the surveillance and control of the confinement facilities.