HEALTH AND SAFETY AREA

BIOSAFETY MANUAL
(BSL2)
BIOLAB FACILITY

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Issued by	*Health & Safety Area*
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1 INTRODUCTION

Biosafety is defined as a set of measures applied to prevent and protect from possible unintentional exposure to biological agents for both, people and the environment.

This manual has been developed with the objective of **establishing biosafety standards at an institutional level, in relation to the various analytical and research activities carried out in the facilities of the Biolab of the ICN2**. These standards are applicable to all research activities involving the use of biological agents, genetically modified or not, and derivatives and products that contain them with the aim of ensuring a level of biosecurity appropriate to the risks associated with these materials.

People who work in laboratories can only guarantee their own safety and that of their colleagues and the community if they know the biosafety norms and how to apply them. This manual contains definitions and general and specific requirements that must be considered when implementing and maintaining biosecurity in the Biolab. These requirements cover the types of microorganisms and biosafety levels required for their manipulation and safety regulations relating to the handling, transportation, conservation and disposal of potentially harmful substances for personnel and the community.

1.1 Legal framework

There is specific legislation contained in various European directives that regulates all activities involving the use of genetically modified organisms (GMOs) and / or pathogens that can pose a risk to both health and the environment. One of the consequences of this legislation is that any organisation intending to carry out activities involving potentially harmful GMOs or patheogens must notify the authorities and/or receive authorization in advance. This applies both to activities that are carried out in a confined regime (laboratory, greenhouse, animal ...) and those performed in the environment (field trials, clinical trials, etc.). The administrative procedures to follow are detailed in the following legislation:

- Law 9/2003 of April 25, which establishes the legal regime for confined use, voluntary release and commercialization of genetically modified organisms.
- RD 178/2004. General regulation for the development and execution of law 9/2003, April 25.
- Decree 62/2015, which establishes measures for the exercise of the powers of the

Generalitat of Catalonia in matters relating to genetically modified organisms.

- RD 664/1997, of May 12. Protection of workers against risks related to exposure to biological agents during work.

It should be noted that RD 664/97 has a list of biological agents classified according to the level of danger and defines minimum containment levels for each of the risk groups. In the case of GMOs, RD 178/2004 also establishes minimum requirements regarding facilities, equipment, work standards and waste treatment for activities with different risk groups.

Therefore, any principal investigator who intends to use pathogenic organisms and / or GMOs, must perform the following steps prior to the start of activities:

- Determine the level of risk taking into account the safety, pathogenicity or environmental risk of the recipient organism, and in the case of GMOs, the genetic material of the donor used, the vector, as well as the resulting GMO itself.
- Send a notification or request for authorization to the competent authority, **after verification and approval by the Institutional Biosafety Committee of the UAB (IBC-UAB).**
- Register and keep the information for at least 5 years after the end of the activity.
- Present any information required by the competent authority.

In order to determine the level of danger of a certain biological agent, it is necessary to refer to classification lists of available human, animal or phytopathogenic pathogens. For example, in the case of human pathogens, the classification list is contained in Annex II of Royal Decree 664/1997 of 12 May, which covers the protection of workers against risks related to exposure to biological agents during work, and subsequent modifications.

The activities of the confined use of GMOs are classified into four types, depending on the prior assessment of the risks to health and / or the environment that may arise, thus determining the degree of confinement and the administrative regime:

- Type 1: activities of "zero or insignificant risk" with a level of containment type 1.
- Type 2: of "low risk" with a level of containment type 2.
- Type 3: of "moderate risk" with a level of containment type 3.
- Type 4: "high risk" with a level of containment type 4.

This Biosecurity Manual has been prepared and adopted by the ICN2 Health and Safety Area and validated by the Biosafety Committee (BSC) of the UAB. The Biolab **complies with the legal requirements for the application of activities with biological agents of risk group 2 (RG2) or lower, genetically modified or not, carried out in a confined facility of Biological Containment Level 2 (BCL2)**.

This manual will be updated whenever there are changes in working conditions that may affect the level of risk, especially the development of new projects, the implementation of new methods, the handling of new biological agents, the acquisition of new relevant equipment for biosecurity, expansion or modification of spaces, etc.

It will also be reviewed biannually by the Supervisor, and validated by the IBC-UAB, which will introduce the necessary changes or corrections to ensure its adequacy for the activities carried out and current legislation.

1.2 Institutional Biosafety Committee of the UAB (IBC-UAB)

The ICN2 belongs to the UAB-CEI sphere, a collaborative space where all the institutions linked to the UAB share resources and synergies that result in consequent improvements in sustainability and competitiveness.

In accordance with the recommendations established in Royal Decree 178/2004 (Annex II), regarding the accepted international standards and the criteria used by the National Biosafety Commission, it was considered necessary to join the Biosafety Committee of the UAB.

This committee offers the Institute advice and reviews and approves facilities and activities related to the confined use, transportation, commercialization, storage, destruction and / or elimination of biological agents, modified or non-genetically modified, and derivatives and other products that they contain.

The IBC-UAB ensures and guarantees that these activities comply with current legal and internal regulations and records both the delivery and reception of pathogens, GMOs and / or their derivatives classified as risk group 2 or higher.

1.3 Notifications and authorizations

In order to guarantee compliance with the aforementioned requirements, a system of mandatory communication and authorization has been established, which must be carried out or obtained prior to the start of the activity in question.

Accordingly, if you intend to develop an experimental procedure that involves the use of biological agents (microorganisms, cell lines, tissue or fluid samples, GMOs, etc.) in the

facilities of ICN2, you must submit an on-line declaration of proposed activities to the Biosafety Committee of the UAB by completing the proposal form developed by the IBC-UAB.

This requirement is mandatory for all those activities that meet any of the requirements established for evaluation by the IBC-UAB.

2 OBJECTIVES AND SCOPE

The purpose of this document is to establish the communication procedures as well as the safety instructions necessary for the safe handling of material with a biological risk within the ICN2's Biolab.

The biological material handled in the Institute's Biolab includes agents associated with human diseases, which are rarely serious, and for which there are usually preventive or therapeutic measures. However, safety standards must be established to minimize the risk of exposure to them.

The various work instructions, attached as an appendix to this manual, have been adapted to the characteristics of the installation and are, together with other documents, internal standards of work safety, environmental protection and quality of the Institute and the UAB.

The Supervisor of the Biolab, as the person responsible for the installation, together with the principal investigator of a given experimental procedure must recognize their responsibility towards the health of the users or researchers and ensure that the appropriate security measures are applied for each of the activities that are carried out. All personnel who make use of the spaces and equipment of the Biolab must know, accept and comply with the requirements and recommendations contained in this manual.

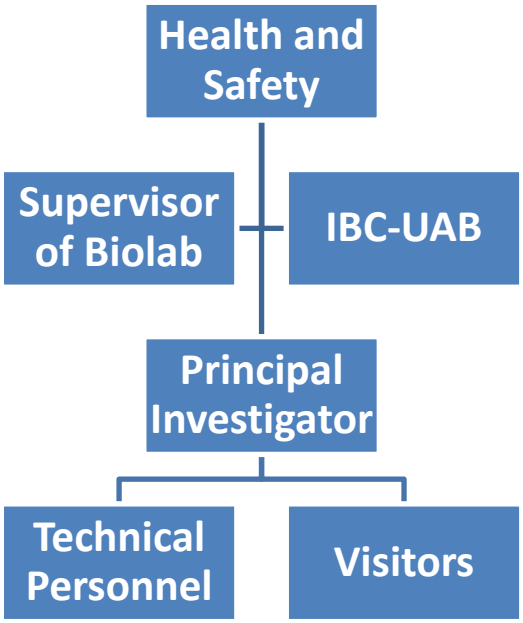
This requirement is applicable to all research personnel within the different research groups of the ICN2 who work with and manipulate biological agents within the laboratories.

3 ORGANIZATION

The Catalan Institute of Nanoscience and Nanotechnology (ICN2) aims to exercise comprehensive control of the risks associated with the use and manipulation of biological material in its facilities.

The ultimate objective is to guarantee the protection and health of all personnel that form part of the center, to portect the environment and to build and promote a preventive culture in the center.

3.1 Organizational chart



3.2 People & organisations responsible for biosafety

The director of the Institute is the person ultimately responsible for biosafety and he/she exercises this function by delegating responsibility to the Supervisor of the Biolab, the Health & Safety Area of ICN2, the principal investigators and, ultimately, to all external users, visitors and students of the center.

The functions of the IBC-UAB are set out in article 3 of its operating regulations.

The functions and responsibilities of people and organizations, in terms of Health and Safety within the ICN2, are defined internally in the Health & Safety Manual approved by the Director on June 3, 2019.

Representatives of personnel that may be involved in biosafety activities and their specific functions and responsibilities are as follows:

a. The principal investigator (PI) of each project or line of research is responsible for the safety and health of all personnel involved in their project. The PI must submit an on-line declaration of proposed activities to the IBC-UAB by completing the proposal form created for this purpose.
b. The Biolab supervisor, as the the person responsible for the installation, has the necessary authority and support to assess the risks, establish safety policies and procedures, ensure adequate training of research staff and the correct maintenance of the installation and equipment.
c. The biosafety adviser (IBC-UAB) is responsible for advice regarding the handling of biological agents and carrying out the general management of the UAB biosafety program.
d. The technical staff, students and researchers are responsible for knowing and applying the procedures and codes of good practices established by the ICN2, as well as ensuring the use of the protection measures provided and communicating any dangerous conditions, accidents and incidents.

3.3 Description of the installation

The Biolab is located in spaces 2091 and 2093 of laboratories on the second floor of the main building of ICN2, within the campus of the Autonomous University of Barcelona.

The installation has been designed in accordance with UNE-EN 12128 standard with materials and construction appropriate for these types of rooms, ensuring adequate containment and robustness and with an approximate area of 36 m^2, distributed between the laboratory and the decontamination transfer chamber (Safety Access System, SAS).

Both the walls and ceilings of the room are composed of panels containing lacquered galvanized steel sheets separated with an insulating core of rock wool. The panels are joined in such a way to allow correct sealing without the need for a sealant.

The outer walls adjoining the rest of the laboratory have 4 windows for observation of the interior from the outside. These windows have a lacquered metal frame, with double laminated glass and UV filter, forming a compact block of the same thickness as the wall.

The outer access door has the same composite covering as the inner access door to the laboratory. In the transfer chamber there is an interlock safety system that prevents simultaneous opening of the inner and outer doors and has an unlocking system in case of electrical or mechanical failure. The outer access door has a restricted access system by means of a fingerprint that controls the electric opening of the door lock.

The floor is waterproof and covered with continuous non-slip material with no joints in order to prevent the accumulation of particles. The floor is resistant to wear and chemicals and is easy to clean and decontaminate. There is a sink in the laboratory as well as an eyewash unit.

The ventilation system is designed to generate negative pressure both in the work laboratory and in the SAS and consists of a 50W fan that drives air through type H5 and M14 filters. The extraction system consists of a 450W fan with an EC motor and a type H14 HEPA filter.

The room has two detectors: O_2 (measuring range 0%-5% Vol) and CO_2 (measuring range 0%-25% Vol) both mounted on the surface. There is a control unit with an alarm.

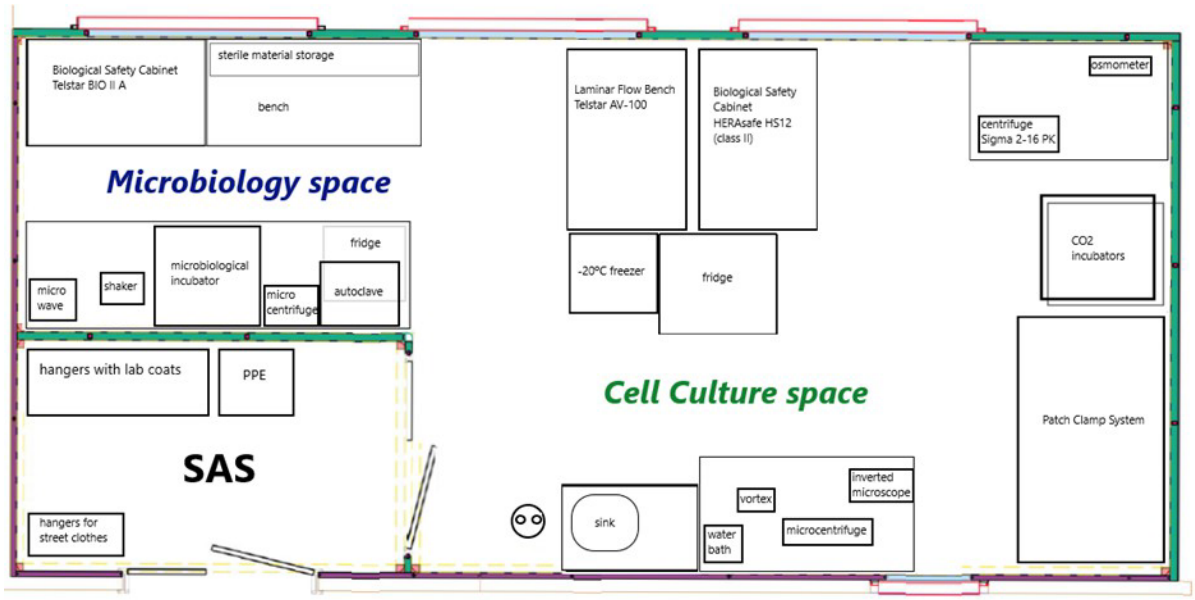
The laboratory also has two disinfection systems, one based on UV lamps equipped with a germicidal emitter and the other a decontamination system using hydrogen peroxide (VHP).

The Biolab is adequately equipped to fulfil minimum basic quality specifications for the type of work that will be carried out. The equipmenmt includes:

- 2 Biological Safety Cabinets (class II)
- 1 Laminar Flow Cabinet

- 2 CO_2 Incubators
- 1 Cryogenic tank
- 1 Microbiological incubator
- 2 Bench-top microcentrifuges
- 1 Refrigerated centrifuge
- 2 Autoclaves
- 1 Inverted optical microscope
- 2 Orbital shakers
- 1 Cell density meter
- 1 Portable vacuum aspiration system
- Water bath, vortex, pipettes
- 2 Refrigerators, 1 freezer and 2 ultra-freezers

The location of the equipment is shown in the following plan:



4 RISK AND BIOLOGICAL CONTAINMENT

Microorganisms can be classified into different risk groups depending on factors, such as pathogenicity, virulence, transmission routes, concentration, host range, etc.

Currently, there are four defined categories or risk groups. This classification presupposes the existence of normal circumstances, the possible effects on healthy individuals, or culture volumes typical of experimental or diagnostic procedures.

Those that rarely cause disease belong to group 1 risk. Pathogenic organisms are classified within groups 2, 3 or 4, depending on the degree of pathogenicity, transmission route and existence of prophylaxis or effectiveness of treatment. Annex II of RD 664/97 contains a list of human pathogens classified by risk group, although there are also other classification systems defined for human and / or animal pathogens (viruses, bacteria, fungi, parasites), plants and cell lines.

The classification of biological agents, according to risk group, provides the user with an indication of the confinement necessary to safely handle the microorganism in question. In addition to the characteristics inherent to each microorganism, the classification relates the handling of specific agents to a number of intallational, operational, technical and physical requirements. Thus, four levels of biological containment are defined.

4.1 Evaluation of biological risk

The concept of risk implies the likelihood of harm, injury or illness. If we talk about evaluating biological risk, we are basically talking about preventing infections or releases to the environment. When it comes to activities that involve working with infectious, potentially infectious or GMO material, risk assessment is a way of entifying the risks and assigning a level of biological containment (facilities, equipment and practices) **that minimizes the risk of exposure or release to the environment.**

When carrying out a biological risk assessment, it is necessary to take into account **certain risk factors** that may affect the final result. This evaluation process begins with an analysis of the characteristics of the biological agent, the experiment and protocols, the equipment and controls, as well as the personnel and environmental conditions of the laboratory where said agent will be used.

Cell lines do not pose a risk by themselves in the sense that they cannot spread, transfer genetic material by accident and it is difficult for them to survive in non-sterile environments.

However, they can be carriers of pathogenic microorganisms (by contamination, by transformation or recombination) and the fact that they are cultivated in conditions different from those found in the organism (pH, temperature, culture supplements, etc.) can modify the expression of oncogenes or surface proteins. This can cause them to express latent viruses or cause genomic rearrangements. **That is why an evaluation of risk associated with each line is essential.**

Cell lines can be contaminated by bacteria, fungi, mycoplasmas, viruses and prions and, especially, by bloodborne pathogens. Therefore, all non-fixed tissues and cells of human origin and / or primates should be considered as infectious and should be handled at BSL2 as a minimum. Tumor cells also represent a potential danger in the case of autoinoculation.

The result of the evaluation determines the **control measures** that must be implemented to reduce the risk to an acceptable level.

Risk assessments have to be revised or carried out again whenever significant changes are introduced, such as: introduction of new biological agents, structural modifications of space or equipment, changes in work procedures, the appearance of an accident or incidents , detection of non-conformities with the established norms or new legal requirements.

4.2 Design of facilities and physical containment

Biological laboratories are special work environments and it is very important to consider at the design stage, all the necessary preventive and protective measures that contribute to the protection of both laboratory personnel and the environment in general.

Both Annex IV of RD 664/1997 (human pathogens) and Annex II of RD 178/2004 (GMOs) contain the minimum specifications required for the different confinement levels for laboratory activities. There are also other requirements that, although not mandatory, should be taken into account according to the result of the risk assessment.

4.2.1. Microbiological laboratory

The design and construction elements of a laboratory (secondary barriers) contribute to the protection of both the laboratory personnel and the environment, against biological agents that can be released accidentally.

In laboratories with biocontainment levels 1 and 2, the risk of infection is mainly due to direct contact or exposure to inadvertent contact; therefore, the secondary barriers provide separation of the work area, control of access by the public, and mid-decontamination and hygienic facilities.

When there is a risk of infection from exposure to a bioaerosol, it is necessary to implement a higher level of containment and multiple secondary barriers to prevent biological agents from being released into the environment. This includes:

- Specialized ventilation systems to ensure unidirectional air flow (from outside to inside)
- HEPA filtration of the extracted air.
- Controlled access areas, locks or SAS room.
- Waste treatment systems.

4.2.2. Cell culture laboratory

The main requirement for a cell culture laboratory, in addition to the characteristics of a microbiological laboratory, is the maintenance of a high level of asepsis. The growth rate of cells in culture is much lower than that of the usual contaminants (eg bacteria, viruses, fungi, etc.) and the entry of these contaminants into the laboratory must be minimized.

The cell culture laboratory is therefore isolated from the rest of the activities, away from passageways and is dedicated exclusively to the cultivation of cells with laminar flow cabinets that maintain a sterility gradient, from the exterior medium to the interior of the flow cabinets and the incubators where the cells are handled.

These laboratories are classified according to their pathogenicity and the entrance of air into the room is designed to produce an increase in pressure inside the room (between 15-20 Pa) that prevents the entry of contaminants.

In addition, in order to prevent the accidental release of biological agents, the air is filtered and generates a pressure deficit.

The following table details the minimum requirements for cell culture laboratories, depending on the level of containment assigned, taking into account that containment level 1 corresponds to a basic laboratory, without special requirements.

RD 178/2004: Table 1A

Containment measures and other protective measures for laboratory activities

Containment measures		Containment Levels			
		1	2	3	4
1	Laboratory suite isolation[1]	Not required	Not required	Not required	Not required
	Laboratory: sealable for fumigation	Not required	Not required	Required	Required
3	Independent entrance and exit	Not required	Not required	Required	Required
Equipment					
4	Surfaces impervious to acids, alkalis, solvents, disinfectants and decontamination agents and easy to clean	Required for bench	Required for bench	Required for bench	Required for bench , floor, ceiling and walls
5	Entry to laboratory via airlock[2]	Not required	Not required	Optional	Required
6	Negative pressure relative to the pressure of the immediate surroundings	Not required	Not required	Required with the exception of[3]	Required
7	Extract and input air from laboratory must be HEPA-filtered	Not required	Not required	HEPA filters[4] required to extract air with the exception of[3]	HEPA filters[5] required for entrance and extraction of air
8	Enclosure or microbiological safety alarm	Not required	Optional	Required	Required
9	Autoclave	Required on site	Required in the building	Required in the laboratory suite[6]	Double ended autoclave required in the laboratory

[1] Isolation: the laboratory is separated from other areas of the same building or in a separate building.

[2] Lock: entrance must be made through an airlock isolated from the laboratory. The clean side of the airlock must be separated from the restricted side by showering facilities and preferably by interlocking doors

[3] Activities in which transmission is not carried out by air.

[4] HEPA: high-efficiency particulate absorber

[5] Where viruses are not retained by HEPA filters, extra requirements will be necessary for air extraction

[6] The safe transport of material to the autoclave outside the laboratory is allowed by means of procedures validated and with an equivalent level of protection.

System of work

10	Restricted access	Not required	Required	Required	Required

11	Biological warning signs on door	Not required	Required	Required	Required
12	Specific measures for the control of formation and diffusion of aerosols	Not required	Required	Required	Required
13	Shower	Not required	Not required	Optional	Required
14	Protective clothing	Suitable protective clothing required	Suitable protective clothing required	Suitable protective clothing and footwear required	Complete change of clothing and footwear before entry and exit
15	Gloves	Not required	Optional	Required	Required
16	Effective control of vectors (for example rodents and insects)	Optional	Required	Required	Required

Waste

17	Inactivation of GMMs in effluent from handwashing sinks and showers and similar effluents	Not required	Not required	Optional	Required
18	Inactivation of GMMs in contaminated material and waste	Optional	Required	Required	Required

Other measures

19	Laboratory to contain its own equipment	Not required	Not required	Optional	Required
20	An observation window or alternative to be present so that occupants can be seen	Optional	Optional	Optional	Required

Table 1. Safety requirements for laboratories according to containment level..

Section 3.3 of this Manual describes the characteristics of the ICN2 facility for the confined use of biological agents.

4.3 Declaration of activities with biological agents

The risks of a specific activity or experimental procedure with biological agents and the need for notification and / or administrative authorization are determined during the previously described stage of risk planning and evaluation.

Accordingly, before starting an activity, principal researchers who develop specific projects within the Biolab must notify the proposed use of biological agents to the IBC-UAB via a proposal form created for this purpose.

The notification of first use of biological agents in the facility must be made in accordance with the internal procedure developed by the IBC-UAB.

Both notification procedures are described in a practical guide issued by the IBC-UAB. The supervisor of the Biolab can also offer help with the notification process.

The ICN2, through the head of the Biolab, will also inform the IBC-UAB about the completion of a specific activity.

4.4 Experimental procedures and inventory of biological agents

The IBC-UAB keeps a record of all the notified experimental procedures to be carried out in the Biolab, to which the supervisor and principal investigator also have access through the proposal form created for this purpose.

Likewise, there is an updated list of the biological agents stored in the Biolab, with the corresponding pathogen technical sheets (PTS), which are available for consultation.

5 SAFETY MEASURES AND RULES OF CONDUCT

5.1 Conditions of access and safety signs

The most important element of biosafety is the strict performance of standard microbiological practices and techniques. The application of good practices avoids or minimizes most of the causes of accidents, injuries and / or infections. Generally, these causes relate to human error, the application of incorrect techniques or the misuse of equipment. All people who work with infectious agents or potentially infected materials must know the potential risks, and be expert in the practices and techniques required to handle these materials safely.

5.1.1. Rules of access

- The Biolab has a access system restricted by fingerprint that controls the electronic opening of the door lock and is limited to those people who have been previously authorized by their supervisor.
- Access to the Biolab is separated from the free-circulation corridor by means of an SAS (Safety Access System) room where personnel must leave their street clothes and personal belongings. Protective clothes and equipment are available and a security system prevents both doors from being opened simultaneously.

5.1.2. Safety signs

On the door there is a biological risk warning sign, specifying the level of biosafety and the name of the laboratory supervisor who controls access to it, as well as indicating any special conditions of entry into the area.

Inside the installation there are also biological risk warning signs:

- Biohazardous waste containers as well as those intended for the transport and / or shipment of biological agents (BA).

- Refrigerators and freezers that contain BA of RG2, human blood or other potentially infectious materials.

- Incubators, waterbaths, stoves and BSCs.

5.2 Safe work procedures

From the reception of the samples, to the elimination of the generated waste, **all operations carried out** in the Biolab must be duly systematized in accordance with the following guidelines, to ensure optimal safety conditions.

5.2.1. Biosafey rules

Human error, incorrect laboratory techniques and the misuse of available equipment are the cause of most laboratory accidents and related infections.

This section lists good practices and safe techniques aimed at avoiding or minimizing the most common accidents caused by these factors. The safety standards applied to the Biolab are based on the "Principles of good microbiological practices" according to the laboratory biosafety manual published by WHO, and are described below:

• General precautions regarding the Biolab

- All laboratory personnel must comply with established safety standards, and you must know all the risks and existing measures before beginning any task.
- Access will only be allowed to authorized personnel.
- Personal items (backpacks, wallets, mobile phones, etc.) must remain in the SAS room, far away from the areas where biological agents are handled or stored.
- Daily cleaning and disinfection of all work surfaces should be done with an adequate and effective product, as well as whenever a spill occurs.
- The laboratory must remain clean and orderly and material, or equipment must not be storred in the corridors. There should always be adequate space to evacuate the Biolab in case of emergency.
- Within the Biolab, there must be negative pressure conditions that allow a unidirectional flow of air from outside to inside, reducing the risk of external contamination in the event of a spill or accident.
- Biological safety and warning signs must be placed on incubators, readers and refrigerators where the biological agents are handled or stored.

- Personal hygiene rules

  - Wounds and injuries should be covered with waterproof dressings before starting work. If the lesions cannot be adequately covered, there should be no exposure until they are cured.
  - Remove rings and other jewelry. It is advisable to tie up long hair.
  - Avoid skin contact with potentially infectious materials.
  - It is forbidden to leave the laboratory wearing a gown or gloves.
  - After removing your gloves, and therefore after each manipulation, wash your hands using antiseptic soap.
  - Access to the laboratory must be done wearing safety glasses. Contact lenses should not be used.
  - It is forbidden to eat, drink or smoke or use of cosmetics in work areas. Likewise, storage of food or drinks is prohibited.
  - People who will have contact with blood of human origin and its derivatives are advised to be vaccinated against hepatitis B.

- Precautions while working

  - Upon receipt of any biological sample, whatever its nature and type of laboratory, it should always be handled with gloves, and you must wash your hands afterwards. If you suspect that the sample may contain unexpected or unknown infectious agents, it must be opened in the BSC.
  - Always avoid the use of books and stationery in the work area, since contaminated paper is difficult to decontaminate.
  - It is strictly forbidden to pipette with the mouth. Pipetting must be carried out with devices specially designed for this purpose, and staff must be adequately trained in their correct use.
  - The use of hypodermic needles and syringes should be limited, only units already assembled should be used.
  - Do not put the cover back on a needle and needles should not be bent or separated from the syringe.
  - Used needles and syringes, as well as scalpels, should only be disposed of in special containers designed for this purpose.
  - In order to avoid accidental cuts, the use of plastic material is preferred to glass. In the case of glass slides, they should preferably have blunted edges.

- The use of eye and dermal protection within the Biolab is established as mandatory.
- The risk of autoinoculation must be minimized, especially when generating aerosols from tasks carried out in the Biological Safety Cabinet.
- When centrifuging potentially infectious biological material, closed tubes should be used. The centrifuge should have rotors or containers to contain the possible generation of aerosols.
- The accidental rupture of a tube and its discharge into the cuvette must be immediately reported to the person in charge of the laboratory, followed by safe disinfection of the equipment.
- Centrifuges should not be used if they do not have a safety locking system to prevent opening while they are forming aerosols.
- Spills and accidents, such as cuts and skin punctures, must be reported immediately to the person in charge of the laboratory and to the medical team of the institute.
- Human cell cultures, samples of human origin, infectious agents, etc. should always be handled in a biological safety cabinet.

5.2.2. Transport of biological material

The internal transport of biological material must be carried out with the following precautions:

- The transport of samples within the Biolab or between laboratories must be carried out in such a way that, in the event of a fall, the spillage is contained.
- Material must be transported in hermetic boxes or portable coolers. These boxes or refrigerators must be rigid and resistant to shocks, have absorbent materials inside and be easy to disinfect.
- Samples must be labeled and identified and may not be used for other purposes.
- Under no circumstances must samples be transported by hand or in pockets.

All transport of biological materials external to the ICN2 and to the UAB campus must be carried out by companies that comply with the international and national regulations in force in the matter of the transport of dangerous goods.

In certain cases, it may be necessary to obtain licenses or special permits for the export / import or transport of biological materials such as human pathogens, animal pathogens, non-commercialized GMOs, etc.

These requirements must be verified sufficiently in advance as their processing may require time.

The guide for the transport of biological materials, issued by the IBC-UAB, contains a detailed description of the specific requirements for the different situations that may arise when sending any type of biological material from the UAB.

5.2.3. Storage of biological material

Following the requirements of RD 664/97, biological samples must be stored inside the Biolab in resistant and hermetic containers, taking into account restricted access to the installation, and must always be identified with appropriate biological warning signs.

Storage in liquid nitrogen freezers should be carried out using vials that can withstand the low temperatures of the medium without breaking. In the case of breakage, the container must be emptied and the liquid nitrogen must be allowed to evaporate and the the container then thouroughly cleaned.

When handling the material stored in these types of freezing containers, always use thermal protection gloves and face shields to protect against possible splashes of liquid nitrogen.

5.2.4. Individual Protection Equipment

Personal protection equipment (PPE) such as gloves, respiratory protection, etc. and work clothes (lab coat, footwear, etc.) are the first line of defense (primary barrier) when handling hazardous biological materials and must be worn when isolation of the contamination source is not possible.

In most cases, the combination of PPE, e.g. gloves, with biological safety cabinets should be practiced. It should not be forgotten that the maximum containment of biological risk can only occur when appropriate work techniques are used in conjunction with laboratory design according to the level of risk assessed.

Individual protection equipment that is mandatory for tasks carried out in the Biolab are as follows:

Protection gloves

The use of gloves is mandatory for the handling of biological agents and material or objects contaminated by them.

In general, nitrile gloves should be worn for work with infectious agents as certified according to EN 374-3: 2004 against biological risk. It is important to bear in mind that gloves reduce the risk of hand contamination, but do not avoid skin punctures or cuts caused by needles, other sharp instruments or glass or broken plastic. Consequently, the use of gloves is intended to complement, and not replace, good working techniques and proper infection control practices, in particular the correct washing of hands.

In relation to the use of gloves, the following general precautions should be taken:

- Wear gloves for all handling of potentially dangerous material.
- Dispose of the gloves whenever they are thought to be contaminated and then use a new pair.
- Do not touch your eyes, nose, mucous membranes or skin when wearing gloves.
- Do not leave the laboratory or walk through the common areas (corridors, dining room) wearing gloves. Wash your hands after removing your gloves.
- Once used, gloves must be removed aseptically and then the hands washed.

Eye protection

It is mandatory to use protective eye and face equipment to guard against the splashing or spraying of infected aerosols, contaminated water, blood or droplets of infectious cultures.

Respiratory protection

In cases where there is a risk of exposure through the air, such as spraying aerosols or possible splashes during the collection of an accidental spill outside the biological safety cabinet, respiratory protection with FFP3 type filters must be used.

Laboratory coat

The use of a laboratory coat is mandatory within the Biolab. Do not wear street clothes that increase the exposed body surface (shorts, skirts, sandals, etc.).

For this reason, it is forbidden to work with sandals; you should always wear closed shoes. The laboratory coat is designed to protect clothing and skin from infectious substances while working in the laboratory, so it is essential that it is always fastened.

It is forbidden to use protective equipment outside the laboratory, for example in the Institute's dining room, offices, meeting rooms and bathrooms, because if it has been contaminated with chemical products or biological agents, it may contaminate other clean areas. For the same reason, your coat must remain in the laboratory and can be periodically cleaned by using the laundry service provided by the ICN2.

5.2.5. Biological safety cabinets

The biological safety cabinet (BSC) acts as a primary containment barrier that allows safe working with biological agents. It is a device designed to prevent the exit of bioaerosols from the work area and, therefore, protects both personnel and the environment from possible exposure.

The Biolab has 1 laminar flow cabinet and 2 Class II biological safety cabinets.

In order to work correctly with a BSC, it is necessary to follow a set of procedures during and after an activity. The general recommendations can be found in the document Use and Maintenance of Biological Safety Cabinets

5.3 Training and information on Biosafety

Human errors and bad practices can jeopardize the effectiveness of preventive measures. As a result, good information on the identification and control of risks is key to the prevention of accidents and continuing education is therefore essential.

The supervisor of the Biolab must ensure that all personnel working under his/her direction have received the appropriate training in relation to their responsibility and the type of activity to be performed. The degree of training required will be proportional to the risk, type of activity and the level of experience of each person.

Under current legislation, all personnel accessing the Biolab must know the risks associated with the handling of biological agents, the correct way to perform procedures, how to prevent exposure and the action to take in the case of accidents.

In accordance with the ICN2's policy on training and information in relation to biosafety, the ICN2 provides training for those personnel who have been authorised to work in the Biolab. This training consistis of an institutional online training course on biosafety (accessible through the ICN2 eLearning training platform) including an exam, followed by a 2-hour practical onsite training in the Biolab.

Once the training is over, staff receive a certificate that serves as an accredition of the training received, which is required by the ICN2 Health and Safety Area and the IBC-UAB. You can then be registered for fingerprint access to the facilities of the Biolab.

5.4 Decontamination

Decontamination, understood as cleaning and disinfection of surfaces, depends directly on the organisms involved, the method used and the time of application.

Within the Biolab this operation is established as mandatory for reasons of occupational safety, experimental (cross-contamination) and environmental effects in terms of proper management of biohazardous waste.

To carry out disinfection of any kind, the following factors must be taken into account:

- The range of disinfectant tasks that the product can be used for.
- The concentration required for the task.
- The contact time with the surface to be decontaminated.
- The biological agents that have to be eliminated.

The disinfectant product will have a broad spectrum of tasks with a fast and irreversible action.

As for sterilization, this is done by moist heat under pressure (autoclave). The material to be sterilized is placed in suitable closed bags, and left for 20 minutes at 121ºC in a saturated atmosphere devoid of air. For some agents, other conditions may be necessary.

In the Biolab there is a specific Cleaning and Disinfection Plan that requires you to document the applied disinfection procedures of all surfaces that require them. Likewise, any equipment (eg refrigerator, freezer, incubator, centrifuge, BSC or similar) used in the handling or storage of BAs must be decontaminated prior to any repair, maintenance or transfer activities.

5.5 Waste management

The handling of GMOs or natural pathogens generates waste that must be inactivated and eliminated in such a way that it cannot cause damage to living beings or the environment.

The ICN2 has a Waste management procedure for the entire center and a Waste management plan specifically for the Biolab.

Waste is collected every Thursday from the different laboratories of the center and moved to a specially equipped warehouse.

The ICN2 has an external waste manager, Stericycle, which provides the appropriate containers for each type and removes these residues biweekly for treatment in a plant.

5.5.1. Classification of biological waste

Biological waste within the center is classified as follows:

Non-hazardous biological waste.

Inert and non-special waste or contaminated with biological agents considered non-hazardous. They have a very reduced capacity to induce or produce an infection, an allergy or a toxicity to humans, animals or plants, and do not pose any danger to the environment. They are similar to group II of Decree 27/1999, of sanitary waste management.

This classification includes the cultures of biological agents of risk group 1, non-GMOs (non-genetically modified organisms).

Example: cultures and samples of biologically innocuous tissues, and single-use material that has been in contact with these agents, e.g. non-human cell cultures.

Biohazardous waste and contaminated sharp material.

Biohazardous waste is any liquid or solid waste that is or contains biological agents of risk group 2 or higher (see Annex II of RD 664/1997 for human pathogens), and therefore, with the capacity to produce an infection, an allergy or a toxicity to humans, animals or plants or that are dangerous to the environment. Genetically modified organisms (GMOs) of any risk group are included. Also included in this classification are cultures of biological agents of group 2, 3 or 4 and contaminated material and cultures of biological agents of any risk group that is a genetically modified organism (GMO) and contaminated material.

Example: blood, blood products and tissues of human origin, live and attenuated vaccines, cultures of microorganisms or human cell lines genetically modified or not, contaminated non-sharp material, etc.

5.5.2. Treatment

Biological waste that is classified as non-hazardous or group 1, must be neutralized with bleach and diluted with water whenever possible, for subsequent drainage in the sanitation network. Solid waste is treated as municipal waste (in opaque bags with a minimum thickness of 55 micrometers).

Waste classified as biohazardous, as well as material that has been in contact with it, is treated as sanitary waste. Solid waste must be deposited in black containers, specifically labelled as special biohazardous waste for further treatment by the waste management company. Liquid waste should be placed in a closed plastic canister, specifically labelled as special biohazardous waste for further treatment by the waste management company. In no case should these products be poured into the drains, nor the material that has been in contact with them put into the normal wastebaskets.

This group also includes sharp contaminated material: needles, glass slides, scalpel blades, etc.. These materials must all be deposited in yellow Chemo-Box containers, which should not be used for other types of materials such as gloves, plastics, etc.

5.6 Selection and maintenance of equipment

All the equipment in the Biolab must comply with safety regulations. That is why all new equipment must come supplied with both a declaration of conformity and an instruction manual. Both documents are filed in an internal register for consultation.

The Biolab equipment is regularly checked and decontaminated to ensure quality of results and protection of people and the environment. A preventive maintenance plan of the facility is available. Internally, the rules of use for each existing piece of equipment are documented and available to all users.

6 EMERGENCIES

The ICN2 has an emergency plan for the main building where the Biolab is located. In addition, there is a specific Emergency Plan for the Biolab installation, which defines potential events and the action to be taken in emergencies.

All Biolab users must know the protocol and the guidelines for action in the event of an emergency, a fire or an evacuation of the building and also the meaning of the different alarms and the emergency telephone numbers and contact persons according to the type of event. This information is available in the Biolab and the corridors and reception of the ICN2 building and can also be acccessed on the center's intranet.

The supervisor of the Biolab is responsible for maintaining the containment conditions, as well as ensuring that personnel working in the Biolab are adequately trained and that they comply with the safety standards and protocols associated with the Biolab.

The Health and Safety Area trains personnel on fire extinguishing, emergency telephones, alarm systems and evacuation routes as well as managing the maintenance of such systems. The external prevention service, through the department of occupational medicine, carries out specific medical examinations (initial and periodic) of personnel working in the Biolab.

6.1 Types of events

6.1.1. Incidents in the Biolab

These refer to situations where there is a risk to staff in the Biolab but not to the rest of the staff outside the Biolab (eg: small spills of biological substances) and which can be resolved by the Biolab staff.

6.1.2. Accidents in the Biolab

These occur where the risk increases significantly (eg: escape of aerosols, ignition of fire, etc.) to include a risk to and action by staff working outside the laboratory. These accidents must be reported to the Health and Safety Area as well as to the IBC-UAB.

6.1.3. Emergencies in the Biolab

Where there is a failure of the containment systems and a risk to the laboratory staff and those in nearby areas (e.g. a gas leak, fire, flood, etc.) that requires intervention from the external emergency services and those services available in the center.

6.2 Action instructions

In the event of incidents and accidents that involve biological and/or chemical contamination of surfaces and/or generate possible contamination of the exposed personnel due to inhalation, absorption through the skin or inoculation, the following general rules must be followed:

All users must know the mode of action, situation and use of equipment and means of protection.

All incidents and accidents must be reported to the supervisor of the Biolab as well as to the Health and Safety department for registration and subsequent investigation.

If the emergency situation is caused by spills, or near-misses, that requires a rapid and effective intervention to prevent it from becoming a more serious emergency, you should activate the ICN2's Emergency plan.

6.2.1. Fire

Within the Biolab, there are four types of fire according to the fuel that can be produced (A, B, C and D). There are two types of extinguisher:

- ABC polyvalent dust extinguishers, used to suppress class A, B and C fires. They act by interrupting the chemical reaction of the fire. They are the most commonly used.
- CO2 extinguishers for extinguishing electrical fires. They displace or eliminate the oxygen of the chemical reaction of the fire creating an inert atmosphere and also diminish the heat due to the cooling caused by the expanding carbon dioxide.

In the case of needing to extinguish the burning clothes of personnel, you can use the emergency shower or a fireproof blanket, whcih can also be used to quench liquids that burn on a surface.

The action in the case of a fire is as follows:

- The Biolab staff are responsible for containing and extinguising the fire using the available portable fire extinguishers.
- Before using said extinguishers, you must press the alarm button closest to the installation to alert Reception, the Head of Emergencies and thereby the rest of the personnel in the institution.
- The alarm will initaiate the action procedure as ordered by the Head of Emergencies.

6.2.2. Contamination of surfaces, equipment and personal exposure

In the case of any spill, the first step is to immediately clean it up, trying to avoid direct contamination with the spill.

If the spill occurs inside a BSC or outside of it, the surface must be decontaminated following these guidelines:

- Use personal protection equipment (gown/coat, double gloves and goggles together with respiratory protection type FFP3 if the release of contaminating aerosols is expected.) Disinfectant, tweezers and disposable paper will be required.
- Remove any broken glass with tweezers. Cover the affected area with disposable or absorbent paper, avoiding the formation of aerosols.
- Add disinfectant (eg. Virkon) on top of the spill and disposable paper and let it act for at least 20 minutes.
- Contaminated material and disposable paper must be disposed of in biosanitary waste containers.
- Before changing clothes, treat hands and any exposed area with disinfectant. In case of suspicion of splashing to the eyes, the eyewash should be used for at least 15 minutes.
- Discard dirty clothing, gloves, etc. in the right container. Wash hands with antiseptic and change clothes.
- Hermetically seal the biosanitary waste containers.

Personal contamination can occur in four different ways; respiratory, skin or mucous, digestive and parental (skin punctures, cuts, etc.).

If staff have received an injury, open wounds, etc. you must be fully protected before assisting

them.

If contamination occurs, proceed as follows:

- Remove contaminated clothing.
- Wash hands and affected area with warm water and disinfect thoroughly, paying attention to nails, skin folds, etc. In the case of eyes, use the eyewash for 15 minutes.

In both cases, the incident must be reported to the laboratory supervisor and the Health and Safety Area.

6.3 Health Surveillance

In accordance with article 8 of RD 664/1997, the ICN2 must guarantee an adequate and specific health monitoring of people at risk of exposure to biological agents.

The medical area of the External Prevention Service of ICN2 in colabotarion with Health and Safety Area manages individualized medical records with the health data of personnel at risk of exposure. Therefore, the Health and Safety Area through whom the medical surveillance program is carried out, must be kept informed of any relevant changes and operations that may pose a risk to health.

The Biolab supervisor will notify these changes to the Health and Safety Area before starting work, as well as the personnel who are exposed (through a list of updated authorized personnel) so that the information can reach the Health Services monitoring department.

The doctor will contact the staff involved and conduct, as appropriate, a health assessment to assess the degree of fitness for work. The acceptance or rejection of this assessment by the person is recorded and filed in a digital format.