CELL CULTURE SERVICE

OPERATING GUIDELINES FOR CELL CULTURE LABORATORIES

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Content

1. OBJECTIVE:

Ensure that all personnel who directly or indirectly work with experiments where cell cultures of wild type mammals or stably or temporarily transformed (GMO) are used, are aware of the regulations and the appropriate way of working in the service.

These instructions will include at least training in aseptic work techniques and in the biosecurity of the organisms used in the experiments, allowing the understanding and assumption of the biological risks that may arise.

The purpose of these guidelines is to guide staff with the aforementioned instructions, as well as to know who to ask for advice and / or help in case of need. To publicize the structure and characteristics of the SCT-CC laboratories, the basic rules of work and use of the devices, as well as the responsible persons to whom to contact in case of breakdowns, contamination, accidents or suggestions.

The facilities of the Cell Service of the UdL-IRBLLeida are accredited for working with type II GMOs and therefore meet the requirements of biological containment level 2 laboratories (BSL-2) with the consequent characteristics of this level and compromising compliance with standards and protocols by users of the service.

2. AREA OF APPLICATION:

These guidelines apply to users of UdL-IRBLleida Cell Culture laboratories.

3. AUTHORIZED PERSONNEL

Only **authorized personnel** may enter the cell culture laboratories.

Each user will have a responsible researcher who will be in charge of their experiments and compliance with the regulations and good practice within the service. The IP will fill out a group form where they will mention the members of their group who will use the service, which cells they will work with and the level of biosafety they require, and in which project they are linked to be able to do the billing . On the first day of entering the service, new users will fill out a form with their personal data (and will receive an explanation of the service guidelines. Likewise, new users will have to prove that they have understood and accepted the regulations through a short test. The service reserves the right of admission to the facilities in case of not complying with the regulations established in them.

Data of those responsible:

	Name	Location	*e*-mail	Ext. Tel.
Scientific coordinator	Judit Ribas	Lab. B2.2 Bio. I	judit.ribas@mex.udl.cat	2936
Technical support	Marta Rafel	Office 2.19 Bio. II	mrafel@irblleida.cat	2953 12953
Technical staff	Laia Beà	Office -1.18 Bio. II	laia.bea@udl.cat	3758 12953
Technical staff	Rosa Vaquera	Office -1.18 Bio. II	rosa.vaquera@udl.cat	3758 12953

4. INTRODUCTION

4.1. What is cell culture?

Mammalian cell culture is the process or set of techniques that allow the **growth of tissue fragments** of different species in an artificial environment "*in vitro*", to examine and manipulate cell behavior while maintaining its physiological, metabolic, biochemical and genetic properties to the maximum. , etc. Cell culture laboratories work with the culture of cells that could become from immortalized cell lines or primary cultures.

The main characteristic, which defines the cell culture laboratory, is the **maintenance of asepsis** since the growth rate of the cells in culture is much lower than that of the usual contaminating microorganisms (fungi, yeasts, bacteria and viruses). Therefore, for the maintenance of the culture it will be vital to avoid the appearance of any unwanted microorganism.

4.2. Cell Culture Service of the Biomedicine building, Lleida

The Culture Service (SCT-CC) is in charge of ensuring the proper functioning of the different spaces and equipment for working with cell cultures, as well as training its users in good practices, preservation of asepsis and **biosafety**, advising them on case of need both in technical matters and in the design of some experiments.

It is a service attached to the Department of Basic Medical Sciences (CMB) of the UdL and all researchers from the different departments of the University of Lleida and IRBLleida can be users, as well as external researchers, who will be able to cultivate and manipulate all types of Mammalian cells (both primary cells and stable cell lines) that have a Biological containment level 2 (BSL-2) or lower, allowing to study and characterize them at the molecular and cellular level.

Users can work on a self-service basis or by hiring the support of service technicians (see rates to know the services offered). The service also offers the possibility to use a BSL-2 lab to manipulate culture human samples. The service does not accept and does not have the necessary facilities to cultivate or isolate dangerous biological agents of risk 3 or higher that require a containment level 3 or higher, as occurs with the SARS-CoV-2 virus.

If you need to manipulate human samples and / or risk 2 biological agents, you must be previously authorized by the Biosafety committee (comitebioseguridad@irblleida.cat).

Before working with any putatively infected culture, the Biosafety requirements of the Biological Agent (BA) present or possible in the culture must be checked (in case of doubt you can consult official pages such as RD 664/1997 (https://www.boe.es/eli/es/rd/1997/05/12/664/dof/spa/pdf) or others like https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html or on the web: *https://www.insst.es/databio-fichas-de-agentes-biologicos* where it specifies the level of biosafety necessary to work with those cells. All human or primate cells are biological agents of at least level 2 risk and therefore require handling in biosafety hoods.

The SCT-CC is located in the Biomedicine building at IRBLLeida and has 6 rooms with Containment Level 2 with specific characteristics for the use of cell cultures, a dissection room and a cell cryopreservation area with liquid Nitrogen tanks.

1. **Lab -1.3:** Laboratory to work with stable lines.
2. **Lab 1.9:** Laboratory with positive pressure and independent air circulation with HEPA filtered air inlet for working with stable lines.
3. **Lab 2.9:** Laboratory with positive pressure and air circulation with filtered air inlet with HEPA for working with primary cultures.

4. **Lab 2.16**: Laboratory of cultures with positive pressure, double door independent air circulation with entrance and exit of filtered air with HEPA for delicate cultures (stem cells), of long duration and free of mycoplasma and viruses.
5. **Lab 3.9:** Laboratory with positive pressure and air circulation with filtered air inlet with HEPA for working with Primary cultures
6. **Lab 3.16:** Negative pressure, double door, independent air circulation culture lab with HEPA filtered air inlet and outlet for manipulating or culturing cultures of BSL-2 human samples and / or lentivirus production.
7. **Lab 4.11:** Dissection laboratory.

SERVICE EQUIPMENT:

The SCT-CC has:

17 CO2 INCUBATORS	15 LAMINAR FLOW HOODS	7 BIO SAFETY HOODS IIA	2 refrigerated CENTRIFUGES (1 with ANTI-AEROSOL covers)	4 RT CENTRIFUGES (1 with ANTI-AEROSOL cover)
10 THERMOSTATIC BATHS (1 of metallic beads)	1 HYPOXIA CABIN	1 FLUORESCENCE LOUPE	6 DISSECTION LOUPES	6 FLUORESCENCE INVERTED MICROSCOPES WITH CAMERA
4 INVERTED MICROSCOPES	5 VORTEXES			

5. DESCRIPTION OF CELLULAR CULTURE, RISKS AND LABORATORIES:

5.1. Definition Primary Culture and Line Crulture

Cell Culture involves cellular disintegration, either by enzymatic or mechanical methods. The cell suspension that is obtained can be cultured as an adherent monolayer or in suspension in the culture medium. The cultivation of cells allows their propagation, notably increasing the cell mass of the culture throughout the generations.

Cells that are grown directly from a subject are known as primary cells. Most primary cell cultures have a limited life span, that is, after a certain number of divisions the cells enter senescence and stop dividing, generally maintaining viability. Occasionally, a primary culture is kept for more generations than expected. This fact is due to the appearance of immortal cells in the culture. The reason for the immortalization of these cells is, most of the time, unknown, but it is believed that this ability is related to cell control pathways, that is, for a primary culture to establish itself as a stable line it is directly related to its genetic variability.

An established or immortal cell line is that one which has acquired the ability to proliferate indefinitely

There are differentiated laboratories by primary cultures and by line cultures in SCT-CC:

Primary Laboratories: Intended to work with primary cell cultures and cells from lines contaminated with mycoplasma or not tested.

Line Laboratories: Intended to work with stable mycoplasma-free cell lines.

All SCT-CC culture laboratories are accredited as level 2 containment rooms with these requirements:

- Independent rooms from the rest of the building
- Independent ventilation system with HEPA filtration.
- Trained users to handle biological agents.
- Restricted access to facilities.
- Use of biosafety cabinets for handling and / or culturing biological agents with BSL2 requirement, especially human samples and cultures.
- Minimization of aerosols and splashes production. Both working inside the biosafety cabinet and with the use of centrifuges with anti-aerosol covers.

5.2. Main biological risks of cultures:

Mammalian cell cultures not contaminated with other biological agents generally do not present a significant risk to the handler; possible dermal inoculation of this culture causes only local inflammation. However, these cultures can contribute substantially to the risk of the handler in exposure to other biological agents that can act as a basis or aid the survival or replication of opportunistic agents.

Human and primate cell cultures may contain pathogenic viruses including: Hepatitis B and C virus, HIV, human leukemia virus, Epstein-Barr virus, cytomegalovirus, Herpes simplex 1 and 2, SARS-CoV-2. Non-human animal or primate cell cultures may contain Hanta virus, lymphocytic chori meningitis, influenza virus, etc. As well as other parasites such as toxoplasmosis or mycobacterium tuberculosis that could be present in human lung tissues, etc.

Other primate cells and tissues also pose risks to laboratory personnel. Potential hazards are presented by cells transformed with viral agents such as SV-40, EBV, or HBV, as well as cells that contain viral genomic material. Carcinogenic cells are also potentially dangerous as a result of self-inoculation.

The highest risk cell cultures are those that come from humans and primates, especially if they are derived from peripheral blood, lymphatic, and nervous tissue.

(Biological risk: evaluation and prevention in work with cell cultures. NTP 902. 2011)

Some human and primate lines present a special risk and are classified in the Biological risk group greater than 1. Therefore, it is very important to evaluate the biological risk of each one by looking at the biosafety specifications in which each cell line has been classified. and their procedures and protocols before working with them.

For example, risk 2 HeLa cells have DNA-integrated papillomavirus and mRNA from the virus capsid has been observed (Xiao et al. 2015). In the case of 293T, also classified as risk 2, it has been observed that they express the large T antigen of the SV40 virus, which helps to produce lentiviral particles, making it a risk 2 line. this antigen and the production of tumors in case of infection with these cells in hamsters and rats, however, in humans there are discrepancies in this regard. Some research suggests that they could cause certain types of cancer, while others consider that there are not enough dates to confirm it (Xiao et al. 2015;Stepanenko &Dmitrenko, 2015; Eddy et al. 1961; Eibl etal. 1994; Lowe et al. 2007).

Cuadro 1. ***Clasificación de los microorganismos infecciosos por grupos de riesgo***

Grupo de riesgo 1 (*riesgo individual y poblacional escaso o nulo*)
Microorganismos que tienen pocas probabilidades de provocar enfermedades en el ser humano o los animales.

Grupo de riesgo 2 (*riesgo individual moderado, riesgo poblacional bajo*)
Agentes patógenos que pueden provocar enfermedades humanas o animales pero que tienen pocas probabilidades de entrañar un riesgo grave para el personal de laboratorio, la población, el ganado o el medio ambiente. La exposición en el laboratorio puede provocar una infección grave, pero existen medidas preventivas y terapéuticas eficaces y el riesgo de propagación es limitado.

Grupo de riesgo 3 (*riesgo individual elevado, riesgo poblacional bajo*)
Agentes patógenos que suelen provocar enfermedades humanas o animales graves, pero que de ordinario no se propagan de un individuo a otro. Existen medidas preventivas y terapéuticas eficaces.

Grupo de riesgo 4 (*riesgo individual y poblacional elevado*)
Agentes patógenos que suelen provocar enfermedades graves en el ser humano o los animales y que se transmiten fácilmente de un individuo a otro, directa o indirectamente. Normalmente no existen medidas preventivas y terapéuticas eficaces.

Ref: NTP 233: Biological safety cabinets

Recommended Practices according to the manual on Biosafety in Microbiology and Biomedicine Laboratories. 4th edition. CDC NIH:

Both human and other primate cells should be handled using Level 2 biological containment practices. All work should be done in a Biosafety cabinet and all material should be decontaminated before being disposed of.

All workers who work with human cells and tissues must work in accordance with the policies and guidelines established by the institution's control plan. Workers should take a baseline serum sample, be given the opportunity for Hepatitis B immunization, and undergo an evaluation with a healthcare professional after an exposure incident.

6. BASIC RULES OF CELLULAR GROWTH ROOMS

1. Eating, drinking, smoking, chewing gum, makeup, handling contact lenses, and storing food or drinks are prohibited in the laboratory.
2. The laboratory will remain orderly, clean, and free of materials not related to work. The entrance with coats jackets, bags or backpacks is not allowed.
3. Users must wear a clean long-sleeved gown exclusively for work into cultures laboratories.
4. It is recommended to wear long pants and closed shoes.
5. Gloves must be used in all cases where cells are handled. In the case of BioIIA, a category III double glove must be used following the UNE EN ISO374-5: 2016 standard. The pair of work gloves will only come out of the cabin to be thrown into the biological container.
6. Used gloves must be removed aseptically and hands must then be washed. Users should never leave the lab waring using gloves. Example of how to remove gloves correctly: https://www.youtube.com/watch?v=pM8SEp5cLo8
7. Users cannot touch the laboratory or the doorknob wearing used gloves.
8. Most room equipment must be reserved using the Supersaas program on the following website with a maximum of 24 hours and a minimum of 15 minutes in advance. Fluorescence microscopes should be reserved when no flow hood is being used at the same time.
   - Laminar flow cabinets, fluorescence magnifying glass and dissection magnifying glasses: https://www.supersaas.es/schedule/SCT_CC/Campanes_flux_laminar.
   - Hypoxia chamber: https://www.supersaas.es/schedule/SCT_CC/Reserva_Hipoxia__SCT_CC
9. Users with live cells have preference in the microscope over those who have fixed cells.
10. Work surfaces (Hoods, microscopes, stands ...) will be disinfected at the beginning and at the end of each use with Propane-AF (mixture Isopropanol and EtOH)
11. All material used must be collected at the end of the task and to let the working area empty, clean and disinfect.
12. All materials, samples and contaminated cultures should be decontaminated before to throw them away. Cultures to eliminate must first be neutralized with bleach, and aspirated. The cell plate will be removed within the solid contaminated waste.
13. Contaminated solids waste must be thrown into the black bio-waste containers (one under each hood).
14. Waste containing cytotoxic drugs or substances is thrown into the blue cytotoxic container. In case of liquid cytotoxic residues, they are thrown into the liquid cytotoxic container, if they also contain biological agents, they will be previously inactivated with bleach. Inform the service personnel of the composition of this liquid waste to take the appropriate safety measures.
15. CLEAN and NOT CONTAMINATED PAPERS, PLASTICS, POREXPAN, etc. must be deposited in the RECYCLING CONTAINERS (located inside the labs or in the hallways).

16. **Any user** can close a black or yellow container if they are full and take an empty one (inside the room). In the case of liquid waste, should be first neutralized with bleach or Virkon and then emptied into the liquid carafe cytotoxic residues (on the side of the sink).

17. All technical procedures (tube vortex, pipetting, centrifugation ...) must be performed in a way that minimizes the formation of aerosols and droplets. Examples: Long pipettes must not be hooked to the pipettor avoiding leakage and droping; vortex eppendorfs and centrifuge tubes especially if they carry infectious substances must be with well close tube and carefully. Biological samples containing virus or human fluids must be centrifuged in the centrifuge with antiaerosoles covers whenever possible.

18. Once the work in the hood is finished, the suction tube must be cleaned first with bleach to neutralize contaminants or liquid media culture (colour change from red-phenol of the medium transparent) and with Propano-AF to remove remaining disinfectant of the tube. The vacuum must be closed to avoid the constant use of the pump.

19. Any accidentally liquid pour into the waterbath, must be reported to the laboratory supervisors. To check the danger of the dropped product, to inactivate it if it is necessary and clean and change the water of the thermostatic bath must be done.

    The water bath should be switched off when no user needs it. It is important to prevent from being turned on overnight; the resistance can burn and cause a possible fire.

20. **Material should never be taken from Primaries labs to Lines labs, as it could be a source of contamination for cell lines**.

21. All spills, accidents and overt or potential exposures to infectious materials must be reported to the laboratory supervisor. A written record of such accidents and incidents should be maintained

22. When you defrost a maintenance line, write down the line name and date in the incubator's log. The service technicians will mark when the sample has passed the mycoplasma test.

23. When viral productions or infections are carried out, it points to the corresponding registry with which virus it is working and which gene it overexpresses or suppresses.

24. Cell transfer between laboratories is not allowed without the consent of the service itself.

25. All spills, accidents and / or direct (or potential) exposures to infectious materials must be reported to the person in charge of the service. In each room there is a register where users must write down such accidents and incidents.

26. The laminar flow cabinets and the BioIIA are NOT smoke extractor cabins nor do they have active carbon filters, so the use of volatile toxic products will be previously assessed with the service personnel, in case of absolute necessity and taking into account the risk. biological that the samples may have. The use of these substances in a non-extractor cabin will be under the responsibility of the IP as well as any repair of this due to the use of these products.

27. In the case of the creation of a GMO in a human line (hs line transformed in a stable way through lentiviruses, CRISPR, etc.), contact the Biosafety committee (**comitebioseguridad@irblleida.cat**).

28. All cells will have to be written: the owner or the group to which they belong, the date of creation and the cell line with which they are working. Failure to write down these data can make it difficult to identify them. If the service considers that there is a culture to be thrown, it has the duty to ask its researcher, as long as the plate is clearly identified.

29. In the case of unidentified plates / flasks, the service reserves the right to remove abandoned or contaminated plates from the incubator.

30. To avoid spillage, the cells seeded in plates will have to be placed on trays. In the case of screw cap jars, the trays will be optional. Each group must have their trays, which can be metal or plastic. The size of these trays should be less than half the surface of the incubator shelf.

SANITIZING SOLUTIONS FOR GROWTH ROOMS:

Propane-AF: 52% ethanol + 12% isopropanol. Immediate effect, must be removed next. Do not use on methacrylate.

Bleach 70%, the bleach loses its disinfectant effect as it comes into contact with air and light. The recommended use for disinfection in liquids with microorganisms is 2% sodium hypochlorite. Commercial bleach contains 5-6% sodium hypochlorite, to last an average of 3-4 days, it is prepared with 70% bleach, which will remain in 4% sodium hypochlorite.

Virkon between **1% - 4%:** Commercial viricidal, sporicidal, fungicidal and bactericidal disinfectant product based on monopersulfate and acid. sulfamic. Spray on surfaces. Only in use in the 3.16 lab.

7. SERVICE APPARATUS: INSTRUCTIONS FOR USE.

Content:

7.1. LAMINAR FLOW CABINS:

a. Definition:

They are stainless steel worktables, which are supplied with laminar flow air previously filtered by HEPA filters, conferring an aseptic work surface avoiding the entry of unfiltered turbulent outside air.

Its function is to maintain an area free of particles, especially of possible contaminants (bacteria, yeasts ...) that could access the culture.

Depending on the type of cabin, it will protect:

- harmful agents (chemical or biological) to personnel.
- to the product of the different biological pollutants.
- to the environment (external in the cabin) of biohazard agents

b. Cabin type:

Vertical laminar flow cabinets

The vertical laminar flow cabinets ensure good protection of the product, and according to their design, also a partial protection of the manipulator by not receiving all the air coming from the interior of the hood directly.

- Laminar flow cabinets are not considered biosafety hoods. Type I biosafety hoods are those that protect the handler, but not the sterility of the product

Biosafety IIA Cabinets

The class II biosafety cabinet protects the product, the handler, and the environment. In type A, 30% of the air is eliminated in each cycle previously filtered by HEPAs and the remaining 70% recirculates. Inside the cabin, work is done with negative pressure, giving the manipulator greater safety. It is the most suitable for working with biological agents of risk 2 or that require an BSL-2.

Examples of use of Biosafety cabinet 2:

a. Production of lentiviral viruses, retroviruses, others.
b. Infection of cell lines or primary cultures with risk 2 viral particles.
c. Primary cultures and manipulation of human cells.
d. Handling human samples
e. Cultures of human lines classified as type II biological risk. Especially HeLa or 293T cells.

TYPE OF CABINS ACCORDING TO RISK:

		CLASE I	CLASE II TIPO A	CLASE II TIPO B	CLASE III
AGENTES BIOLOGICOS	GRUPO RIESGO 1	(1)	(1)	(1)	(1)
	GRUPO RIESGO 2	(1)	(1)	(1)	(1)
	GRUPO RIESGO 3	(3)	(2)	(2)	(1)
	GRUPO RIESGO 4	(3)	(3)	(3)	(1)
PRODUCTOS DE ALTA TOXICIDAD CANCERIGENOS SENSIBILIZANTES OTROS		(2) (*)	(1) (*)	(1) (*)	(1) (*)

(1) Totalmente indicada (2) Puede utilizarse (3) Uso no recomendado

Ref:	NTP 233: Biological safety cabinets

BIOSECURITY IN THE DIFFERENT TYPES OF CELL CULTURE:

CELLULAR CULTURE CONTAINMENT

Well characterized cell lines of human or ape origin. Well characterized, non-human and non-simian cell lines with low risk of endogenous infection with human pathogens.	Containment level 2 and use of biosafety cabinet
Cell lines or strains not fully characterized or authenticated	Containment level 2 and use of biosafety cabinet
Cells with endogenous pathogens and deliberately infected cells	Adequate containment of the pathogen
Human blood cells, lymphoid cells, nervous tissue of human or simian origin	Adequate containment to the potential risk.

SOP OF WORK IN THE CABINS

7.1.1. LAMINAR FLOW CABIN

PREVIOUS OPERATIONS

1. RESERVATION OF THE BELL ON THE WEB:
https://www.supersaas.es/schedule/SCT_CC/Campanes_flux_laminar
2. PREPARATION OF THE MATERIAL
3. PREPARATION OF SANITIZING SOLUTION
4. PLACEMENT OF EPIS

a) Clean the surfaces of the cabin with alcoholic solution (Propane-AF, (mixture of ethanol and isopropanol) or 70% ethanol) present in the room, using the sweep technique. On methacrylate screens, use diluted bleach. **Ethanol breaks down methacrylate**!

b) Introduce the material previously sprayed with alcoholic solution in the cabin as centrally as possible and properly separated so that the ultraviolet rays can reach all the material and leaving the air extraction grids free for a good maintenance of asepsis inside the hood.

c) Work with a long-sleeved gown, hygienic mask (min.) and gloves.

GOOD PRAXIS INSIDE THE HOOD

d) Separate the work area: clean area, work area and dirty area.

e) Introduce dry media, samples and buffers out of the bath and previously sprayed with alcoholic solution.

f) Work slowly and with concentration to avoid sudden movements that cause turbulence (→ point of contamination) and aerosol production.

g) Do not pass your arms over open samples→ point of contamination

h) Do not put notebooks, calculators, cardboard, or paper boxes inside the hood,→ point of contamination.

i) Do not touch the sterile material that will come into contact with the cells with gloves. In case of doubt, discard the material or identify the plate for follow-up.

j) Open the sterile material in the hood. The reinsertion of the material already used in the culture medium or buffer should be avoided.

k) To aspirate liquids, turn on the aspiration pump and connect a Pasteur pipette to the tube (if different liquids are to be aspirated, it is good to add a yellow pipette tip and change it so as not to change the Pasteur pipette each time).

l) Avoid leaving the caps of the bottles and tubes directly on the surface of the hood (insert and remove them each time by holding them by hand). If it is necessary to leave the cap on the surface face up, leave it face up and away from the work area, so as not to put your arm over it.

WASTE

m) Throw the material that has been in contact with the biological sample in the biological waste container.

n) If you need to remove cells, aspirate the medium, add diluted bleach or Virkon, allow it to act for 5-15 minutes, aspirate it, and discard the plate in biowaste.

o) Pour the aspiration Pasteur pipette into the biological waste container and disinfect the aspiration tube first with 70% bleach or Virkon and then with alcoholic solution until no remains of the culture medium remain in the tube and the liquid waste container changes color neutral (no color).

p) Cytotoxic residues are thrown into the container by cytotoxic (blue) or into the jug present in the room.

ENDING

r) Clean the surface of the booth and disinfect with Propane-AF or Virkon (considering safety measures).

7.1.2.BIOSECURITY II CABIN (BIOIIA)

PREVIOUS OPERATIONS

1. PREPARATION OF MATERIAL
2. PREPARATION OF SANITIZING SOLUTIONS
3. PREPARATION OF MATERIAL DECONTAMINATION AREA WITH DISINFECTANT
4. PLACEMENT OF EPIS

a) Before performing any procedure, the hood surfaces will be cleaned with the alcoholic solution present in the room for 80% min. in alcohol, Virkon using the sweep technique.

b) Until the flow has recirculated and carries the proper flow, it is not safe to work inside the hood. All the material will be introduced into the hood

c) Work with a long-sleeved gown, hygienic mask (min.) And gloves. In case of performing procedures with aerosol production or with risk of splashes, use eye protection glasses and double long-sleeved gown, disposable gown, or sleeves.

d) To work in the BioIIA, a double glove will be used. Gloves in contact with the skin will be made of nitrile. Those of category III are recommended complying with UNE EN ISO 374-5: 2016 and ISO16604: 2004 (substitute for EN374: 2003 virus specification or 374-5). Work will be done at about 10cm inside the hood, avoiding covering the air extraction grills to allow good user safety.

e) If it is necessary to leave the hood to perform another task, the outer gloves should be changed before touching any other surface. Once used, the first pair of gloves will be removed aseptically. Inside the hood there must be the minimum essential material previously thought, to avoid having to enter and leave it while working.

f) While not working, the interior of the hood must be empty. The Bio-II-A only keeps a yellow container for sharps waste and a bottle of soap and water for long pipettes.

ACTIONS TO BE TAKEN - PROTOCOL in the BIOIIA

g) Divide the space of the hood into a clean area, a work area, and a dirty area.

h) The material should not accumulate at one point, but will be distributed to achieve a good distribution of the flow and avoid turbulence.

i) Work slowly, concentrated and avoiding sudden movements that could favor the production of aerosols.

j) If the protocol is for virus production, these viruses should be stored in the Biomedicine -80ºC freezer for storage, where they will be properly hermetically sealed and with indications of biological danger.

WASTE

k) Discard the small contaminated material (tips, tubes ...) in the yellow waste container or in a zip-lock disposable bag / bottle and the sharp waste in the yellow waste container located inside the hood. The aspirated liquid must be neutralized. The tubes that have a lid will be launched closed with its lid. After the job is finished, close the plastic bag tightly and throw it in the bio-waste container.

l) Throw the long pipettes into the beaker with soapy water that you can find inside the hood. When finished, take the long pipettes, wait until all the neutralized liquid has drained off, and throw them into the biowaste container.

DECONTAMINATION AND CLEANING OF THE HOOD AND WORK AREA

m) All contaminated or active materials, samples and cultures will need to be decontaminated before disposal. In the case of liquids that need to be disposed of, (if they cannot be enclosed in an airtight bag) they will first be neutralized with bleach, placed in a jar with a lid and the plate / jar will be disposed of in the solid waste container. In the case of volumes requiring aspiration, they must be neutralized with a product whose performance is not affected by the amount of organic matter . asking for permission from those responsible for the room.

n) When you finish working, remove the reagents from the hood by externally decontaminating them with 70% Ethanol or Virkon, decontaminate the work material and residues by leaving it inside the hood sprayed with 70% Ethanol. The waste will be thrown into its container and the work material will be removed.

o) Any material that has to enter and / or exit the hood must be cleaned externally with a paper impregnated with a disinfectant solution.

At the end of work, all the material used must be collected and the booths should be left empty, clean and disinfected after use.

7.1.3. USE OF GERMICIDE IN BIOSECURITY II HOODS

OBJECTIVE

Regulation of the use of germicide in Biosecurity II hoods

DEFINITIONS

Following the current regulations' recommendations:

Royal Decree 486/2010, 23 April, about users' health protection and safety against risks related to exposition to artificial optical radiation

Technical guide for the avaluation and prevention of risks related to artificial optical radiation

Regulate the use of germicide in Bio II hoods

SCOPE OF APPLICATION

All Bio II hoods from the University of Lleida's Cell Culture Service

PROCEDURE

1) Identify at lab's door that germicide is being used in order to avoid anyone to enter

Reference No.

ISO 7010-W027

Referent

Warning; Optical radiation

Function

To warn of optical radiation

Image content

Star with 11 points with circle in centre

Hazard

Optical radiation (such as UV, visible radiation, IR)

Human behaviour that is intended to be caused after understanding the safety sign's meaning

Taking care to avoid injury to eyes and skin when in the vicinity of optical radiation

Additional information

Test data obtained according to ISO 9186-1 are not available from more than one country. Find from national testing, however, showed that in one country the safety sign did not reach the cri of acceptability. Consequently, a supplementary text sign shall be used to increase comprehens except when the safety sign is supplemented by manuals, instructions or training.

If necessary, a supplementary sign shall be used to give further information about the kind of o radiation (e.g. UV, visible radiation, IR).

2) When switching on/off the germicide, the user has to cover the body and wear optical radiation protective glasses
3) The hood's door has to be closed during the use of germicide
4) The user is responsible of switching off the germicide and give access to the lab with the corresponding placard when he finishes

7.2. CO2 INCUBATORS:

a. **Definition:**

The CO2 incubator is a chamber that maintains the cultures in constant and optimal atmospheric conditions for their growth, at the service of cultures they are programmed by the optimal conditions in the growth of mammalian cells.

- 37 ° C temperature: physiological temperature of mammalian cells
- CO2 concentration (5%): to maintain the appropriate pH for its growth. The culture medium acts like blood in animals. CO2 dissolves in water forming bicarbonate, most of the CO2 present in the blood is in the form of bicarbonate (HCO3-), this acts as a pH buffer, allowing fluctuations of gases, nutrients, and metabolites without causing dangerous changes in the blood pH).
- High humidity (to avoid evaporation of water from the culture medium).

To maintain these constants, it has two doors, an exterior and an interior glass which must be tempered. The transparent door allows the location of the plates and avoid prolonged openings. Inside the incubator there is a tray with distilled water and forced air recirculation is produced, facilitated by the holes in the shelves.

b. **Instructions for use:**

1. The plates should be placed on trays to facilitate the depositing of plates on the shelves of the corresponding incubator (primaries, lines, viruses) and to avoid spillage in the incubator. Trays are owned by each group and are required for plates (not for jars).
2. Care must be taken not to open the incubator door for too long so as not to destabilize the atmospheric constants inside. Close the door carefully.
3. The plates must be handled with gloves or ethanolized hands to avoid contamination to the cultures.
4. The culture plates must be handled with care to avoid spillage by holding them in such a way that the lid does not open and it does not lose its horizontality.
5. The trays where the plates are left should be washed regularly by the user to avoid the growth of fungi or bacteria. In case of spillage on the tray, it must be absorbed with paper moistened with bleach and, finally, disinfected with Propane-AF. If it occurs in a virus experiment, leave the bleach or Virkon to work for 15 minutes. It must be reported in the incident register and the service technicians.
6. When the spill is inside the incubator, the same procedure as point 4 would be applied, being the communication of the incident to the service technicians absolutely mandatory.
7. If one of the cultures becomes contaminated, it must be immediately removed from the incubator and recorded in the contamination log of the laboratory.
8. Incubators are automatically calibrated daily. While the incubator is being calibrated, the door can NOT be opened to avoid performing an erroneous calibration by falsifying the configuration of the constants.

7.3. MICROSCOPES AND LOUPES

- **General instructions:**

  If you need to use the microscope for a long time, please reserve it in advance in the program. Live cells have a preference for use under the microscope vs. the fixed ones.

1. Clean the eyepieces with paper and Propane-AF and the plate where we deposit the sample to be observed, thus avoiding contagion problems between users and cultures.
2. Place the sample on the plate, set the necessary magnification and focus it to visualize the samples.
3. Every user should ask for help and training when there is any unknown microscope / magnifier function. The user should NOT manipulate unfamiliar functions of the microscope.
4. Remember to close the microscope and loupe lamp when you finish working.

7.3.1 Inverted phase contrast microscopes:

a. **Definition:**

They allow the morphological control of living or fixed cells within the culture container.

The fact that the samples to be observed are in thick containers (plates or flasks) means that a conventional microscope is not able to focus and observe the cells, so microscopes have been developed in which the illumination source and objectives are They are inverted with respect to the plate of a conventional optical microscope.

The second characteristic that determines the optical instrument is the absence of color in the sample, because it is living cells that have little contrast and cannot be stained without damaging them. To alleviate this, the microscope is equipped with a phase contrast device, thus increasing the contrast of the image and the quality obtained is much higher.

7.3.2 Inverted microscopes or fluorescence loupes:

a. **Definition:**

They are microscopes or magnifying glasses like the previous ones but that have a lamp that emits at different wavelengths allowing the visualization of images that have fluorescence or that are marked with fluorochromes.

They have a camera assembled and connected to a computer to capture images to document the state of the cultures.

b. **Instructions for use:**

- In the microscopes of laboratories 3.16 and 3.9, to extend the life of the mercury fluorescence bulb, once it is turned on, it cannot be closed for 15 minutes; and once it is turned off, you must wait 10 minutes before turning it back on. To manage the maintenance of the fluorescence lamp, users must record the number of hours that the bulb has worked on the registration form that they will find next to the microscope.

- Fluorescence microscopes and stereomicroscopes in other rooms have halogen or LED bulbs.
- The cameras associated with them can be used to take photos of the experiments carried out in the tissue culture rooms. Any user with live cells will have priority over any user with fixed cells, even if the user has reserved the microscope in register.
- It is necessary to follow specific instructions for each software. Ask the service technicians if in doubt.

7.3.3 Dissecting Loupes

a. **Definition:**

Stereomicroscopes magnify the sample 4-50 times to facilitate tissue dissection. They are used in primary culture laboratories. It is designed for observing a sample at low magnification, typically using light reflected from the surface of an object rather than transmitting it through it.

- During the dissection, it must be monitored with the sharp material and the biological risk that it entails.

7.4 CENTRIFUGES:

a. **Definition:**

In the culture laboratory, a centrifuge is needed for the precipitation of cells in suspension, obtaining cell types by gradients, concentration of buffers or viruses, etc.

b. **Instructions for use:**

- Put the tube or plate inside the rotor adapter and counterbalance with the same volume to the symmetrical position of the opposite band.
- Choose the appropriate program and start the device.
    - The most common program to centrifuge cells is: 1000 rpm 5 'at Room Temperature.
- If a tube breaks inside the centrifuge without an aerosol cap: before opening, wait for any aerosols that may have formed to settle at the bottom (15 min). According to the biological risk of the samples, vacate the room from the rest of the users and proceed to clean the spill: Absorb the liquid material with absorbent paper, remove the broken material with gloves and tweezers to avoid punctures and inactivate the spill with disinfectant (bleach or Virkon). Leave on for 15 minutes. The broken material must be eliminated with a double glove, sterilizing it with bleach or Virkon, depositing it in hermetic bags and discarding it in the biological waste container. Once inactivated, clean with alcoholic solution.
- **Centrifuge with anti-aerosol lid**
    - Remove the cubes with tube adapters from the centrifuge and open them in the BioIIA hood.
    - Insert tubes into tightly sealed adapters.
    - Close the buckets with the spray cap and reinsert them into the centrifuge.
    - Use the correct program.
    - Once the centrifugation is finished and the centrifuge opened, it is necessary to take the closed bucket to the BioIIA hood. It must be opened inside the cabin, the tubes removed and the bucket returned to the centrifuge, checking that no spillage has occurred.
    - Remember to touch the buckets with clean gloves and change them once you have touched the samples inside the safety hood and have to return the buckets to the centrifuge.
    - If a spill occurs inside the buckets due to a tube break, this spill will be cleaned up and inactivated inside the BioIIA biosafety cabinet. The broken material should be removed with a double glove, and tweezers sterilized with bleach or Virkon (or in an airtight bag in the biological waste), Add disinfectant and leave it inside the BioIIA hood with ultraviolet light for 20 minutes. All material must be neutralized and the centrifuge, rotor, bucket, and tube adapters cleaned and sanitized before reuse.

7.5. THERMOSTATIC BATHS:

a. **Definition:**

Thermostatic water baths are made up of a bucket where the water is deposited and the head, which heats the water with a resistance helps, and is distributed once it is heated through a rotor.

Thermostatic baths are used to heat culture media or buffers, run chemical reactions, or thaw frozen samples. They are programmed to keep hot water at 37ºC.

They are filled with non-sterile water and, since the temperature that the water reaches is ideal for the growth of contaminating microorganisms and algae, the precaution of drying well and ethanolizing all the materials introduced into the bath will be an important point to avoid contamination in hoods.

Instructions for use:

- The cuvette should be filled with distilled water until it covers the resistance. Some thermostatic baths are blocked if they are turned on if there is not enough water; in this case, add more water and reset the head before turning it on again.
- Turn on the bath, they are programmed to work at 37ºC. Do not change the temperature without the permission of the laboratory supervisors, it takes approximately 15 minutes to reach the temperature.
- The tubes and bottles that must be introduced into the bath must be securely fixed, with floats, racks or weights, to prevent them from spilling and contaminating the bath and the product.
- When there are no bottles and tubes in the bath, it should be turned off.
- In case of spillage of any reagent / cells in the bath, you have to notify it at the technical staff to proceed with the bath decontamination.

There are also dry metal bead baths designed to replace water in laboratory water baths without circulation and without agitation. Compared to water, metal beads are more resistant to bacterial growth and therefore can help prevent the spread of contamination throughout the laboratory.

This bath is located in the room with the greatest bioprotection: at 3.16.

In this case, the bath can be left on for the entire working day without danger of burning out the resistance.

7.6. LIQUID N2 TANK (-196ºC) :

a. **Definition:**

Tank containing liquid N2 that has a high refrigerating power (-196ºC) and is used to store cell lines. To freeze the cells in suitable conditions, review the designated protocols for each cell line that allow them to maintain their viability for a long time (years) as long as the cold chain is not broken.

b. **Instructions for use:**

- Liquid nitrogen must be handled with gloves and it is advisable to protect the eyes and wear covered shoes to protect the feet from possible splashes. Direct contact with the skin causes burns.
  - The tanks are closed and users must make an appointment with the service technicians to open them and collect or leave samples.
  - In case a user has permission to open a tank alone, the following must be taken into account:
    - The user must know where the samples are located before opening the tank to avoid excessive evaporation of N2. N2 gas displaces oxygen; Users should read the safety regulations before use (PNT-CC-18).
  - You should never have a tank with levels less than 15 cm of N2 (l). Keep in mind that nitrogen displaces oxygen, so the person in charge of filling them must be at a certain distance from the tank while filling the tanks or dewars.

c. **Accidents and safety:**

In case of an emergency due to a Liquid Nitrogen spill, ventilation must be imperative; the exterior doors should be opened to facilitate the removal of nitrogen gas. If there are burned areas of the body, act according to the severity of the affected person, call 112 or contact the mutual depending on the type of contract you have (see chapter 12).

d. **Biosecurity:**

In case of breakage of a transformed cell vial, we will collect it with gloves while it is still frozen, place it in an airtight tube or neutralize it with bleach, and reject it in a bio-waste container. In the event that the tube is already thawed and there is a spill of biological agents, we will act following the spill protocol PNT-CC-19 and we will write it down in the registry.

7.7. HYPOXIA CHAMBER

The hypoxia chamber is a hermetically closed cell culture cabinet that allows the control and regulation of the concentration of oxygen, CO2, temperature and humidity. They have the characteristic that the samples can be introduced, incubated, examined and removed without losing the pre-established environmental conditions.

The hypoxia cabin is not a Biosafety cabin; the air it expels is not filtered or laminar flow. Before using it for the first time, contact the service technicians.

It must be reserved each time it is needed through the Super SAAS program and for the duration of use. If you need it for more than a day or in advance, contact the service technicians.

8. CELL CULTURE CONTAMINATION

In vitro cell cultures can easily become contaminated since their growth rate is slower than most microorganisms and viruses that occur in their environment. Most cell cultures become contaminated with bacteria, fungi, and viruses. Large bacteria are easily detected as some media have color indicators for pH and can change color more quickly than usual from red to yellow when the medium is acidified, which means nutrient consumption. You can detect a specific odor or through a microscope see the type of bacteria that determines the contamination. Small bacteria such as mycoplasma (0.2 µm) are not easily detected as there are no very obvious visible symptoms (see point 10 of the guidelines). The detection of fungi can be determined by means of microscopes if the fungi have the ability to bud and create filamentous organelles. Virus detection can only be assessed by molecular or antigenic techniques. Yhtui

8.1. Source of contamination

a) One of the main sources of contamination in the cell culture laboratory is the user himself.

In cell culture laboratories it is very difficult to work completely free of microorganisms, but we can reduce their concentration taking into account the source of contamination:

Cultures are often contaminated with microorganisms that come from the hands, mouth, face, or clothing. To avoid this, you should wear a hygienic mask, gloves, a long lab coat and wash your hands with soap every time you want to work, as well as disinfect all the gown material and sleeves.

b) Another major source of contamination is clothing. The user should wear a clean lab coat before working in the lab and wear different footwear to greatly help reduce the number of germs in the room. Wearing woolen clothing while working in the booths is a source of contamination. The service has hangers or cabinets (depending on the room) where you can store an exclusive gown for the grow room.
c) Another very important source of contamination is the remains of bath water introduced into the hood and touched by mistake. If we doubt a contaminated medium, we analyze an aliquot of medium, incubating it in the incubator room for bacteria (room 4.9) so as not to put other cultures or other users at risk In case the contamination is low and it is a culture After a very important experiment, the medium will be changed with a new one and the dose will be increased and / or the antibiotic in the medium will be changed.

Basic methods of good practice to avoid contamination:

- ➔ The user must wear a clean lab coat (specific to work in the cell culture room).
- ➔ The user must wear aseptic gloves while working.
- ➔ It is recommended to spray hands and sleeves with alcoholic solution.
- ➔ The user should avoid contact with sterile material that comes into contact with the culture.
- ➔ The user should avoid passing his hand or arm over the open material that will be in direct contact with the sample.
- ➔ The user must work in the central part of the cabin distinguishing the work zones in: clean zone, work zone and dirty zone.
- ➔ Once the user is working in the booth, they should not answer the phone, touch the doorknobs, scratch their hair, nose, etc. Gloves should be changed if one of the above occurs.
- ➔ The surface of the hood must be clean and empty before working, avoiding that the material is too close to each other.

➔ When removing any tubes or bottles from the bath, they should be blotted dry and wet and dried again with ethanolized solution.
➔ Users must ensure that airflow can pass between all objects in the hood. Any porous material such as cardboard boxes should not be put inside the hood. The surface of the hood must be free of material agglomeration.
➔ To avoid or minimize the formation of aerosols and cross-contamination that can occur during work, the user:

- You must use different bottles / tubes of medium, buffers, and reagents, for each cell type.
- It should not work in parallel with more than one cell line in the hood.
- You should not work with non-sterile or contaminated (or questionable) material.

➔ Another important point is how to transport the plates from the hood to the incubator or microscope. This point is especially important if biohazard material is transported:

At first, the outer part of the plates containing the culture is sterile, but when it is removed from that environment it is no longer sterile. The user must be careful with their handling avoiding openings by mistake:

- The jars or plates should be taken gently, avoiding strong movements that could spill the medium or cause it to come into contact with the lid, but firmly at the same time to ensure that the lid is not accidentally opened outside the hood.
- The jars or plates should be touched with clean gloves or hands washed with soap and sprayed with ethanol.
- Plates should be placed on a tray inside the incubator.
- When looking under the microscope, the user must ensure the cleanliness of the plate before placing the plate / bottle.

➔ Old cultures should not be forgotten in the incubator, they can be a source of contamination for other cultures.

9. INSTRUCTIONS IN CASE OF CONTAMINATION:

- When a culture is contaminated or we suspect it, all reagents used with this culture must be replaced or quarantined. For the second case, 1 aliquot of medium will be incubated in the bacteria incubator room (room 4.9) (so as not to endanger the cultures of other users).
- When a cell culture plate / flask is contaminated, it should be noted in the contamination log and should be disposed of:
  - The contaminated plate must be taken to a BioIIA hood, minimizing its opening and adding decontaminating product to the plate (bleach / Acril).
  - The decontamination of the product must be carried out for 10 minutes to ensure its correct decontamination.
  - The neutralized liquid must be aspirated with the vacuum system or placed in a hermetically closed tube.
  - The plate / bottle must be rejected in a bio-waste container.

If the experiment is irreplaceable, the user can try to change the medium, change all the reagents, and add specific antibiotics to try to save the culture without jeopardizing the cultures of other users. The culture must be sealed with Parafilm®.

The cultures contaminated with mycoplasma in the Lines laboratories should be discarded or transferred to the primary laboratories.

10. MYCOPLASM CONTROL:

- Mycoplasma is a bacterium that belongs to the Mollicutes family that includes more than 180 different species, but in cell cultures 95% of these are *M.orale, M.arginii, M.fermentans, M.salivarum, M. hyorhinis and A.laidlawii.* It is the smallest self-replicating organism with a measurement of between 0.2-0.8 µm. It does not have a cell wall and is usually attached to the surface of the cell membrane of other organisms, taking advantage of its hosts to absorb nutrients. In nature it is found as a parasite in humans, mammals, reptiles, insects, and plants.
- Mycoplasma grows little by little, it does not kill cells, but affects different cellular parameters such as an increase in the sensitivity of apoptosis inducers, chromosomal aberrations, disruption of DNA synthesis, alterations in the efficiency of transfections, inhibition of the cell growth, etc.

To guarantee that the line rooms are free of Mycoplasma, periodic controls are carried out in which the lines with which the users work are tested. In order to carry them out, users must provide us with samples following this protocol:

- Collect 500-1000µl of cell culture supernatant that has been in contact with the cells for at least 48h (preferably 72h) in a 1.5ml eppendorf that closes properly and withstands temperatures of 95ºC. Samples infected with active viruses will not be accepted. Check that the tubes are well marked with the user's name and the name of the sample.
- Freeze the sample and place it in the culture freezer in room -1.3
- Fill in the application form and delivered via email or downloaded from page. IRBLleida website (https://www.irblleida.org/ca/serveis-cientifico-tecnics/cultius-celulars/). And send via email:
  - line name; cell type; origin (stocks, maintenance, ...);
  - Name of the person and group;
  - Pick up date.
- PCR analysis is performed once a week.

- The result will be made known by e-mail. If the result is positive and if the user does not choose to destroy the cells, the service technicians are authorized to demand the transfer of the cell line to the appropriate primary room.

11. INSTRUCTIONS IN CASE OF FAILURES AND INCIDENTS:

- Any incident that occurs, the person who finds it must assess its urgency, write it down in the incident record and report it to the supervisors of the service laboratory, in person, by email, internal phone call No. 2953, 3758 or on mobile 12953 or 664340756.
- An incident is considered urgent resolution when failure to act puts the work of the users or the viability of the cells in the incubator at risk. Laboratory technicians must take appropriate action and monitor resolution.
- When the incident is urgent and the user cannot find a service technician, it must be analyzed what action could correct the incident and carry it out, examples:

Incidence	**Performance**
One of the incubators is damaged	Move all plates from all users to other properly functioning incubators.
All incubators in a single lab are off, or have a CO2 fault	Try to reset the electrical system (control box on the sink side). If this is not solved, move all plates to another incubator in another room.
All incubators in the building are off, or have a CO2 fault	Seal the incubators and prevent anyone from opening them to maintain their parameters.
Other equipment (bath, centrifuge, microscope, ...) fails	Users can exceptionally use the equipment in the other rooms, with the exception of room 3.16, and always advising the cultivation service technicians
Electrical failure in the room or building.	Urgent incidence in the OTI via web http://www.udl.cat/ca/serveis/oti/formulari/salut/

12. GENERAL INSTRUCTIONS IN CASE OF ACCIDENT:

- Handler accident with biohazard agents:

An accident can be a spill of biohazard material affecting a person superficially or with injuries.

Clothing that has been contaminated should be removed and placed in a plastic bag. They should be washed with bleach or similar and at a high temperature. Depending on the severity of the agent, the contaminated clothing will be thrown into the biological container.

Wash the skin and / or wound well with tap water (and soap, depending on the severity of the injury).

Disinfect the affected area with 70% ethanol.

If it is an open wound, apply povidone iodine (Betadine) and cover the wound. Depending on the severity, the injured user should go to the appropriate emergency hospital.

SPILLS

In the event of a liquid spill with biological material, follow the protocol that you will find in the Spill Kit present in the room:

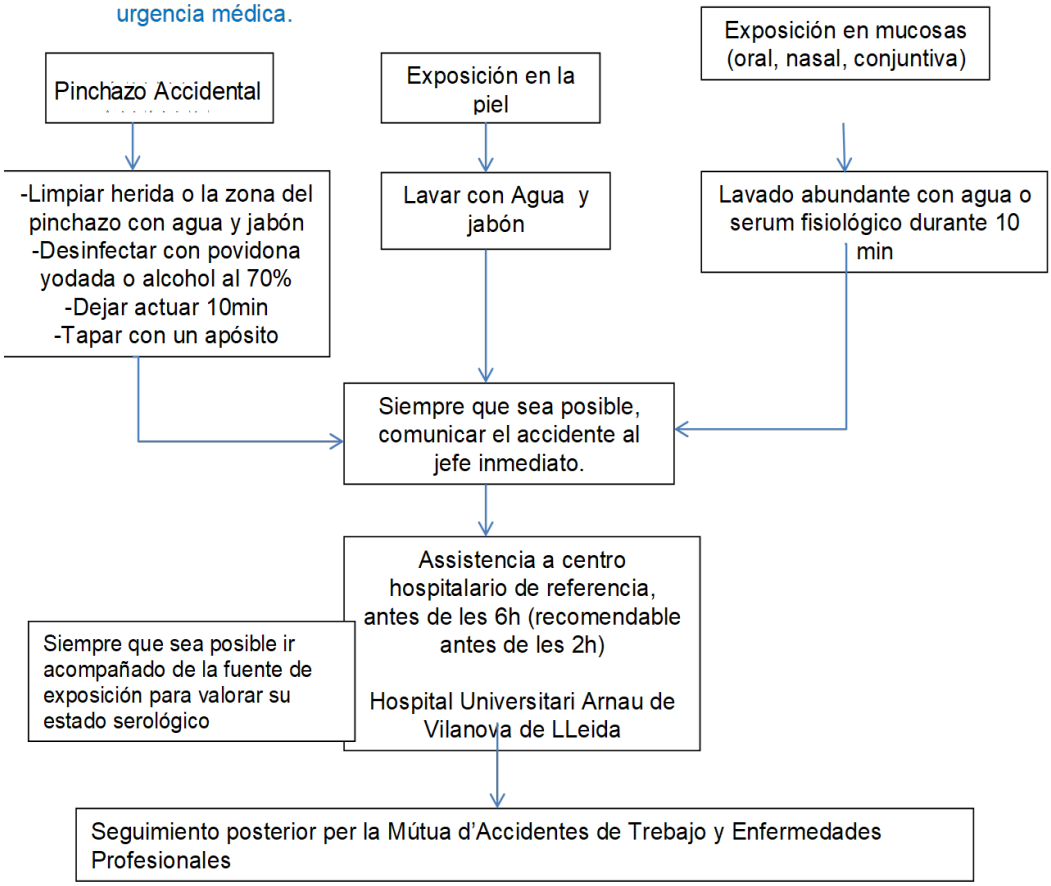
1. Cover the liquid with absorbent paper or vermiculite and immediately neutralize with concentrated bleach for 10-15 minutes.
2. Throw absorbent material into bio-waste containers.
3. Disinfect surfaces with Ethanolized.

Accidents with biohazard material must always be reported to the IP responsible for the researcher and the medical service to follow the traceability of a possible disease.

- **GENERAL NORM**: Most of the risk agents handled are inactivated with sodium hypochlorite (depending on the load of organic matter contained in the sample). In addition, depending on the agent, they are also inactivated with alcohol or soap. In consecuense:
- ON SURFACES: apply bleach, leave to act for 10-15 min. and dry with filter paper that will be thrown into the biological material container later, disinfect with 70% ethanol.
- ON NON-RESISTANT SKIN, CLOTHING OR SURFACES:
    - Remove clothes first.
    - Wash under running water without rubbing the skin.
    - Apply hand soap and rinse for a long time under running water.
    - Disinfect with ethanol at 70%.
    - If it is an open wound, wash with soap and water and then apply iodine solution and dressing.
- FINALLY:
    - IF NECESSARY, CALL EMERGENCIES (tel: 112) and / or GO TO THE INDICATED MUTUAL DEPENDING ON THE REGISTRATION TO THE IRB OR UdL
    - NOTIFY THOSE RESPONSIBLE FOR THE SCT AND COMPLETE AN ACCIDENT NOTIFICATION FORM.

ACTUACIÓN (Qué Hacer?)

Una exposición a sangre o a cualquier otro material biológico de riesgo es una urgencia médica.



PAS, PDI : http://www.prevencio.udl.cat/export/sites/Sprl/ca/.galleries/Accident/Que-fer-cas-accident-PAS-PDI.pdf

ESTUDIANTES : http://www.prevencio.udl.cat/export/sites/Sprl/ca/.galleries/Accident/Que-fer-cas-accident-PAS-PDI.pdf

IRBLleida: http://www.quironprevencio.com/es

13. TRANSPORTATION OF BIOLOGICAL RISK SAMPLES

The transport of biohazard samples is the route that the samples take from the place of origin to the IRBLleida facilities for handling. The transfer of biohazard samples to the different rooms of the building should also follow these strategies following the UN3373 standard:

The samples must be kept in a leak-proof primary container and properly labeled in relation to its content.

This primary container covered with absorbent material to collect possible spills, must go inside a secondary container "protective", that is, robust, watertight, leak-proof and resistant to chemical disinfectants and / or blows.

In the case of regulated transport (between centers), a tertiary container that complies with the regulations is necessary according to the type of biological material being transported (UN3373 Cat B / UN2814 Cat A) correctly identified with the sample it contains in accordance with the regulations transport of biohazard samples.

In liquid samples:

Disposable hermetic tubes or bottles containing the sample inside plastic boxes (eg cryoboxes) that allow keeping the tubes in a vertical position. These boxes, in turn, must be covered with absorbent paper and inside large, well-closed plastic containers.

In solid samples:

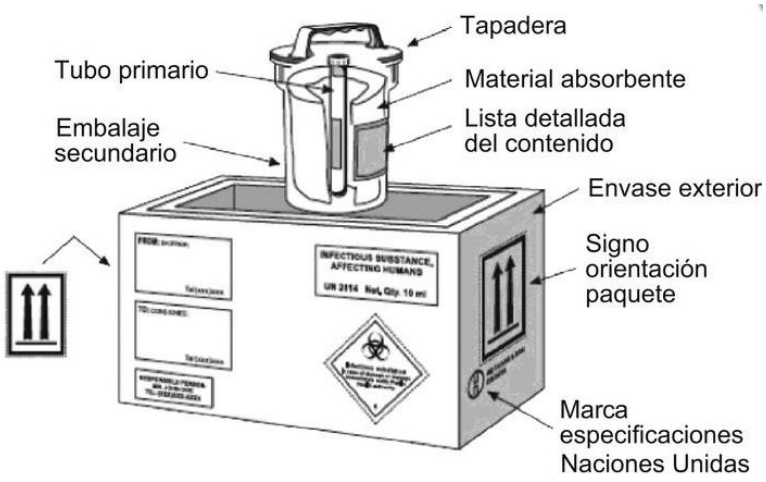
Disposable hermetically sealed plastic bottles or containers with the sample inside, inside watertight plastic boxes, covered with absorbent paper and inside large and well-closed plastic containers that follow the regulations for the transport of biohazard substances.

Tubs o flascons hermètics i contenidors: Falcon tubs de 15-50mL, tubs d'extracció de sang ...

Contenidors Primaris:

Contenidors secundaris:

Regulated shipping:



13.1 Example of how to transport lentivirus correctly:

The viral particles that are present in the culture medium are collected in 15 or 50 ml tubes. These tubes must be hermetically sealed and will be transported in a second airtight container made of impact resistant plastic and with absorbent paper. This minimizes the risk of dropping and breaking the virus tube.

The tubes are transported to a heated room for freezers (room 3.14). The room will be restricted with card access for authorized personnel. A -80ºC freezer is properly marked and is where the virus tubes will be placed. These tubes must be well labeled as defective or non-replicative lentiviruses, the date of production, the name of the inserted gene, the species of origin, and the name of the producing researcher.

For use, the tubes are collected from the -80ºC freezer and transported again to a second hermetic plastic container with absorbent paper to a Biosafety II-A cabinet, where the cells to be studied will be infected at containment level 2.

14. 3.16 LABORATORY

To be able to enter to the 3.16 lab, a specific training given by the SCT-CC is required. Previously it has to be given, also by the SCT-CC, the general training about culture.

In order to work in the 3.16 lab, the protocol has to be approved by the Biosecurity Committee (comitebioseguretat@irblleida.cat), who will inform the SCT-CC. The template that has to be sent to the Committee can be found in the newt webpage: https://www.irblleida.org/ca/sobre-nosaltres/comites-i-comissions/

15. SANCTIONS

The UdL SCT-CC wants to implement a system of faults and penalties to ensure compliance with existing working rules in the rooms managed by the SCT-CC. SCT-CC gives specific eye training to all users according to the level of biocontention they require. Users perform an exam that accredits them to be users of any room (except 3.16) and receive a "SCT-CC work training accreditation". In the case of room 3.16 users (Biocontention 2+), a specific formation is performed followed by the signature of a "declaration of responsibility". Based on the above, it is considered necessary to implement a system of offences and sanctions that ensure compliance with the rules and the safety of users, SCT-CC personnel, service personnel present in Biomedicine and general population.

These sanctions will be previously arranged by the Research Comission and Government Board.

16. CITATION IN PUBLICATIONS

In the publications, doctoral theses or final Master's works obtained through the use of the service, a sentence will be included in the acknowledgments and it will be communicated to the service. As an example:

The human sample manipulation were performed in the Cell Culture Sientific & technical Service, Universitat de Lleida, Lleida, Catalonia, Spain, or The cell culture experiments were performed in the Cell Culture Sientific & technical Service from Universitat de Lleida, Lleida, Catalonia, Spain .

17. RATES

The prices to use the facilities of the Service are determined by the scientific-technical cell culture service approved by the UdL Research Commission and the Governing Council annually. They can be consulted at:

http://www.udl.cat/export/sites/universitat-lleida/ca/recercaNew/serveis-cientific tecnics / .galleries /2021/2021-LABORATORIO-DE-CULTIUS-CELLULARS.pdf

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ANNEX 1. Maintenance tasks for equipment and laboratories

- Laminar flow booths should be thoroughly sanitized at least once a year or whenever there are spills. These undergo an annual check of the flow and the level of particulate. Its surface and walls should be cleaned and disinfected with alcohol or Virkon each time they are used. The lower part of the cabins will be cleaned once every 15 days.
- The hypoxia chamber is reviewed once a year. Oxygen should be calibrated four times per year.
- Culture labs should have a daily basic cleaning, as well as a more thorough cleaning once or twice a year to minimize the accumulation of dust and other particles.
- Liquid nitrogen tanks should be periodically checked ensuring a liquid phase (min 15-20cm) and a vapor phase inside the tanks where the cells are kept. These will be filled as needed.
- The levels of CO2, N2 and synthetic air gases should be checked periodically to ensure supply to the incubators and the hypoxia chamber.
- Incubators they must receive CO2 at a pressure of 0.8-1.5 bar. They must be thoroughly disinfected (filter change, disassembly ...) once a quarter or as needed. During the rest of the year, the base, the doors and the water tray should be cleaned periodically.
- Thermostatic baths should be periodically cleaned with soap and water and filled to cover the resistance with distilled water. The bath head, if the alarm sounds it may be due to overheating, you have to add more water to the bath and reset the head (small button on the back which must be accessed with a pen tip). Algaecide and fungicide product can be added to the water to extend its maintenance.
- Mercury microscopis lights, magnifying glasses and fluorescence lights should be changed when they burn out or when they have exceeded their maximum number of hours of life. At this point, the microscopes should be checked and the new lights are properly centered.
- Microscopes need to be dusted periodically following the maintenance protocol.
- Biological and cytotoxic waste in the containers must be closed when they reach 75% capacity and taken to the corresponding waste room where they will be managed with the contracted company.
- For any incident or breakdown, you should contact the technical service of the specific device.