

	DOCUMENTS SCT-CC			
	GUIDELINES FOR WORKING IN CELL CULTURE LABORATORIES			
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1. OBJECTIVE:

The main objective of the guideline is to promulgate the SCT-CC laboratories structure and characteristics, the basic work rules and equipment usage, as well as to know the technicians to whom address for notifying breakdowns, contamination, accidents or suggestions.

2. SCOPE OF APPLICATION:

UdL-IRBLleida cell cultures laboratory users.

3. INTRODUCTION

The Cell Culture Service (SCT-CC) is a type II service that belongs to the Department of Basic Medical Sciences (CMB) from the UdL and serves all users from different departments from the University and to external users.

Mammalian cell culture is the process or set of techniques, that allow the growth of tissue fragments of different mammal species in a “*in vitro*” environment to examine and manipulate cell behaviour, keeping up their properties (physiological, metabolic, biochemical, genetic, etc.).



Cell culture laboratories are working with growing cells coming from immortalized cell lines or primary cell cultures (tissue obtained from murine stable, human tissue...).

The main feature that defines the cell culture laboratory, is the **maintenance of asepsis**. This is because of the growing rate of cultured cells is much lower than usual contaminating microorganisms (fungi, yeasts and bacteria). Therefore, for the maintenance of the cell culture is vital to avoid the appearance of any unwanted microorganism.

The SCT-CC is located into **Biomedicine (IRBLleida)** building and it has seven cell culture laboratories and one **cellular cryopreservation zone** with liquid nitrogen tanks.

1. **Lab -1.3:** Cell culture lines Lab
2. **Lab 1.9:** Cell culture lines Lab
3. **Lab 2.9:** Primary Cell culture Lab
4. **Lab 2.16:** Cell culture lab to work with delicate cells (stem cells) or long time cultures free of mycoplasma and viruses.
5. **Lab 3.9:** Primary Cell culture Lab
6. **Lab 3.16:** Cell lab to culture primary human samples and/or lentivirus production
7. **Lab 4.11:** Dissection room

The SCT coordinators and scientific assessors are Judit Ribas and Serafí Cambray. The technicians from the service are Marta Rafel (IRB), as responsible senior technician and Iván Hidalgo (UdL), as qualified support technician.

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4. AUTHORIZED PERSONNEL

Only authorized personnel will be allowed to enter in the cell culture laboratory.

The first day on the cell culture laboratories, the new users will fill up the personal data sheet and the SCT-CC technicians will give an explanation about laboratories and guidelines. Likewise the new users will have to prove to have understood and to accept the regulation of the service through a small test. The service reserves the right of admission to the facilities in case of not complying with the regulations established in it.

4.1. SCT-CC TEAM INFORMATION:

	Name	Localitzation	e-mail	Ext. telph.
Coordinator and	Serafí Cambray	office 1.11 Bio I	scambray@irbllleida.cat	2482
Scientific assessor	Judit Ribas	Lab. b2.2	judit.ribas@mex.udl.cat	2936
Technician Responsible	Marta Rafel	office 2.4 Bio. II	mrafel@irbllleida.cat	2953 12953
Qualified support technician at partial time	Iván Hidalgo	office 2.4 Bio. II	ivan.hidalgo@udl.cat	2953 12953


5. DESCRIPTION OF CELL CULTURES LABORATORIES:

5.1. PRIMARY CULTURES LABORATORY:

Designed to work with primary cultures and/or cells infected with mycoplasma or not tested.


5.2. CELL LINES LABORATORY:

Designed to work with stable cell lines *Mycoplasma*-free.

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6. GENERAL LABORATORI SAFETY PROCEDEURES AND RULES

1. Is forbidden to eat, drink, and smoke; also to chew gum, to make up or handle contact lenses, store food or beverages in the laboratory.
2. The lab should be maintained tidy, clean and free of materials not related to the work.
3. Users must be wearing clean long sleeves lab coat to go inside the room, and to use it only for tissue culture labs.
4. Working surfaces must be disinfected with ethanol or fagetrial (taking in consideration the safety procedure) at the beginning and at the end of each usages, and after all potentially dangerous material spills (environment, cells, viruses ...).
5. Laminar hoods users must spray with ethanol sleeves and hands (if they are not wearing gloves).
6. No notebooks, calculator, papers or cardboards boxes should be placed inside the hood; they could be a focus of contamination.
7. Users **must wear gloves in all cases of cell manipulation**. In case of working with potential risk material in BioIIA hood, users must wear double gloves.
8. The gloves will be thrown in an aseptic manner once they are used, and after the user will proceed to clean his/her hands. Never will leave the lab wearing dirty gloves.
9. Be care to don't touch surfaces of the lab neither devices with used gloves, even if they are not contaminated.
10. At the end of the task, remove and tidy up all material used from inside the hood and let it clean and disinfected.
11. While no user is working in the hoods, they **should be empty**. To avoid risks from BioIIA hood residues, they can contain the yellow container, the water-soap soaked pot for long pipettes, and the vacuum dispenser bottle.
12. All **contaminated material**, samples and cultures must be **decontaminated before to throw** them away. In the case of cell cultures, they will be neutralize with bleach and the resulting media will be aspirate. The empty plate will be thrown within the solid contaminated residues.
13. The **solid contaminated residues** will be thrown into the **black containers** (under each hood).
14. The Residues containing some **cytotoxic or drug substance** should be thrown into the **blue container** (one in each lab).
15. DO NOT throw clean solid residues (plastic envelopes, paper, etc.) into the **black containers**. YOU CAN **RECICLATE CLEAN PLASTIC; PAPER, POREXPAN** into adequate containers available in the corridors.
16. If any needed container is full, any user can close it properly and to take a new one from the room. If what is full are the vacuum dispenser bottle, first neutralize the liquid with bleach and empty it into the liquid cytotoxic residues garraf (close to the sink).
17. **BioIIA residues**: throw the tips, syringes, pasteurs and small stuff into the yellow container. Aspirate all liquids in the vacuum dispenser bottle. Let the pipettes inside the water-soap soaked pot.

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18. Once the work in the hood is finished, clean the vacuum tube first with bleach and after with a bit of ethanol until the liquid has been neutralized. Finally **switch off the vacuum** to avoid extra consume of the general pump.
19. **Bath:** Do not throw dirty liquids into the bath. If something spill-in contact any person from the service.
Switch off the bath once nobody is using it. If it rest on and the resistance goes out of the water it can burn and to produce fire.
20. Never bring material and cells from primary cell lab to lines lab. It could be a source of contamination for the clean lines.

7. OPERATING INSTRUCTIONS OF THE DEVICES:

The basic instructions to follow for each device are the following:

7.1 LAMINAR FLOW HOOD

a. Definition:

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

Depending on the type of hood it will protect:

- Personnel of harmful agents.
- The product from different contaminants.
- The environment (external to the hood) of contaminating products.

b. Hood types:

Horizontal laminar flow* hoods are very suitable for good protection of the product, but not for the manipulator. It should be taken into account that the distribution of the material in the horizontal flow hood must be different than the vertical flow hood, since the front-line material is not as protected as the one in the bottom.

Vertical laminar flow* hoods ensure good protection of the product, and, according to its design, also a partial protection of the manipulator.

The biosafety class II cabinet protects the product, the manipulator and the environment. In those of type A, 30% of the air is eliminated in each cycle and 70% is recirculated. It is the most suitable for work with particle risk pathogenic agents 2 (biological agents that can be a cause of human illnesses and could become a danger for workers. They rarely cause infections. It is unlikely that they will spread to the community and usually it exist prophylaxis or effective treatments).

* Laminar hoods are not considerate as a Biosafety cabinet. Biosafety cabinate type I are those which protect the user but not the sterility of the product (chemical/smoke hoods).



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		CLASE I	CLASE II TIPO A	CLASE II TIPO B	CLASE III
AGENTES BIOLÓGICOS	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

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.

7.1.1. General rules:

Before starting to work:


1. Book the hood for the time required to perform the experiment in the **registry paper** (P-CC-04) attached to each hood; it is mandatory to reserve the same day (in case of specific necessity speak with the technicians).
2. Clean and disinfect the hood with the appropriate disinfectant:

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- Ethanol at 70% (immediate effect, it must be wiped below. **Do not use in hoods with methacrylate front**, because it causes opacity).
 - Fagetriald 0.5% (leave to act **10 minutes** before to dry it). **ATTENTION:** contain aldehydes (like formaldehydes): High cleaning and disinfection product. Good vs. virus and spores. It works in all kind of material. Apply it in the absence of people because produce toxic vapours.
3. Place all the fungible material to use inside without stacking it, allowing the UV to reach everywhere (previously we will spray it with ethanol, especially in the area of the boards). **WARNING:** The UV of the hood is UVC, high energy, germicides. Avoid contact with eyes and skin.
 4. Switch on the UVs and the flow for about 5-10 minutes before starting to work.
 5. Switch off the UV and switch on the light to begin to work.

Meanwhile you are working in the hood:

 1. Work with lab coat and gloves (spray sleeves with ethanol before starting).
 2. Put all the material as inside the hood as possible: when working, the ventilation grilles must be free.
 3. Never pass your arms over the sample.
 4. Avoid leaving the bottles and tubes caps on the surface of the hood (put them and remove each time holding them with your hand). If it is necessary to leave the cap on the surface, leave it face up and away of the working area, to avoid to pass the arm over.
 5. Be careful not to touch with the gloves the sterile material that will come in contact with the cells. In case of doubt, discard the material or identify the plate and trace it.
 6. Open the disposable material in the hood. If the sterile material accidentally contacts with non-sterile material, do not return it within the culture medium or buffers. It is recommended to change the pipette each time instead of leave it inside the bottle.
 7. To aspirate the liquids, switch on the vacuum and connect a Pasteur pipette to the tube (if you have to aspirate different liquids it is good to add a yellow pipette tip and change it each time).
 8. Throw the material that has come in contact with the biological or cytotoxic sample into the black/cytotoxic container.
 9. Discard the rest clean material (PLASTIC, CARTON, PAPER, POREXPAN, etc. in the recycling bins.
 10. Before throwing the cells, kill them with bleach, then aspirate the liquid and discard the plate into the black container. Do not throw contaminated liquids or cytotoxic in the sink.
 11. Do not move plates with cultures from one laboratory to another, except major necessity.

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To finish working:

- Collect and disinfect with 70% ethanol all the material used, make sure that the surface of the hood is clean of solid and liquid waste and let it empty.
- Throw the Pasteur pipette into the black/blue waste container and disinfect the vacuum tube first with 70% bleach and then 70% ethanol **until there are no remains of the culture medium in the tube, and the liquids waste deposit turns from coloured red-phenol media to neutral (discoloured).**
- Clean the **surface and disinfect it** with ethanol or "fagetrilal" (according to security norms).
- Let UVs running for at least 5-10 minutes (most hoods can be programmed for 20 minutes, by pressing the arrow button in the direction above, once the UV is on).

7.1.2. Bio-II-A:


General activities that are carried out into the hood:

- a. Virus production.
- b. Infection of cell lines or primary cultures.
- c. Primary cultures of human cells (potential transmitters of viric illness)

Work rules:

1. When switching on the UV light, the front cover should not be removed.
2. Work with a long sleeve coat adjusted to the wrists and with double glove.
3. Do not mix with ethanol the interior gloves before to put a second pair (the ethanol may permeabilize them).
4. Throw the contaminated material (tips, eppendorfs, pasteur pipettes, external gloves ...) into the yellow waste container inside the hood.
5. Let the long pipettes to the water-soap soaked glass container inside the hood.
6. Leave the rest of the contaminated material inside the hood spray with ethanol at 70% and switch on UV light about 10 minutes to decontaminate it, and then throw them or tidy them up into the blue/black waste container.
7. Liquids should be aspirate and collected in a container containing **70% bleach**. We will only empty the container when the medium is neutralized and we know that biological samples are neutralized using bleach (red phenol coloured media gets neutralize when its colour turns to uncoloured), therefore:

Medium yellow-white: bleach completely active, we can discard the liquid.

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Orange-pink medium: it is necessary to add more bleach. It is necessary to wait a minimum of 5-10 minutes before to open the vacuum bottle.

8. Therefore, when finished working, the suction tube must be disinfected with **70% bleach** until the liquid in the container is neutralized and then put **70% ethanol** so that there are no remains of bleach in the tube.
9. **To eliminate cells:** put bleach at 70%, let it for 15 minutes, aspirate the liquid, let the plate opened and leave it 10- 15 min under the UV light. Finally, throw the neutralized material in the black container.
10. Biological samples can be removed from the hood once the culture has been lysed or fixed. The outside surface of the tubes, media bottles, plates... must be decontaminated by passing a paper with bleach, drying and then treated with ethanol at 70% before placing them on the centrifuges or any other device, or to take them out of the room.
11. Carefully work preventing spills and aerosol production. Do not keep long pipettes attached to the pipette on top of the work surface to avoid dripping. Take special care when vortexing and centrifuging tubes and eppendorfs, especially if they contain infectious material. Be sure the caps of the tubes are perfectly closed.
12. In the case of spills of contaminant liquids:



Cover the spill with absorbent paper and immediately neutralize it with concentrated bleach for 10-15 minutes. Throw the paper into the container of contaminated waste. Disinfect the surface with 70% ethanol.
13. Spillage in a centrifuge rotor: wait a little bit before opening to avoid breathing the produced aerosols and proceed as the Bio-II-A. Finally, leave the broken material in the hood with the UV for 10-15min.
14. In case of self-spill or accident:

Remove the clothes that could have been contaminated. It will be decontaminated inside the hood with the UV and later, it will be washed with bleach.

Wash the skin or wound with water without rubbing to avoid irritate the skin.

Disinfect with 70% ethanol.

If it is an open wound applied iodine povidone (betadine) and then a gauze dressing.

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7.2 CO₂ INCUBATOR

a. Definition

CO₂ incubator is a camera that maintain the cultures in constant and optimal atmospheric conditions

Optimal Conditions		
T ^a	CO ₂	Humidity
37°C	5%	98%
Cell physiological T ^a	Cellular breathing	To avoid cell water evaporation

To maintain these conditions inside the incubator there is a forced recirculation of air facilitate through the holes of the shelves and a tray with distilled water.


b. Guideline instructions:

1. Use a **tray where to put your plates** to avoid any accidental spill. If an accidental spill affects the incubator:
 1. Absorbed it with bleached paper.
 2. Dry it
 3. Add etoh 70%
 4. Write it to incidence register.
2. Be sure to **close well both doors**, and to minimize the opening to **avoid optimal conditions destabilization**.
3. In case of the **water tray is empty or low**, fill it with **distilled water**
4. **If bacterial/fungal** growing is observed inside the **incubator**, report it to the SCT-CC technicians and write it to **the contamination register**
5. When a plate is **contaminated**, it should be **eliminate** immediately and report it to the **contamination register**
6. When the incubator is under the calibration mode, don't open the door.
7. Never touch the CO₂ manometers or the incubator parameters.
8. Don't open the door of the incubator meanwhile is autocalibrating.

7.3 MICROSCOPE AND STEREOMICROSCOPE

• Types:

- Inverted phase contrast microscopes: Allow the morphological control of the live cells. Objective must be inverted due to the thick surface of the plates. As the sample doesn't have different colours to differentiate between the cells, the microscope must have the phase contrast dispositive increasing like this the quality of the image.

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- Inverted fluorescence microscopes or Stereomicroscope: They contain a source of light that's separate the different long wave allowing to see images marked with different fluorimeters.
- Stereomicroscope: they increase up to 10x the sample to facilitate dissection of the tissue

- **Guideline:**

1. If you need to use the microscopes or Stereomicroscope for long time, remember to book them in advance. You have a register sheet at your disposal. Remember that live cells will have preference even if you have booked the microscope.
2. Microscopes with Hg lamp (lab **3.9and -1.3**)
 - **Beginning and End:** Write your name, date and reading on the UV lamp (P-CC-07).
 - Once the **UV light** is **on**, you must wait to switch it off **15 min** later. Wait **10 min, to switch them on again.**
3. Remember to switch off the light of the microscope when you finish to use it.
4. Don't touch any button you don't know for what it works. Ask to the technicians before.
5. To avoid contamination between users: clean the lens with ethanol 70% and the slide where we place the plate to observe the cells
6. Lab 1.9 and 2.16: Cover the microscopes and the computer screen after 5 P.M. (night UV disinfection protocol damage them).

7.4 CENTRIFUGES

In a cell culture lab a centrifuge is mainly necessary for the precipitation of suspended cells, concentration of buffers or others and culture phase separation.

Guideline:


1. Properly balance the tubes.
2. Accidental spill: before opening, wait for the settlement of aerosols. Clean the inside with a paper impregnated with bleach, and finally spray it with ethanol.
3. If you were preparing virus, proceed as before but leave the bleach to act for 15 min.

7.5 BATH

SCT-CC technicians are regularly checking for the state of the equipment and material of each SCT-CC lab. However, on special occasions, you might find

The water levels are low...

- ▶ How to proceed?
Add distilled water to reach the min level (preventing the motor to burn).
- ▶ Last person to use it is responsible of switching off the bath!

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7.6 LIQUID NITROGEN TANK

Tank containing liquid Nitrogen (-196°C). It is used to store cell lines for long time

- ▶ **IMPORTANT:** use **gloves, long sleeve lab coat, eye protection, and closed shoes** for manipulation.
- ▶ Each group has specific boxes assigned.
- ▶ To get extra space, contact with the SCT-CC members.
- ▶ Don't let the tank cover opened to long time to avoid N₂ (l) evaporation, cause displaces oxygen.

7.7 HYPOXIA CHAMBER

The hypoxia chamber is a cabinet for cell culture that require the control of oxygen, carbon dioxide, temperature and humidity.

The workstation is a sealed chamber where internal temperature, humidity and gas concentration can be regulated, controlled and maintained. And where samples can be introduced, manipulated, incubated, examined and removed without loss of these environmental conditions.

Before using it for first time, you must get in contact with the SCT-CC personal technicians.

8. Cell Culture contaminations

One of the main way to contaminate the plates in the lab is through the own user.


In a cell culture lab is impossible to work completely free of microorganisms, but to minimize the impact is important to take in consideration the source of the contamination.

Most of the time, the cultures are contaminated with microorganisms coming from the user hands or mouth. This is why is very important to use gloves and to clean well the hands with soap each working time, as well as to disinfect all material and sleeves with ethanol.

Another big source of contamination is the clothes. To wear a clean lab coat before to work in the lab and to use different street shoes reduce a lot the number of microorganisms in the room. The wool jumpers inside the hood are a source of contamination.

To avoid contaminations:

1. Wear a clean lab coat, work with sterile gloves and avoid hands contact with any material that has to touch the culture.
2. Never pass your hands, arms... over the opened culture, plate, tips, pipettes, tubs, media, etc. and work centric without blocking the holes of the hood
3. Once you are working inside the hood, don't touch the telephone mobile or any surface of the lab, to scratch the hair, nose, or any other part of the body without removing or changing your gloves.
4. The hood surface must be as empty as possible, with the minimum material to work and avoiding porous material as carton boxes.
5. The UV light should be able to pass between all the objects present inside the hood, and the laminar flow should not have material producing turbulence.

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6. Aerosols produced within the working time with media or buffers can spread contaminations, so is important to use one media for each type of culture.
7. It is high recommended to work in the hood with one cell line per time.
8. When hot media, buffers or cells tubes are removed from the bath, they should be spray with ethanol and to dry the extra liquid before to put it inside the hood.
9. The external part of the plates or flasks are sterile at the first time, but once we move them to the incubator they are not sterile, so it is important to maintain them as clean as possible, using a try to move them from the hood to the incubator and also to use gloves or ethanolized hands in case of necessity to touch the plates or flasks.
10. Another prevention to take is to disinfect the platina of the microscope when there is necessity to observe the cells in there.
11. Take the plate delicately and softly to avoid spills of the interior media.

9. INSTRUCTIONS IN CASE OF CONTAMINATION:


- If your cell culture is contaminated, write it down to the **contamination register (P-CC-10)**.
- Elimination of the culture:
 1. Put 70% bleach on the plate.
 2. Leave to act 10 min.
 3. Aspire the liquid with the vacuum.
 4. Throw the plate in the black waste container.

10. Mycoplasma CONTROL ON LINES:

- Mycoplasma is a bacteria that belongs to the *Mollicute* family and includes more than 180 species, in cell cultures 95% of these are *M. orale*, *M. arginii*, *M. fermentans*, *M. salivarum*, *M. hyorhina* and *A. laidlawii*. It is the smallest self-serving organism with a size between 0.2-0.8 μm . It has no cell wall and is usually attached to the surface of the cell membrane of other organisms, taking advantage of its hosts to absorb their nutrients. In nature it is found as a parasite of humans, mammals, reptiles, insects and plants.
- Mycoplasma growth is slow, it does not kill the cells but it affects different cellular parameters such as an increase in the sensitivity of apoptosis inducers, chromosome aberrations, disruption in DNA synthesis, alterations in efficiency of the transfection, inhibition of the cellular growth and others.

In order to ensure that the line labs are free of *Mycoplasma*, periodic checks are carried out in which the lines of the users work are tested. In order to carry them out, users should provide us with samples following the following protocol:

- Collect the cell culture supernatant that has been at least 48h in contact with the cells (preferably 72h). No samples infected with viruses will be accepted. Mark well the tube to identify the user.

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- Fill the **application form (P-CC-2)** delivered via e-mail or available in the web site of IRBLleida (<http://www.irbllleida.org/es/servicios-cientifico-tecnicos/cultivos-celulares/>) and send it to us via e-mail.
 - Name line
 - Cell type
 - Origin (stocks, maintenance, ...)
 - Name of the person and group
 - Collection date
- PCR analysis will be performed once a week.
- The result will be send via e-mail. If the result is positive and in case the user does not choose to destroy the cells, the SCC technicians are authorized to demand the transfer of the cell line to the appropriate room.


11. INSTRUCTIONS IN CASE OF INCIDENT:

- If any incident occurs, write it down to the **incident register (P-CC-09)**, send an email or call **12953 or 664340756**.
- If it is an **urgent** resolution incident, communicate it directly to the laboratory techniques or to the person in charge of the SCT via ext. telephone or via telephone of incidents 12953 or 664340756.
- The laboratory technicians will take the appropriate measures and will follow the resolution.

If you are alone and you see you can do something to avoid a major lost do it, for ex: an incubator break down, move carefully the plates of all users to another one that works properly.

12. GENERAL INSTRUCTIONS IN CASE OF ACCIDENT:

- **GENERAL RULES:** Most of the manipulated risk agents (most of virus, bacterial or fungi) are inactivated with bleach. In addition, depending on the agent they are also inactivated with alcohol or soap. Consequently:
- **SURFACE DOMAIN:** apply bleach, let act 10-15 min. and wipe with filter paper that will be thrown into the biosecurity container (blue/black), and then disinfected with 70% ethanol.
 - **ON THE SKIN, CLOTHING OR NON-RESISTANT SURFACES:**
 - First remove your clothes.
 - Wash with water without rubbing the skin.
 - Apply soap and rinse thoroughly with water.
 - Disinfect with 70% ethanol.
 - If it is an open wound, wash with soap and water and then apply an iodized solution and filling it.

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• **FINALLY:**

IF IS NECESSARY, CALL THE EMERGENCY SERVICES (PHONE: 112) AND / OR GO TO THE MUTUAL INSURANCE INDICATED ACCORDING TO ADSCRIPTION TO IRBLleida OR UdL.

NOTIFY to THE RESPONSIBLE FOR THE SCT-CC AND FILL AN ACCIDENT NOTIFICATION FORM.

13. ANNEX I. Tasks of maintenance of devices and laboratories

- **Laminar / horizontal flow hood** should be disinfected thoroughly once a year. These pass an annual review of the flow and level of particles. Its surface and walls should be cleaned and disinfected with alcohol every time it is used.
- The **hypoxia hood** passes a review once every year. Oxygen should be calibrated once a trimester.
- **Laboratories of cell culture** must have a daily basic cleaning, as well as a deeper one or two times per year to minimize the accumulation of dust and other particles.
- **Liquid nitrogen tanks** must be periodically checked by ensuring a liquid phase and a steam inside the tanks where the cells are stored. These will be resumed according to the needs.
- The levels of CO₂, O₂, N₂, and synthetic air should be periodically checked to ensure the supply of incubators and the hypoxia camera.
- The **incubator** must receive CO₂ at a pressure of 0.8-1.5 bars. It must be disinfected thoroughly (change of filters, disassembly ...) once or twice a year or depending on the need. During the rest of the year, the base, doors and water tray must be cleaned periodically.
- The **bath** should be cleaned periodically with soap and water and fill it to cover the resistance with distilled water. The head of the **bath** if the alarm rings may be cause it is overheating, more water should be added to the bath and *reset* the head (small button in the back, access with a pen tip).
- **Microscope lamps**, stereomicroscope, and fluorescence lamps must be changed when they are melted or when they have exceeded their maximum number of hours of life. At that time, the microscopes must be checked and check that the new lamps are centred.
- Any incident or failure must be contacted with the technical service of the specific device.