# PCC L2 Laboratories Usage Rules - Version 01

The only persons authorized to work in the L2 lab are those who have been trained by the persons in charge and who have read and sign the present document.

The training in good cell culture practices and the signature of this document lead to the registration on the list of authorized personnel giving access to the cell culture platform and to the booking schedule on GRR (http://grrluminy.marseille.inserm.fr/login.php).

Failure to comply with the rules set out in this document may result in suspension of access to L2 laboratories after warning.

**General instructions:**

- Reservation of MSS on GRR (http://grrluminy.marseille.inserm.fr/login.php) is mandatory and will be invoiced in proportion to the reserved time.

- Users are required to respect their booking slots and to extend them on GRR if required.

- Users are responsible for their PCC access badge and must not lend it to any not authorized user.

- The PCC access door (fire door) must remain closed.

- In case of fire, a visual signal replaces the siren to alert you.

- Any stock problems should be reported to the PCC manager.

- Any material failure, whatever its nature (pump failure, defective or broken pipette, etc...) in a L2 laboratory, must be reported to the person in charge.

- It is mandatory to wear a lab coat (marked with name and date), gloves and overshoes (available in the airlock) in L2 labs.

- People with long hair must tie them.

- The use of mobile phones is forbidden in L2 Labs.

- Culture flasks, plates and Petri dishes, media, etc... must be identified with the initials of the user, the unit to which they belong and the date.

- When leaving the L2 lab, switch off all devices (centrifuges, water bath, microscope, vacuum pumps...) and close the MSS

- DON'T FORGET to switch off the lights!

# Index

# Entry and exit of personnel and equipment

**ENTRY**

Before entering, think of bringing all the equipment you require to perform your experiment.

- Enter in the airlock
- Close the outer door of the airlock before opening another one!
- Clean with 70% alcohol any product coming from the outside
- Tie back long hair
- Put on lab coat (marked with your name, monthly changed)
- Put on overshoes
- Put on gloves
- Put hydroalcoholic gel on gloves
- Enter in the L2 lab
- Close tightly the door behind you

**EXIT**

- Close the MSS and switch off the pump
- Switch off and cover the microscope
- Switch off the centrifuge
- Switch off the water bath
- Leave the L2 lab
- Enter the airlock
- Close the airlock door
- First remove the overshoes and secondly the gloves
- Take off the lab coat and hang it on the coat hanger
- Wash your hands with hydroalcoholic gel
- Exit the airlock
- Don't forget to switch off the lab and the airlock(s)!

# Use of type II MSS

To know: A MSS (Microbiological Safety Station) protects both the handler and the handling only if a few simple rules are respected:

- The enclosure is sterile only once the volume of air in the enclosure has passed through the filter of the MSS: switch on the MSS and wait for the alarm to stop!

- The front edge of the work tray prevents air from escaping or entering the chamber provided if it is not obstructed: do not place any material at this level,

- The flow goes from top to bottom: do not pass any object over an unclosed container,

- The flow must be disrupted as little as possible: only store the necessary material on the work surface (the flow is less disturbed if the objects are spread rather than stacked),

- If the equipment used is not regularly cleaned, being stored under an MSS will not change anything... => Remember to clean the pipettes and racks regularly with 70% alcohol.

Procedure:

- Switch on the MSS and wait for the alarm to stop.
- Clean the work surface with 70% ethanol.
- Bring together the material necessary for handling (flasks, plates, pipettes, media...)
- Install only the material required for handling under the MSS:
    - Do not overload the MSS
    - Clean the media bottles, tubes coming out from water bath, micropipettes... with 70% ethanol
    - Avoid to stack materials (boxes of tips for example, flasks...) since stacks may disturb the flow or at least avoid working close to the stacks
    - Do not obstruct the front edge of the work tray
    - Do not use expanded polystyrene boxes (risk of contamination, porous and crumbly materials). Use easy-to-clean or autoclavable racks

=> During handling :

- Used Pasteur pipettes and tips are placed in the sharpsafe boxes (yellow rigid plastic box) provided for this purpose under the MSS.
- Used pipettes and others wastes are placed in the yellow “DASRI” cardboard container

=> After each manipulation:

- Rinse the pump pipes first with bleach, secondly with water.
- Switch off the pump
- Empty the waste bottle from the aspiration system (see liquid waste disposal). Before reconnecting the bottle to the aspiration system, put 2 tablets of bleach into the bottle (only for glass bottle)
- Put away the material
- Clean the working surface (70% Ethanol)
- Replace the empty Pasteur pipette boxes and empty Eppendorf tube jars. Return the empty ones to the laundry on the 4th floor for refill.
- Seal the full sharpsafe boxes containing used Pasteur pipettes and tips in the “DASRI” waste bin and put new ones under the MSS.

# Waste disposal

## SOLID WASTES

Sharps waste

- Sharp objects (needles, scalpel blades, tips, Pasteur pipettes etc) are collected in the containers provided for this purpose (yellow rigid plastic sharpsafe boxes).
- Once the container is full, close it tightly.
- Place the hermetically sealed container in the “DASRI” waste bin (yellow card)
- Put an empty container

Petri dishes, culture plates and flasks

- Aspirate the culture medium
- Fill the Petri dish, plate, or flask with a small amount of bleach
- Place them on the bench in the area provided for this purpose
- After at least 24 hours, eliminate the bleach by vacuuming and throw the containers in the “DASRI” yellow cardboard box.

  Reminder: Users have to take care of their own flasks after the end of the decontamination process

Other waste products (gloves, paper, pipette packaging, etc...)

- Gloves, paper, pipette packaging, etc... will be deposited in the “DASRI” yellow cardboard bin.
- EPI (dirty or contaminated over-shoes, gloves and lab coat) are thrown in yellow plastic DASRI bag (not cardboard) located into the airlock
- Packing boxes have to be folded before being thrown away.

Disposal of DASRI

When the “DASRI” bins are full, close them, write "PCC + date" and throw them away according to your unit's procedure.

## LIQUID WASTE:

Liquid from the bottles of aspiration systems:

The inactivated liquid is transferred into the provided container in the laundry outside the L2 Lab

Blood treatment:

Incoming procedure...

# L2 Lab maintenance

The weekly cleaning of L2 labs is charged to the users in proportion to the time worked in the lab.

**Procedure:**

- Reservation on GRR (select "Cleaning")
- Reserve on GRR all the MSSs of the L2 lab you clean:
    - You need 2H00 for the big L2
    - You need 1H30 for small L2

Replenishment of stocks :

- In L2 lab *(non-exhaustive list):*
    - Plastics :
        - In the shared cabinet and at each workstation: Tubes (15 and 50ml) - Pipettes (1, 2, 5, 10 and 25ml)
        - In the shared cabinet: flasks (25, 75 and 175 cm2-) - Petri dishes - Plates (6, 12, 24, 48 and 96 wells) - Tips (10, 200 and 1000 µl) - Counting cells - Filter units - etc...
    - Trypan blue under each MSS
    - Bleach (tablets and bottles)
    - Sterile water for incubators
    - Fill the spray bottles with 70% ethanol.
    - Notebooks and pen

- In the airlock *(non-exhaustive list):*
    - Gloves (S, M and L)
    - Overshoes
    - Bottles of hydroalcoholic gel
    - Sterile Eppendorfs tube jars (0.5, 1.5 and 2ml)
    - Pasteur pipette boxes
    - Wizzy boxes of paper towels
    - Sharpsafe boxes
    - Fill the bottles with milliQ water and 70% ethanol.

Cleaning of materials and equipments:

- Store all material outside the MSS: racks, pipettes, etc...
- Vacuum the bleach from flasks, plates, Petri dishes waiting on the benchtop.
- Benchtop: clean with 70% ethanol
- Centrifuge tanks: clean with 70% ethanol, but **neither the rotors nor the buckets**!
- Incubators: clean the inside of the doors with paper soaked in biocidal ZF (no ethanol or detergent!). **Check the sterile water level in the incubators and add some if necessary**.
- **Cleaning of MSS: procedure**
    - Seal full sharpsafes boxes tightly and throw away in the "DASRI" cardboard bins.
    - Close full "DASRI" bins, identify "PCC + date" and throw away *via* your unit according to your procedures.
    - Take out all the equipment being under the MSS
    - Switch off the MSS (prevent absorbent paper from being drawn in by the ventilation!).
    - Clean all walls (inside, outside and glass) with paper towels and 70% ethanol.
    - Lift the worktop and clean the drip tray with paper towels and 70% ethanol.
    - Clean the working surface
    - Switch the MSS back on (alarm for a few moments)
    - Clean all equipment with 70% ethanol before returning it to the MSS.
        - Micropipettes and rack
        - Racks for tubes
        - Pipet-aid
        - Pasteur pipette boxes
        - Tips boxes
        - Eppendorf tube jars
        - Sharpsafe bins
        - Filled bottles containing water and bleach
        - Disconnect the bottle from the vacuum pump, empty it into the waste container of the laundry (if the bottle is made of glass, put 2 bleach tablets in), and reconnect to the vacuum pump.

Floor cleaning

- Required equipment:
    - Broom: In the airlock
    - Bucket, Wipes and Ecodiol: Laundry

Procedure:

- Fill the bucket with warm water and add 2 pods of ecodiol.
- Enter the airlock (don't forget the wipes!) and put on your EPI (personal protective equipment).
- Apply detergent/disinfectant to the airlock floor
- Throw away the wipe in the DASRI trash in the airlock.
- Enter the L2 lab
- Apply detergent/disinfectant to the lab floor while the airlock floor dries.
- Use as many wipes as necessary and Throw them away in the DASRI bins.
- Go out of the lab
- Throw the blouse in the airlock DASRI bin

# Good practices in cell culture

- Mycoplasma detection test:

To know: Mycoplasmas are microorganisms 0.15 to 0.3 µm in diameter (pass through filters 0.2µm) without cell wall. They replicate independently and have the ability to affect host cells in terms of growth, metabolism and functions. Thus, infection of cultures by mycoplasma can leads, for example, to membrane alterations (antigenic characteristics), and/or can affect protein synthesis etc. (see below).

*Table 5.* Effects of mycoplasma contamination on cell cultures

- General effects on eukaryotic cells:
  - Altered levels of protein, RNA and DNA synthesis
  - Alteration of cellular metabolism
  - Induction of chromosomal aberrations (numerical and structural alterations)
  - Change in cell membrane composition (surface antigen and receptor expression)
  - Alteration of cellular morphology
  - Induction (or inhibition) of lymphocyte activation
  - Induction (or suppression) of cytokine expression
  - Increase (or decrease) of virus propagation
  - Interference with various biochemical and biological assays
  - Influence on signal transduction
  - Promotion of cellular transformation
  - Alteration of proliferation characteristics (growth, viability)
  - Total culture degeneration and loss
- Specific effects on hybridomas:
  - Inhibition of cell fusion
  - Influence on selection of fusion products
  - Interference in screening of monoclonal antibody reactivity
  - Monoclonal antibody against mycoplasma instead of target antigen
  - Reduced yield of monoclonal antibody
  - Conservation of hybridoma

Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention Hans G. Drexler∗ & Cord C. 2002

Mycoplasma testing **is mandatory** for all cell cultures grown on PCC.

A test is carried out monthly on supernatant of cell cultures. An e-mail is sent to you beforehand to inform you about the test date and the procedure to be implemented for the harvest of your supernatant.

If your cultures are contaminated, you have to either throw them away or treat them with:

- o BM-cyclin (BM1: 10µg/ml -3 days then BM2 : 5µg/ml - 4 days / repeat cycle twice).
- o Plasmocyne 50µg/ml -10 days
- o Others...

Prevention measures:

- o Do not talk in front of the MSS during manipulation,
- o Use an independent reservoir of culture medium for each type of cultured cells

- Other good practices:

- o Weekly check the sterile water level in the incubators and add some if necessary.
- o Check the remaining level of CO2 in the bottles connected to the incubators (especially on the before WE!). If you change the bottle, don't forget to mark the date of use and to inform the person in charge.
- o In case of contamination in a culture,
  - DO NOT OPEN the flask.
  - Take the flask out of the L2 lab and bleach it in the laundry.
  - Notify the person in charge of the PCC and fill in the contamination declaration form (see APPENDICES).

## In case of incident: procedure

In case of contamination: Remove gloves and lab coat if contaminated and dispose of in the DASRI waste bin, and put some news ones.

(In case of cut: Follow the instructions "What to do in case of accident with biological material" posted at the entrance to the laboratory)

- Put on gloves and a new lab coat

- Absorb the liquid with paper towels soaked in 70% ethanol, then spray the 70% ethanol on the contaminated area.

- Throw the papers away in the DASRI waste bin

- Remove gloves and throw them away in the DASRI waste bin.

(In case of broken glass: do not hesitate to use pliers to pick up the debris and throw it in the DASRI dustbin. - Disinfect the pliers after use -)

- Clean several times with 70% ethanol.

- Check that the liquid has not spilled into the drip tray under the worktop.

- If this is the case, clean the holding tank with 70% ethanol.

- Clean with ethanol all the material present under the MSS

- Remove gloves and throw them away in the DASRI waste bin.

- Put on news gloves and continue the manipulation.

- Report the incident to the person in charge as soon as you leave the laboratory.

- Make a work accident report if there has been an injury, cut or splashed into the eye.

- Record the incident in the RRST logbook.

# APPENDICES: Practical informations

| | | |
|---|---|---|
| | **CONTAMINATIONS DECLARATION FORM**<br>**2020-00** | |
| Monitoring of sources of contamination (equipment, procedures) | Cell Culture Platform | Version 003<br>(octobre 2013)<br>Joël Tardivel-Lacombe<br>Laurence Borge<br>English Version<br>20200211 |

Any contamination of a cell culture must be reported and saved in this contamination form.

The objective of this declaration is to continuously improve the quality of work within the Cell Culture Platform (ISO9001 requirement)

For this, it is necessary to identify sources of problems related to the material, the environment or the gestures of the manipulators, in order to resolve them

No sanction is linked to the identification of contaminations occurring in working conditions in accordance with Good Practices in Cell Culture (GPBC).

| | |
|---|---|
| Declaration date | |
| Cell manipulaton date | |
| Name<br>Research Unit N° | |
| Research Project | |
| Cell type / culture | |
| Antibiotics : yes / No | |
| MSS N° | |
| Contamination type :<br>(Bateria, fungi…) | |
| How was the contamination detected / identified? | |
| Probably cause | |
| What have you done? | |

Health & Safety

What to do

# ACCIDENT WITH BIOLOGICAL MATERIAL

## STRAIGHT AWAY

### Cuts and pricks

- **Wash immediately** with a liquid neutral soap for at least three minutes.
- **Carefully rinse.**
- **Disinfect** for at least 15 minutes with:
  - stabilized Dakin's solution,
  - or 70° ethanol.
- **Go to see the appointed doctor about the risk of HIV and viral hepatitis within two hours (contact details given on the site's sheet on What to do in the event of accidental exposure to blood).**

### Sprayed onto the skin

- **Wash immediately** in running water or use the emergency shower with the diffuse jet for at least 15mn.
- **Go to see the appointed doctor** about the risk of HIV and viral hepatitis **within two hours (contact details given on the site's sheet on What to do in the event of accidental exposure to blood).**

### Sprayed into the eye

- **Wash immediately** in running water for at least 15 minutes, pulling the eyelids widely apart with the head tilted, affected eye down (get help from a colleague).
- **Contact lenses will get washed out but if not, do not try to take them out.**
- **Do not use eye drops** or any type of ocular solution.
- **Go to see the appointed doctor** about the risk of HIV and viral hepatitis within two hours **(contact details given on the site's sheet on What to do in the event of accidental exposure to blood).**
- **See an ophthalmologist as soon as possible.**

## FOLLOWING DAYS

- **Go to see the Prevention Physician** within 24 hours for a risk assessment and report any symptom that you notice in the days following the incident or accident.
- **Fill out an "Accident in the Workplace" form.**
- **Notify the Prevention Assistant** and **report** the incident or accident in the Health & Safety Log.
- **Report the incident or accident** to the Prevention Adviser.

## PREVENTION

- **Handle** biological products in a Class II biosafety cabinet.
- **Wear safety goggles** with side bars, suitable gloves and a buttoned up coat.

Compiled by BCPR and BCMP - Creation and production: Audrey Raudin (DRH - MO) - October 2015

Conduite à tenir

# EN CAS D'ACCIDENT EXPOSANT AU SANG (AES) OU À DES PRODUITS BIOLOGIQUES HUMAINS

**Sites de Marseille**

## IMMÉDIATEMENT

### Piqûre, coupure, projection sur peau lésée

- **Ne pas faire saigner.**
- **Laver immédiatement** à l'eau et au savon neutre pendant 5 minutes.
- **Rincer**.
- **Désinfecter** pendant 5 minutes au moins avec :
  - du Dakin stabilisé,
  - ou à défaut de l'alcool à 70°.

### Projection oculaire ou muqueuse

- **Rincer** abondamment à l'eau courante à faible pression **pendant 10 minutes**, en écartant bien les paupières, tête inclinée et l'œil atteint positionné vers le bas (se faire aider par un collègue).

## DANS LES 4 HEURES

- **Consulter d'urgence le MÉDECIN RÉFÉRENT** afin d'évaluer le risque de contamination hépatites, HIV ou autre. Signaler que l'on vient pour une exposition au sang.

**Service d'accueil des urgences :**
**Hôpital Saint-Joseph : 04 91 80 68 90 / 66 70**
**Hôpital de la Conception : 04 91 38 36 52**
**Hôpital Nord : avant 16h00 service de médecine interne : 04 91 96 89 33**
**après 16h00 urgences : 04 91 96 48 24**

**Appeler avant de venir pour une prise en charge directe à l'arrivée**

- **Le médecin référent décidera de l'opportunité d'un traitement préventif et de sa réévaluation.**

## DANS LES 24 HEURES

- **Faire établir un certificat médical initial d'accident du travail** (cerfa 11138*03) par le médecin consulté en urgence.
- **S'assurer** d'un témoin.
- **Déclarer l'accident dans les 24 h** : les démarches à entreprendre vous seront indiquées par le secrétariat de l'unité ou de la délégation régionale.
- Faire **noter l'accident** sur le registre santé et sécurité au travail.
- **Notifier rapidement l'accident au médecin de prévention pour enquête, suivi clinique et biologique :**

## EN CAS D'URGENCE MÉDICALE GRAVE

- **Appeler le 15**

Santé & Sécurité

Rédaction : BCPR - BCMP - Réalisation : Julie Arqué et Audrey Raudin (DRH - MO) - Septembre 2016

I **declare that I have read the rules for the use of the PCC L2 lab and I undertake to respect them.**

**A Marseille, le**_____

**Name, first name** **Signature**