

P3 practices and procedures

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Working with 1HV is potentially hazardous. This guide outlines some procedures for avoiding dangerous situations, but most of all, **USE COMMON SENSE**. Since not every dangerous situation can be anticipated, it is essential that good judgment be used to maximize safety. Laurent Roux is the Principal Investigator in charge of the P3 facility, and Romaine Stalder is responsible for supervising its day-to-day operation. Any question regarding the equipment, including possible malfunctions, should be addressed to Dominique Oneyser. Safety procedures should be discussed with Romaine Stalder and Laurent Roux. If someone appears not to work safely, it is everyone's best interest that this situation be corrected. If you happen to cause some problem yourself, do not fear the wrath of your colleagues. Instead, come forward immediately and participate in its solution.

ONLY FULLY TRAINED PERSONNEL IS ALLOWED TO PERFORM EXPERIMENTS IN THE P3. Under special circumstances observers may be allowed to enter accompanied by a trained P3 user, but only with explicit permission fromLR.....

Entering the P3

The P3 is entered using the Cerberus access card. The doors to the anteroom and to the P3 main room should not be opened at the same time. A green light above the first door indicates that the second door is closed, and, therefore, that entrance into the anteroom is permitted.

- Upon entering into the **anteroom**, change **shoes**. Alternatively, paper "booties" can be used.
- Put a **first pair of gloves** in the anteroom.
- New gowns are located in the anteroom. Put one on, or put on your previously used gown, stored in the P3 lab right behind the door. Label your gown with your name. Gowns are worn for no more than a few weeks. If there is any suspicion of contamination with infectious material, they should be discarded in trash for contaminated waste.
- After entering the P3 main room, put a **second pair of gloves**. Always wear two pairs of gloves while in the P3. The first goes under the sleeves of the gown, the second over. The second pair of gloves is changed frequently during work, in particular at the tissue culture hood. It should be disposed of in the waste for contaminated material located in the tissue culture hoods. Always change the second pair of gloves when leaving the tissue culture hood. Always change the second pair of gloves before touching the phone.
- Wear shoes that cover your feet, and socks. Shorts are not recommended.
- **Safety glasses** are highly recommended, in particular when working with large quantities of virus.

Working in the P3

The P3 is designed along, a theoretical **gradient of possible contamination:**

- **No infectious** material goes in the **first bay**
- **Infectious** material in closed containers is handled in the **second bay**
- **Tissue culture** takes place **exclusively** in the **third bay**.
- **Vials containing infectious** material (tubes, flasks, etc...) are opened **exclusively** in the tissue culture **hoods**.

- **Sharp objects** (needles, Pasteur pipettes, etc ...) are **banned** from the P3.

- Only **disposable plastic ware** (pipettes, flasks, tubes, etc ...) is used.

Tissue culture work:

- **Change the second pair** of gloves often while working.

- **Trash cans placed in each hood** are for all disposable items that have come in contact with **infectious material** (i.e., everything used in tissue culture). These trash cans are **double-bagged**, with autoclavable red bags.

- **Only plastic pipettes** (not Pasteur pipettes) are used for the aspirator bottles.

- Aspirator bottles should contain at least **500 ml of bleach**, or **150 ml of a disinfectant** such as Rocadyne. **When 2/3 to 3/4 full**, bottles are **emptied down the sink**, while flushing drain with **disinfectant**.

- After **pipeting infectious material**, **rinse** the pipette with **5% bleach**, then discard in the infectious waste bag.

- **Do not let the trash overflow**.

- Before **removing a full bag from the hood**, **seal it first** by closing the inner bag, then close the outer bag. Prior to autoclaving, put on the sterilization **indicator tape**.

- After finishing, **spray** the work area **with 70% ethanol**. Also **spray** or wipe **bottles, pipetmen, flasks**, etc... prior to **removing from the hood**.

- Before leaving the hood, turn off the pipette aid, and make sure that the place is in the very state you would like to find it to start working.

Centrifugation:

- When **removing tubes** containing infectious material for centrifugation, **wipe the outside with 70% ethanol**.

- **Biosafety lids** are used when spinning infectious material in the "low speed" centrifuge (Megafuge).

- **Ultracentrifugation buckets** are **decontaminated** after use by spraying **with 70% ethanol**.

Radioactive material:

One hood and **one incubator** are dedicated to work with **radioactive material**. Monitoring of the working place with a proper detector is performed after each experiment involving radioactivity. Dispose of infectious radioactive waste in containers designed for this purpose. Most radioactive waste can be autoclaved with charcoal filters, and then disposed of like normal radioactive waste.

Waste

- All potentially contaminated waste is disposed of in trash **cans containing double biohazard** bags, in the tissue culture **hoods**.
- Autoclave the waste bags when they are nearly full, do not wait for them to overflow...
- Noninfectious waste such as plastic wrap, paper towels, etc... are disposed of in the big trash cans placed on the floor. These also are autoclaved.

Spills

- **Always warn** all personnel present in the P3 about the **occurrence of a spill**, except when it takes place inside a tissue culture hood and is of a very small volume (a few drops).
- **If a small spill** (a few droplets) of infectious material occurs in the hood, on the bench, in the incubator, on the floor, etc.... stop working, change gloves, and spray the spill and the neighboring area with 2-10% bleach. Wipe up and clean the area with 70% ethanol. Change gloves often while working.
- **If a large spill** occurs, douse the area profusely with bleach. Contain the spill with paper towels. Call Michel Loche so that cleaning procedures can be coordinated. Have everybody evacuate the P3 promptly.
- **If a bottle breaks** in a centrifuge, do not open the machine. Call Michel Loche and/or Didier Trono.
- **Spills involving broken glass are of special concern** because of the potential for puncture wounds. If such a spill occurs, notify Michel Loche and/or Didier Trono. Do not attempt to clean up the area without thoroughly decontaminating the sharp objects with bleach / 70% ethanol. Remember that shattered glass tend to fly long distances. Err on the side of caution.
- **In case of exposure of skin** or clothing to potentially contaminated material: immediately and abundantly spray with 70% ethanol. Take off the contaminated clothing and wash the skin profusely with soap and water. Do not scrub so hard as to break the skin.
- **In case of a spill in the eye**, immediately use disinfectant eye drops placed in spill kit box in the P3.
- **In case of a penetrating wound**, wash immediately with soap and water, then rinse with 70% ethanol. ContactX....., ...LR.....and the Division des Urgences Medico-Chirurgicales (Tel: 28 100). See details in the Post-Exposure Management Program (appendix).

Leaving the P3

- **Remove the outer pair of gloves**, and dispose of it in the infectious waste container in a tissue culture hood.
- **Remove the gown** in the front bay. Hang it inside out.
- Proceed to the anteroom.
- **Remove the inner pair of gloves** and dispose of it in the anteroom trash can. Change shoes. . Wash your hands thoroughly.

Removing infectious material from the P3

No infectious material is removed from the P3 unless:

- **it is inactivated** (autoclaved or detergent inactivated), or
- **it is in a closed container** that is cleaned on the outside with 70% ethanol and packaged for mailing (which involves placing the sample inside several strong containers).

Cleaning

The P3 is cleaned biweekly. Everyone who works in the P3 will be scheduled to clean the facility. Cleaning must be done on the **date posted**, while no one else is in the facility.

List of duties:

1. **Sweep** first, then **mop floor** with proper disinfectant (e.g. **2% Lysol**).
2. **Wipe** down bench surfaces with **70% ethanol**.
3. **Wipe centrifuges** (inside and outside), doors and handles of incubators, refrigerators and freezers with **70% ethanol**.
4. **Wipe phone and microscope** plate and **knobs** with **70% ethanol**.
5. **Empty** floor **trash cans** and **autoclave**.
6. **Wipe** all **door handles** and **clean sink** in the **anteroom**.

HIV testing, HBV vaccine

- When individuals **first join** the laboratory, a baseline **plasma sample** is stored with the Medecin d'Entreprise, HUG, Batiment de Liaison, 2nd floor.
- **HIV testing** is on a **voluntary basis**, but is recommended at a frequency of once **every six months**
- All individuals **leaving** the laboratory will be required to have a **final plasma sample harvested**.
- **Vaccination against the hepatitis B virus is mandatory to work in the P3 facility.**

Training

ALL PERSONNEL MUST COMPLETE THE P3 TRAINING COURSE BEFORE WORKING IN THE FACILITY.

Thoroughly **read the P3 practices** and **procedures** handout.

Receive **introductory training**, given by ...X.....

Observe other workers executing P3 practices and procedures before handling infectious material. **Final authorization** to work in the P3 is given after a discussion with ...LR.... and after individuals **sign a form acknowledging that they have received the P3 training**, and fully understand the guidelines and procedures of the P3 facility.