



**Containment
level 2 – rodent
training**

Introduction

Why Level 2 ?

- Risk analysis for each pathogen → scale 1 to 4
- **Level 2** is required for any pathogen that can cause **human or animal disease** but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available, and the risk of spread is limited.

The risk posed by every biological agent is determined according to a number of criteria including rate of infection, history of contraction in the lab, sample origin, and potential for creating aerosols, among others. A number of national authorities examine the characteristics of each agent and rate them for risk on a scale of 1 to 4, 1 being the least dangerous, and 4 being very dangerous and highly contagious.

Containment level 2 is the standard for working with biological agents that pose a medium risk to human health and the environment. In other words, a CL2 pathogen can make you sick, but there are usually effective preventative measures or treatments available.

Introduction



Why level 2 ?

- We want to confine pathogens in the room
:
 - Protect workers
 - Protect environment and spread to other humans
 - Protect other laboratory animals
- Obligatory Canadian standard (Canadian Biosafety Standard- Public Health Agency of Canada)

Containment level 2 is meant to confine pathogens to prevent their transmission to humans or animals and limit their propagation into the work environment. There are Canadian standards outlining the design of bioconfinement facilities and operational methods, and these standards are sure to be the basis of the training. In December 2015, a new edition came into effect, entitled Canadian Biosafety Standard.

Introduction

Why a training ?

«No biological safety cabinet, installation, or method can guarantee safety if the operator does not use safe techniques, the basis of which is solid information»

- Organisation mondiale de la santé

Requirement of the Public Health Agency of Canada and Canadian Food Inspection Agency



It is important to use the right methods when working with animals, and even more so when that work involves infected animals or animals that carry pathogen. Since the safety of handlers and their work environment rests on a solid foundation of knowledge and technical skills, everyone who needs to work in a CL2 zone in an animal lab at UL or one of its affiliated centres must undergo standard training.

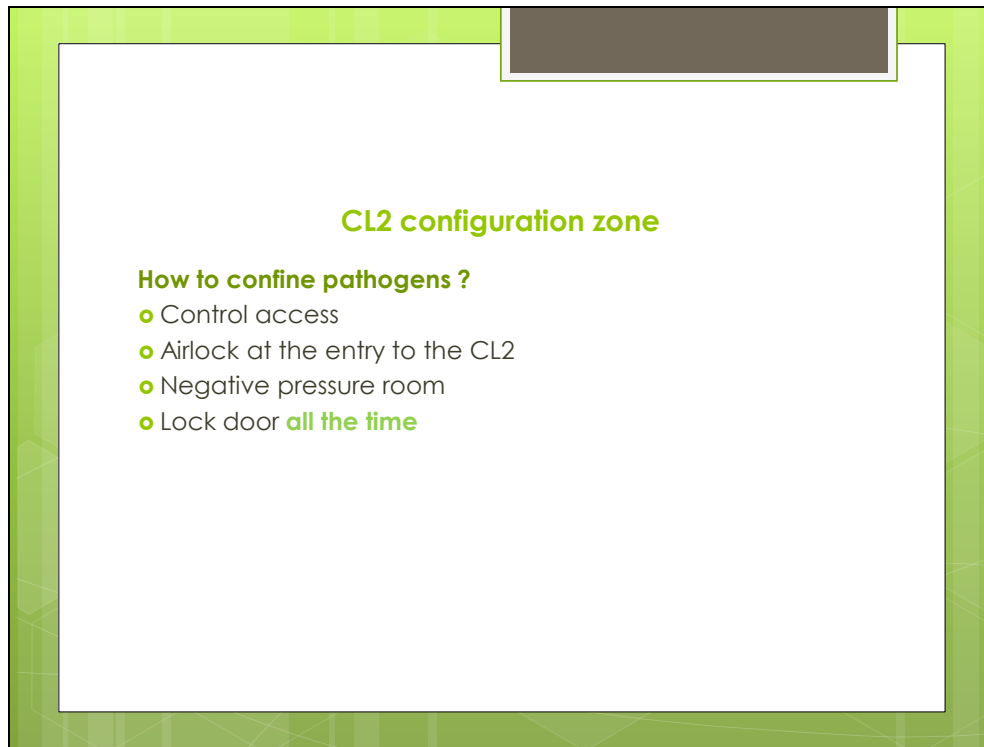
In addition, it is also a requirement of the Public Health Agency of Canada and the Canadian Food Inspection Agency.

Introduction

Why rodent training ?

- Variable and different housing standards
 - Ventilated cages
- Use of biological safety cabinet

This training is specifically for work with CL2 rodents, since handling them is different than handling larger species. Since this is a small species, it is easier to keep in ventilated cages or in a biological safety cabinet, which increases handler safety.



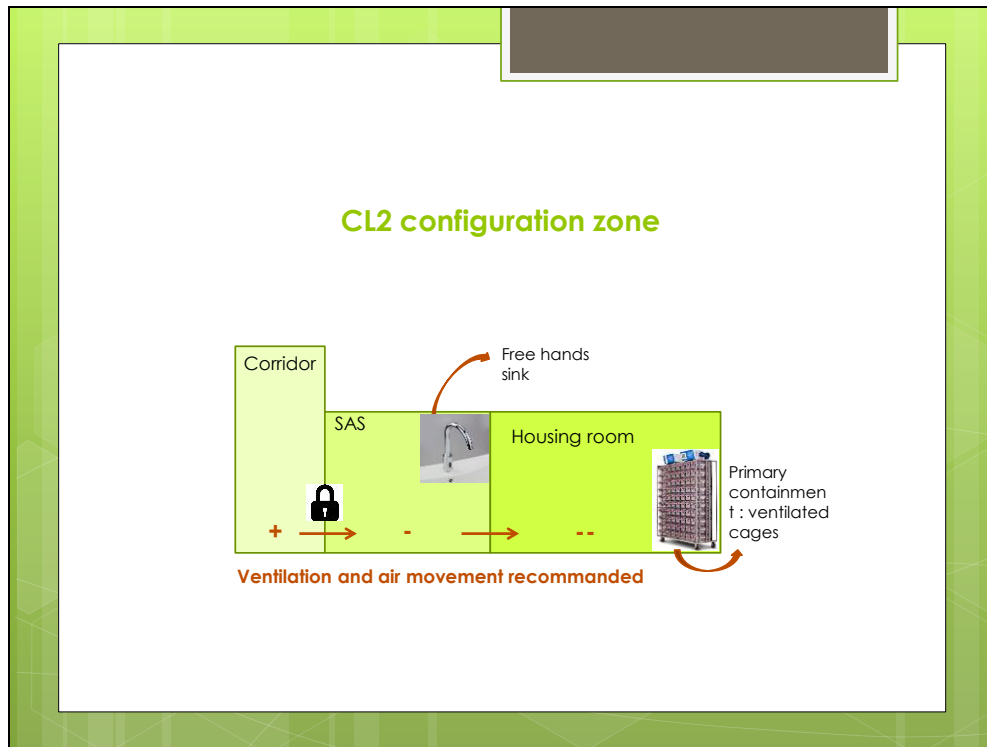
The CL2 zone is designed to keep the pathogens from getting out. First of all, access must be limited to people who need to be there. It is strictly forbidden to share access codes or magnetic keys with anyone. If someone doesn't have access, it must be for a good reason.

Interns, visitors, and workers (e.g., plumbers, electricians), and subcontractors must follow the training **OR** be accompanied by an authorized person **at all times while in the confinement zone** and must comply with the dress requirements.

The airlock at the entry to the confinement zone keeps the PPE separate from the CL2 room. It also balances the air pressure.

Keep the doors closed at all times so that the ventilation remains effective (pressure balance). It is preferable that the airlock not be open at the same time as the door into the room to avoid exposing staff walking by in the hall.

If a key system is used to restrict access to the confinement zone, make sure to lock the door behind you when entering so that access remains controlled.



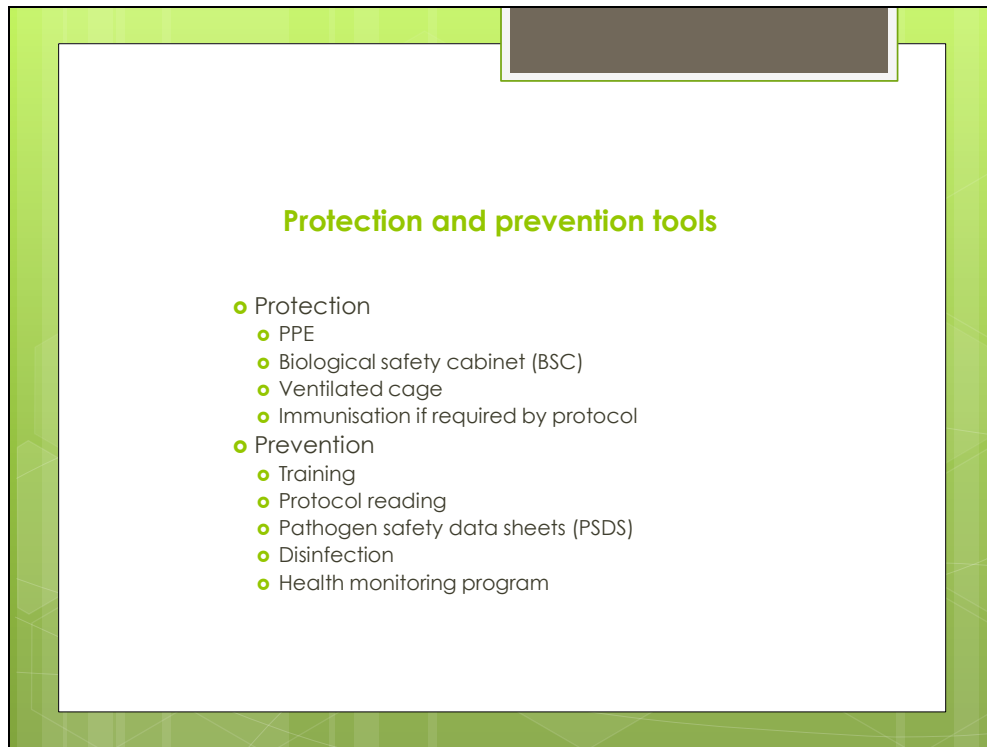
Here is a typical example of a CL2 rodents zone. Notice the locked door at the entry, the inward airflow, and the ventilated cages. This is all done to avoid contaminating the rest of the animal lab. The facilities will be configured differently if the rodents are not kept in ventilated cages. The standards will actually be higher.



Animal facility basic rules

- Basic rules :
<https://www.dsv.ulaval.ca/cms/site/ddsv/accueil/formation/visite>
- In the housing room, consider all material potentially **contaminated**

The basic rules of the animal lab all apply in CL2. Refer to the OH&S animal lab training (see the DVS website under "training" and "animal lab visit") for a refresher.



Different modes of protection are required in CL2.

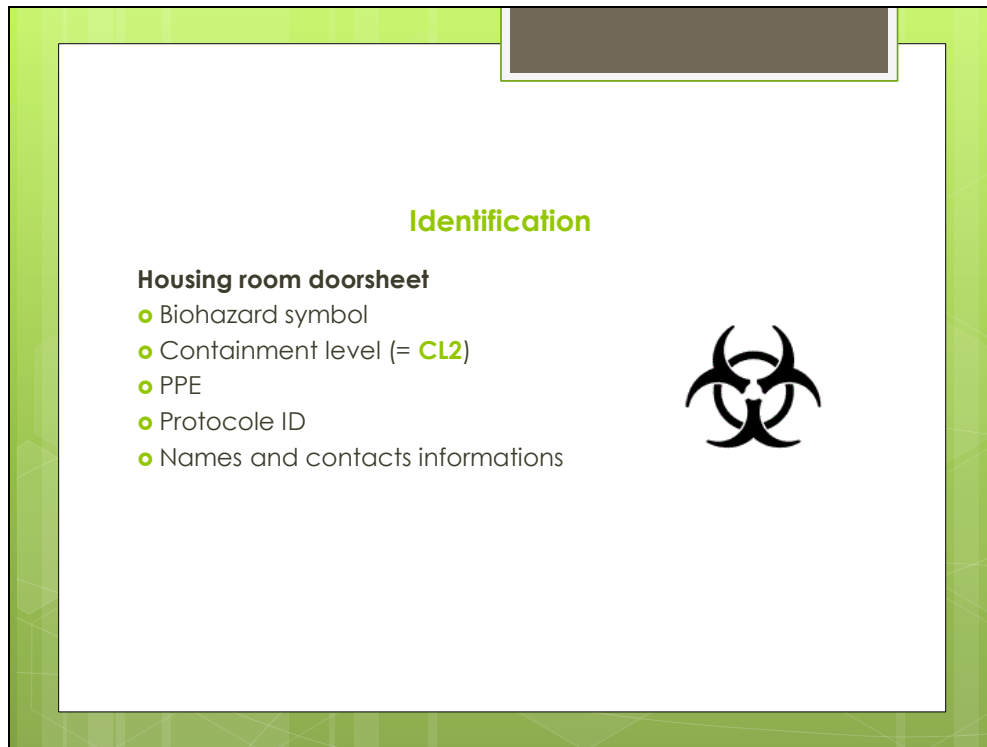
PPE (personal protection equipment) is important when working in a CL2 zone. Respirators, gloves, and other equipment help prevent staff from being infected. It must be in good condition and worn properly.

A **BSC** helps contain the pathogens when opening cages.

A **medical exam** identifies people who are at greater risk when working in these conditions, and specific **immunization** may be required in certain cases (e.g., vaccination against influenza may provide increased protection if you are working with that virus).

It is VERY IMPORTANT to read the protocol before starting any work related to that protocol. You need to understand the protocol and higher-risk activities to be well-prepared.

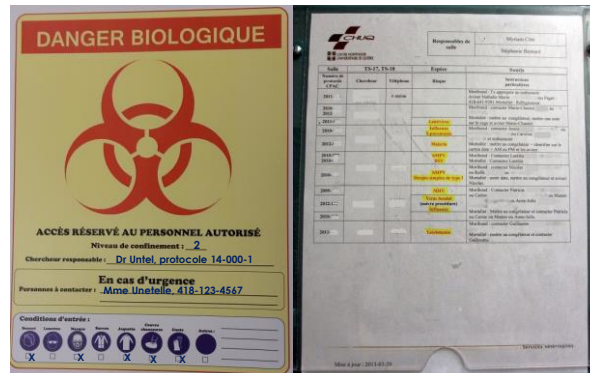
Pathogen Safety Data Sheets (PSDS) are available on the Public Health Agency of Canada website. They provide a summary of a pathogen's mode of transmission, clinical signs, protective measures, and disinfection methods.



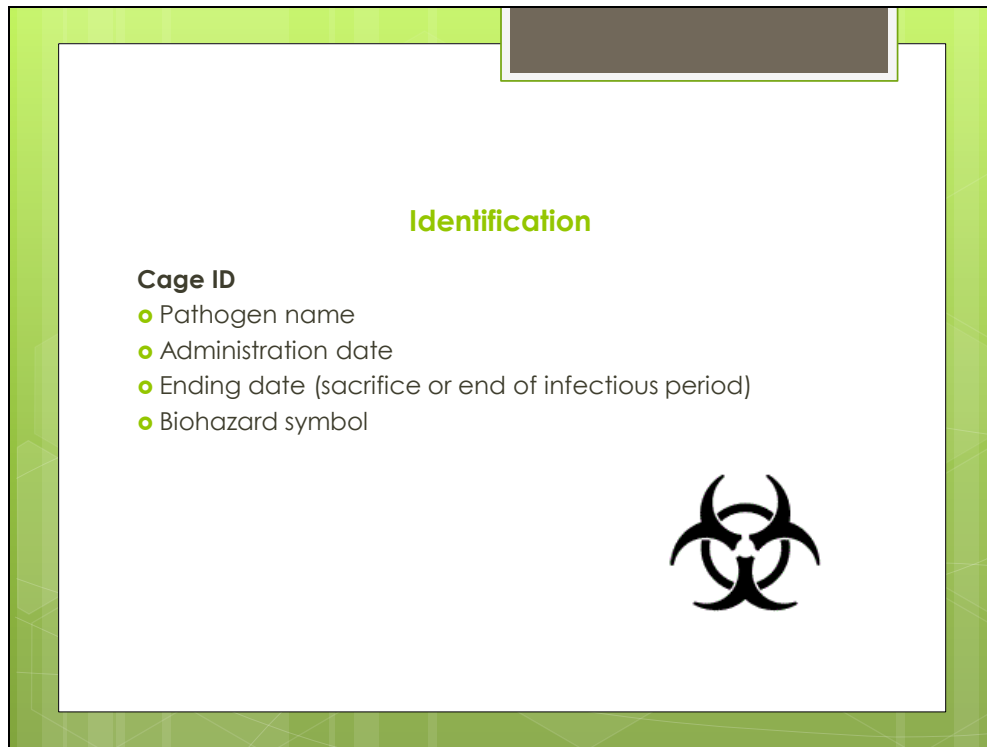
One way to prevent being exposed to risk is to use proper signage. Always check the signs on the door before entering. Different logos indicate which risks are present in the room (e.g., infection, chemical).

Identification

Housing room doorsheet



CL2 rooms are always identified with "Biohazard" signs.



In addition to the signs on the doors, every infectious cage must be identified.
In addition to the usual information, the following must appear on the cage of a confined animal:

- Name of risk
- Date of administration
- End date (infectious protocol or sacrifice)
- Biohazard symbol

Identification

Cage ID

EXPÉRIMENTATION
hMPV

Charles River 03 avr. 13
42705
Souris

Date infect.: *Avril*
Date sac.: *mai 2011*

#	Génotype	Commentaire
1		gr 1 : SACIN
2		
3		8h50
4		
5		

PPE

Other PPE can be require in addition of the standard PPE usually weared in the animal facility.

Ex :

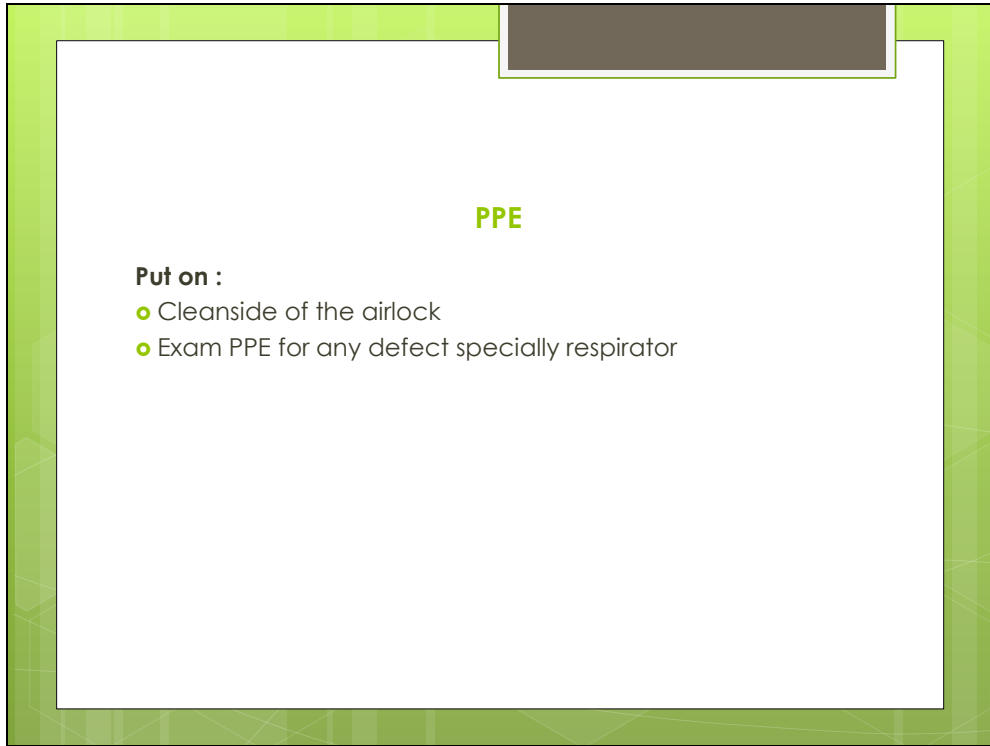
- Single-use gown
- Head cover
- Booties

+ others PPE required by protocols

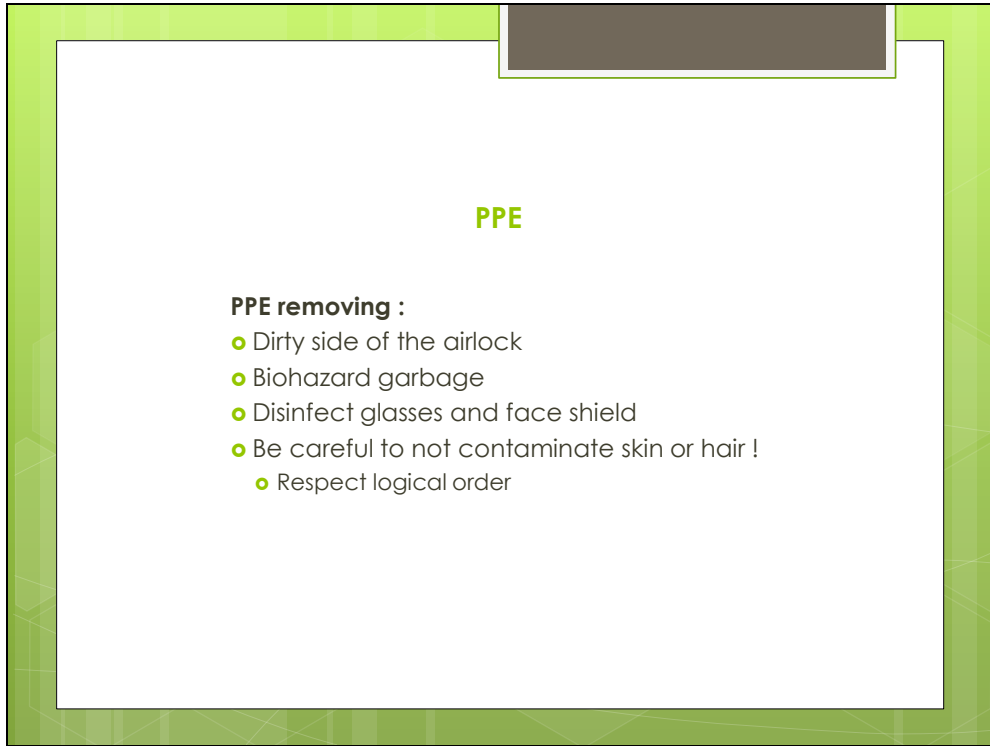
Entering a CL2 zone requires a gown (preferably single-use) and a head cover in addition to the usual respirator and gloves. Booties must also be worn over your shoes or over your first pair of booties.

Protocol may require additional PPE. If a second pair of gloves is required, they must cover your wrists. You could also wear Tyvek sleeves.

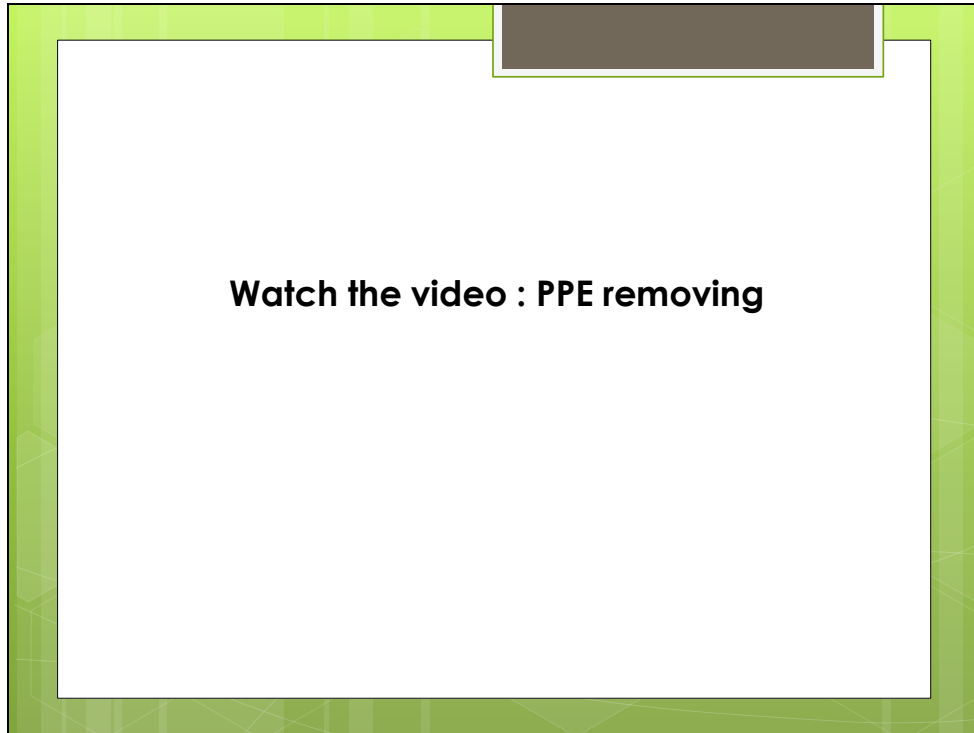
Glasses and a face shield should be worn when there is risk of spatter (e.g., in the laundry area).



Put on your PPE on the clean side of the airlock and examine it for any defects.



Unless indicated otherwise, PPE worn in CL2 is used once and disposed of with the biohazardous waste. Be careful not to contaminate your skin or hair when removing PPE. Remember that the front and sleeves of the gown may be contaminated.



- If you were wearing Tyvek sleeves and/or a second pair of gloves, you have already taken them off inside the biological safety cabinet (if you only wore 1 pair of gloves, you took them off in the BSC).
- Remove your gown carefully, folding it to cover the potentially contaminated sleeves.
- Booties are usually removed when crossing the line between the dirty side and the clean side of the airlock. Here, PPE is removed in the CL2 room, and the door is considered to be the line between the contaminated and clean sections.
- If you are wearing glasses or a face shield, remove them for disinfection at this point.
- Remove your head cover.
- Remove your respirator, keeping it as far from your face as possible.
- Finally, if you were wearing two pairs of gloves, remove the first pair now.



Be very careful not to contaminate yourself when removing gloves. Do not touch the skin of your wrist or forearm with a gloved hand, and don't touch your glove with your bare hand.

In other words, a gloved hand can only touch the outside of another glove, and a bare hand can only touch skin or the **inside** of a glove.

You must always **wash your hands** after taking your gloves off!

PPE



This is an airlock connected to a CL2 room.

In terms of wearing PPE in a CL2 zone, do you see anything strange in this photo?

PPE

Answer :

- **Never** use phone with gloves.
- Be aware of the clean and the dirty zone in the airlock and the housing room...
- PPE needs to be **removed before** circulation in the clean zone.



The telephone in the airlock is considered clean equipment.

Remember that the next person who uses that phone won't be wearing gloves. The same goes for cabinet doors and every other "clean" part of the airlock.

Potentially contaminated PPE must be kept on the "dirty" side of the airlock.

In here, you can wear a gown, but **only if** it is new and you haven't gone into the contaminated side yet!

Infectious material transporting

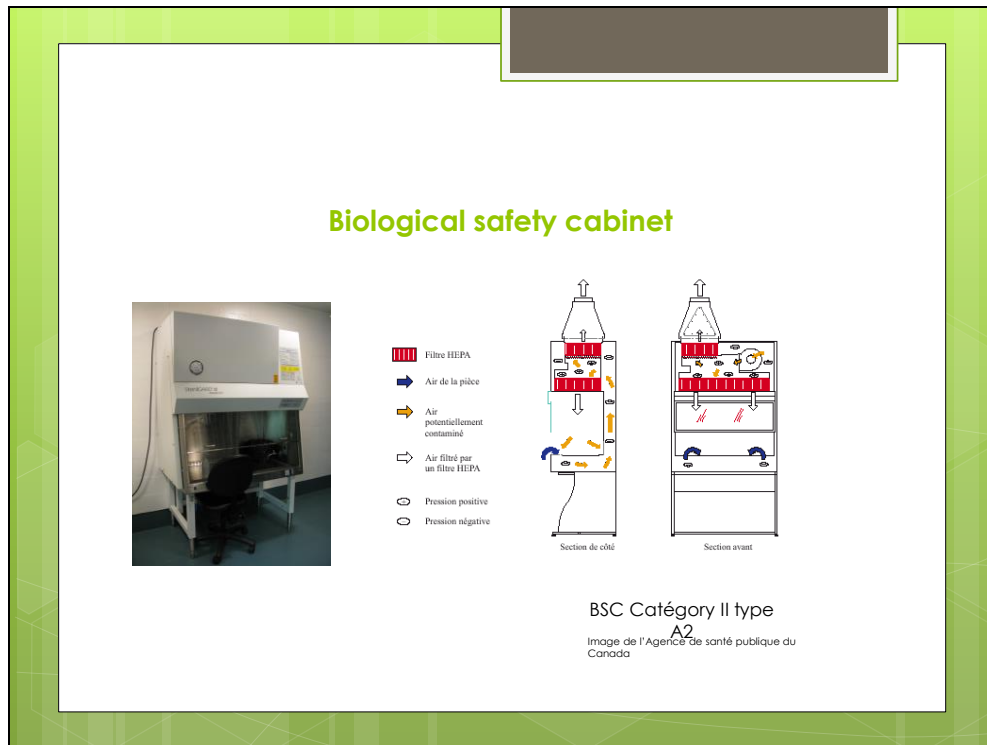
- If the 2 confinement zones are located in different buildings → transport training **required**
 - Contact campus security (biohazard)
- If the 2 confinement zones are located in the **same** building → **no** transport training required



When transporting a pathogen or infected animal organs from a CL2 zone to an animal lab or vice-versa, the rules to follow depend on where the confinement zones are located. Carrying infectious material between 2 confinement zones in different buildings requires training on transporting infectious materials because there are more precautions to be taken. Campus security handles biosafety training. Contact them to make an appointment.

You do not require any additional training to carry infectious materials between 2 different confinement zones located in the same building. However, there are a few safety rules to follow.

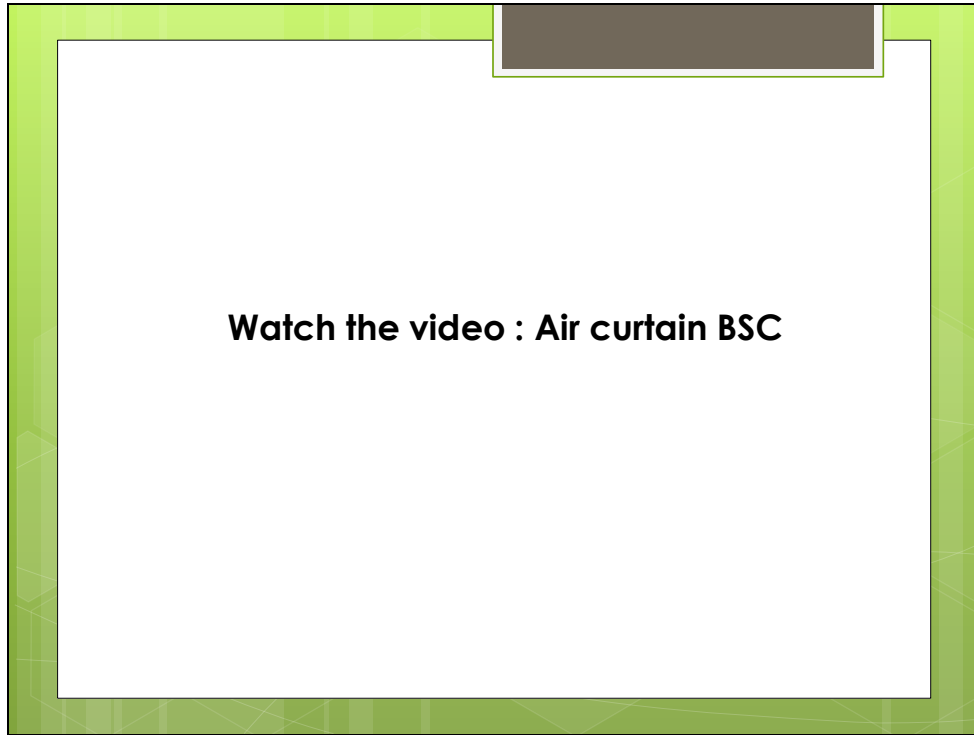
- First, the infectious material must be placed in a sealed primary container before it leaves the CL2 area. Like all other equipment used, the primary container must be disinfected before leaving the biological safety cabinet.
- If possible, use a **shock-resistant** container. A **screw top** is also preferable.
- The container must be **identified**. The biohazard logo and the name of the pathogen should appear on the container.
- If you are transporting several samples at a time, it is better to use a **trolley**. Lay some absorbent material down on the trolley to contain any spills.
- Move slowly and safely and avoid areas where patients are present as much as possible.



In CL1, when you work in a BSC (e.g., a cage switching station), the goal is to protect the animal from external contaminants. You are keeping the animal "clean." With CL2, you still have to protect the animal from external contamination, but you must also protect YOURSELF from the pathogen administered to the animal. That's why you are using a BSC.

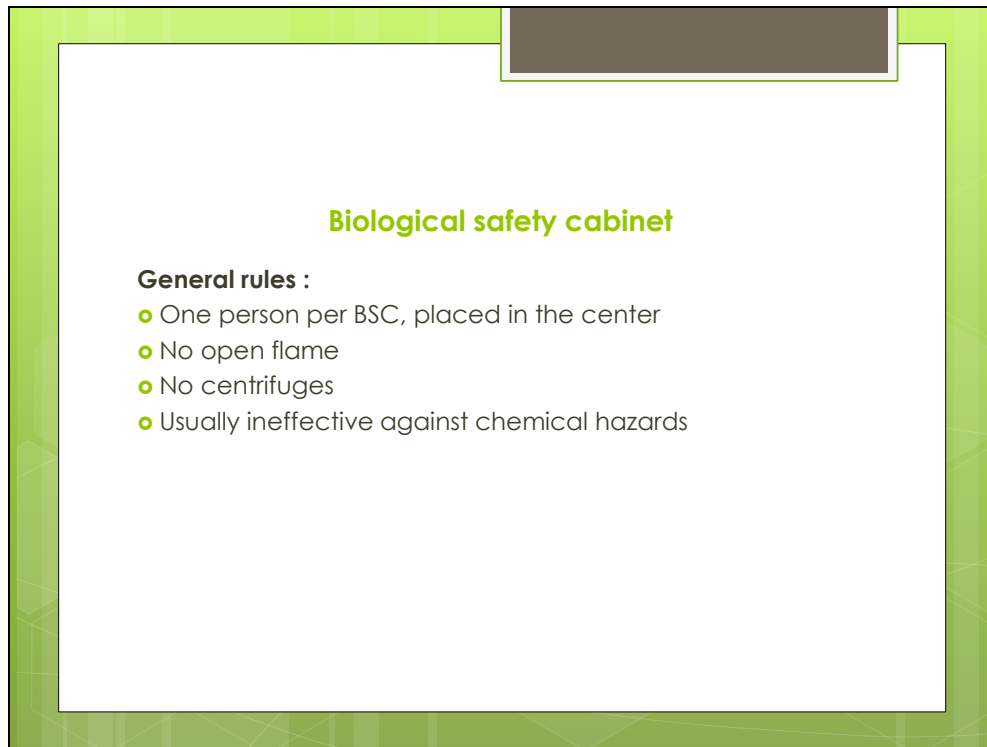
The basic principle of a BSC is to create a curtain of filtered air to provide primary confinement of pathogens and protect the handler. That air is filtered through a HEPA filter before being pushed out of the room. Category II BSCs also filter the air in the room entering the BSC in order to protect the animals inside from exterior contaminants. It isolates them in a sort of "air bubble."

It is very important to understand how the way you work inside a BSC can affect its ability to provide protection.



Watch the video : Air curtain BSC

The protective air curtain created by the BSC is delicate, and the flow can easily be disturbed. Someone walking quickly past the front of the hood, overloading the hood, or making quick motions while working can all disrupt the air curtain. For the hood to do what it was designed to and provide adequate protection, you have to work the right way.



To ensure the BSC works properly, it is recommended that one single person works in it at a time. However, some situations may require 2 people to work inside a BSC at the same time. For example, one person may need to hold the animal while the second person takes measurement. You must be careful to avoid moving quickly and going back and forth needlessly because that can affect the airflow inside the BSC. Obviously, you can't implement 2 different protocols inside the same BSC at the same time and hope to avoid cross contamination.

Do not use an open flame inside a BSC because the heat can affect the airflow and damage the HEPA filter. Do not use mini centrifuges inside a BSC because the velocity at which particles are thrown is too high for the BSC to manage the risk.

Not all BSCs are effective against chemical hazards, so it is important to comply with lab directives at all times.

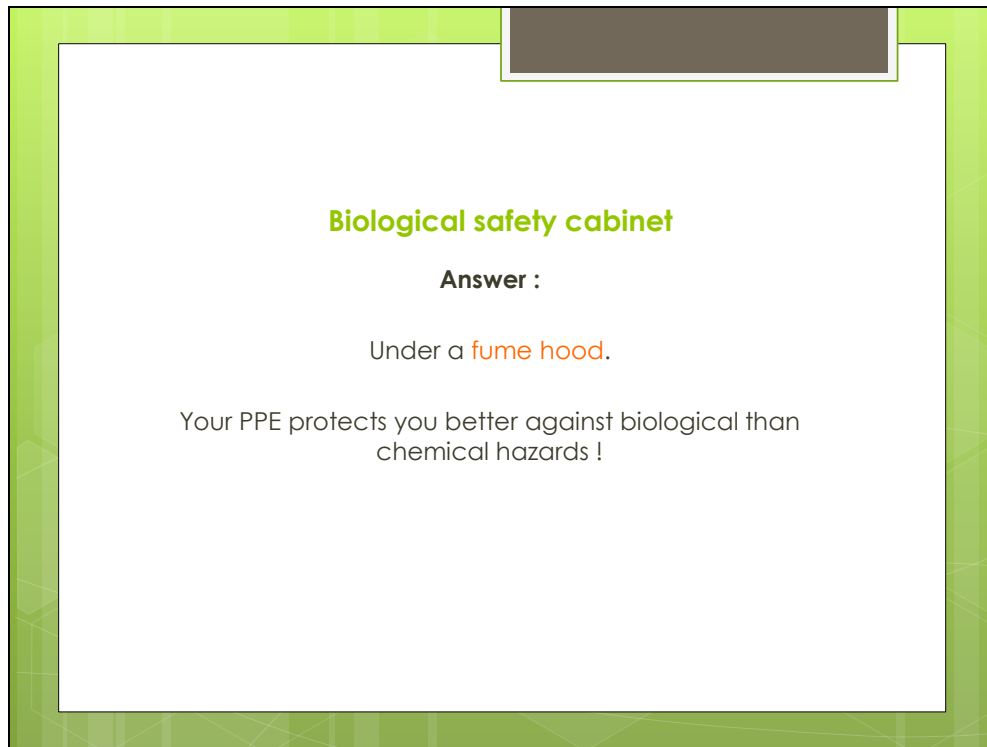
Biological safety cabinet

Question :

You are working with an infected animal (**biohazard**) which you will inject with a dangerous chemical (**chemical hazard**).

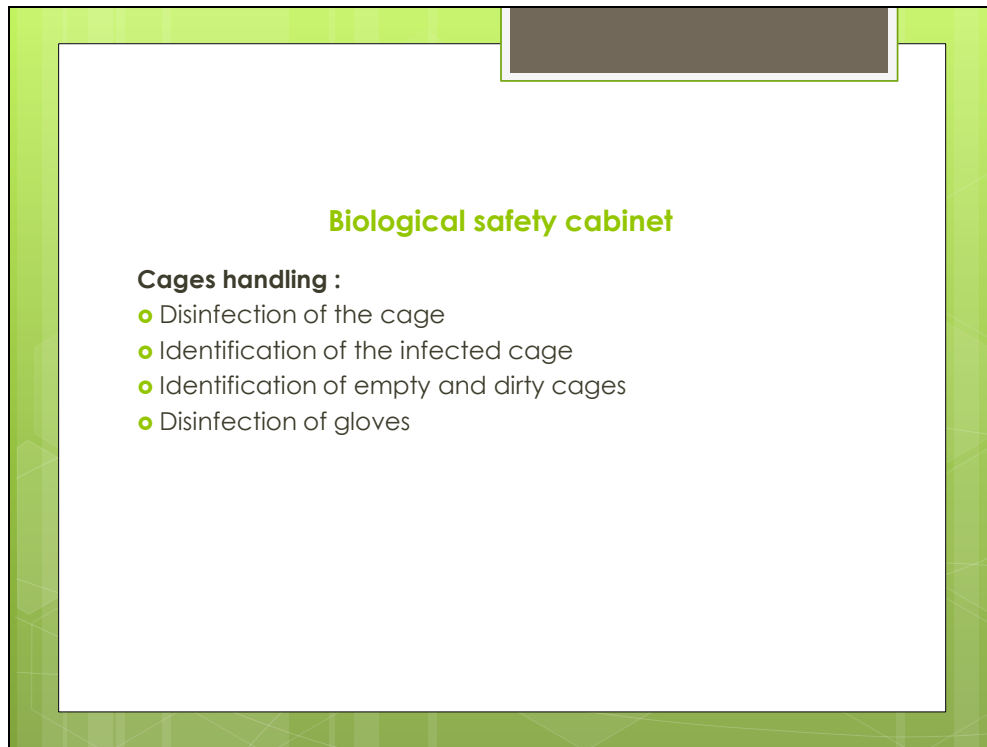
Where should you work ?

You are working with an infected animal (i.e., a biohazard) which you will be injecting with a dangerous chemical (i.e., a chemical hazard). Should you work in a BSC or under a fume hood?



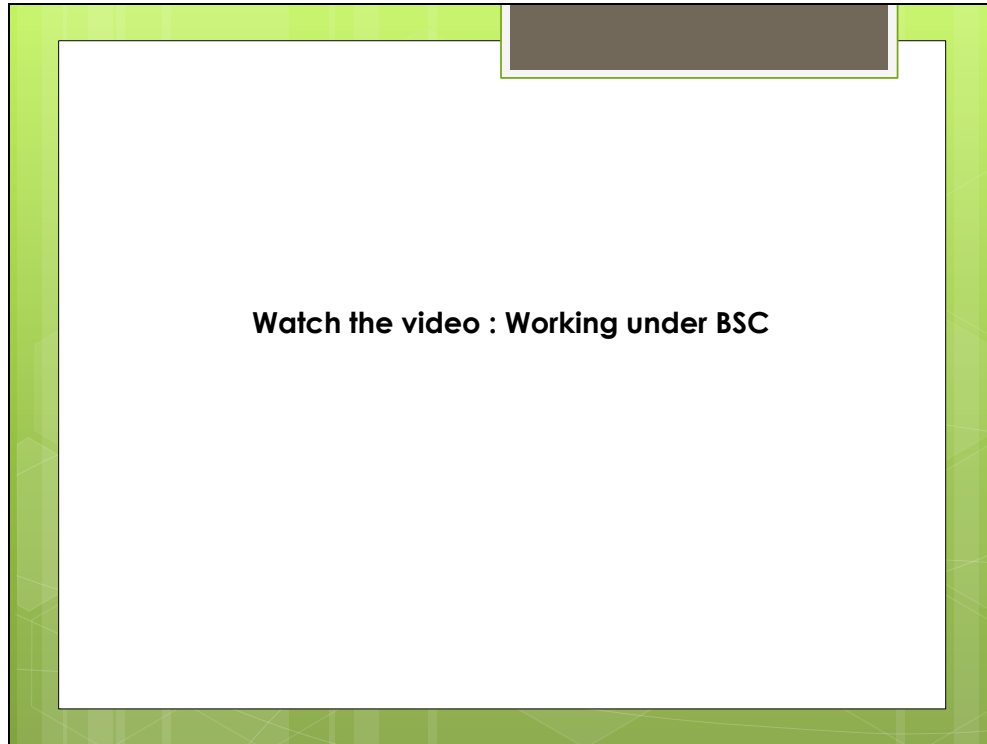
If what you are doing involves both biological AND chemical hazards, it is better to work under a fume hood. The PPE worn in CL2 animal testing labs offers better protection against biological than chemical hazards.

A room with a fume hood must be certified by the biosafety committees of UL and the Public Health Agency of Canada, but you must still follow procedures and lab rules regarding disinfection and handling waste and infected animal carcasses.



NEVER open an infected cage unless it is inside a BSC.

- The interior of a cage is always considered contaminated. You must remove the cover and set it down so that only the exterior side comes into contact with the work surface.
- Infected cages must be properly identified.
- Empty and dirty cages must also be identified so that the cleaning staff is aware of the danger.
- The exterior of the cage (empty or otherwise) must be disinfected before it can be removed from the cabinet and put back on the stand. To disinfect the exterior, use a cloth soaked with disinfectant. Using a spray compromises the seal of the cage's filter.
- You also have to spray your gloves with disinfectant after every time you touch an animal or the inside of an infected cage.
- If you overcrowd your work surface, the cabinet may not work properly. You need to be careful, particularly when changing cages, not to place too many items inside the BSC.



Now let's look at the right way to work inside a biological safety cabinet.

BEFORE :

- Wash your hands.
- The cabinet is not an absolute guarantee of safety. You have to wear full PPE and keep it on until everything is done.
- Make sure there is a box of gloves in your size inside the cabinet.
- Check the last certification date. If the ventilation system isn't on yet, turn it on, as well as the fluorescent light, and let it run for 5 minutes. Make sure the air is flowing inward using a tissue and keeping an eye on the clock. Make sure the air intakes and outlets are not blocked.
- Make sure the alarm system is on and the window is at the right height. Adjust your chair so the bottom of the glass is even with the top of your chest.
- Disinfect the interior and wait 5 minutes. Use a timer.
- Assemble the necessary equipment and place it in the cabinet. Follow the principle of having a clean side and dirty side. Don't block the vents.
- Place an absorbent plastic sheet on the work surface if there is a risk of spatter.
- If you have to work in a sterile environment (e.g., with immunodeficient mice), everything that goes into the cabinet must also be disinfected (usually with hydrogen peroxide). Allow at least 5 minutes for the disinfectant to act.

DURING:

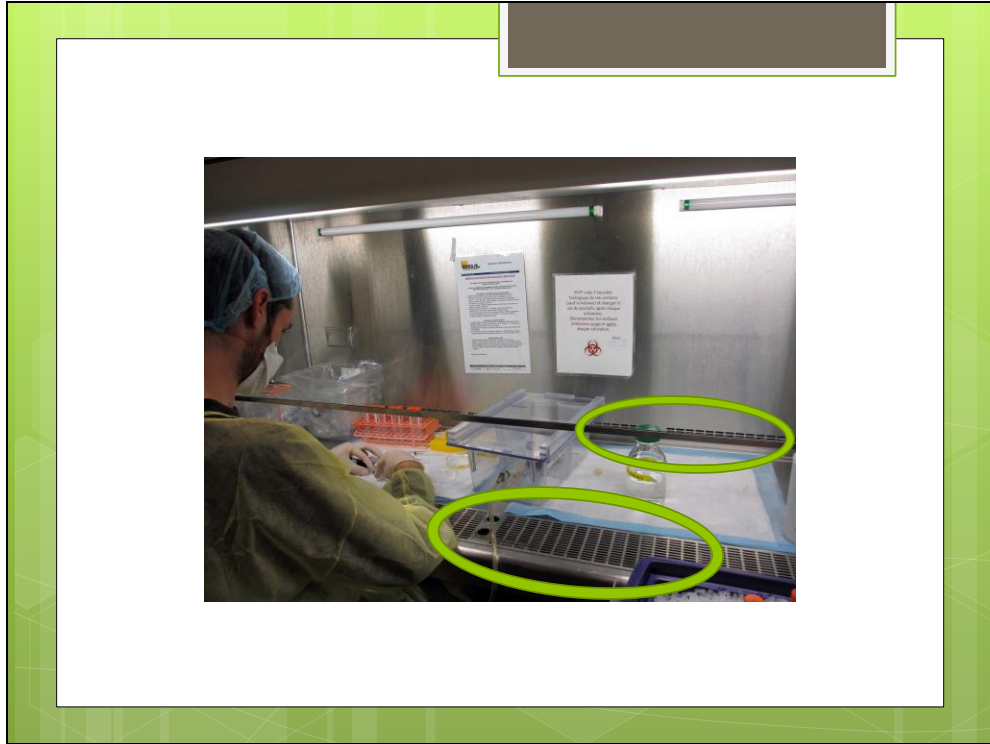
- Once a cage has been opened or the infectious material has been handled, **NOTHING** comes out of the cabinet without being disinfected first. That includes your hands.
- If you need to take your hands out of the cabinet, wait a few minutes first to allow the air to be filtered before disturbing the air curtain. You also need to remember to remove or thoroughly disinfect your gloves **before** taking your hands out.
- Work at the back of the cabinet as much as possible. Slide your hands along the window panel when putting them into or getting them out of the cabinet.
- If what you are doing carries a risk of spatter, try to direct your movement toward the back of the cabinet.
- Move slowly and with precision to avoid affecting the airflow. If you move quickly, wait a few minutes to let the airflow stabilize.
- Keep a bottle of disinfectant inside the cabinet. Remember to spray your gloves with disinfectant after every time you handle an animal and keep movement between the contaminated side and the clean side to a minimum.
- Dispose of all waste immediately, placing it at the back of the cabinet. Be sure to place sharp items in the hard containers. Remember that bags and containers designated for the disposal of used equipment are always identified with the biohazard logo.

AFTER:

- When you are finished, dispose of all waste in the bags and containers marked "biohazard" **inside the cabinet**.
- Then, disinfect all equipment used inside the cabinet and give the disinfectant the required time to act (e.g., 5 minutes for hydrogen peroxide).
- If you are wearing 2 pairs of gloves, you can remove the second pair and dispose of them inside the cabinet. If you are only wearing one pair, you have to change them or disinfect them thoroughly.
- Disinfect the biohazard bags and containers inside as well as the whole interior of the cabinet.
- When you are done disinfecting, leave the cabinet running for 5 minutes to clear the organic load from the air inside.
- Remove your PPE and wash your hands according to procedure.

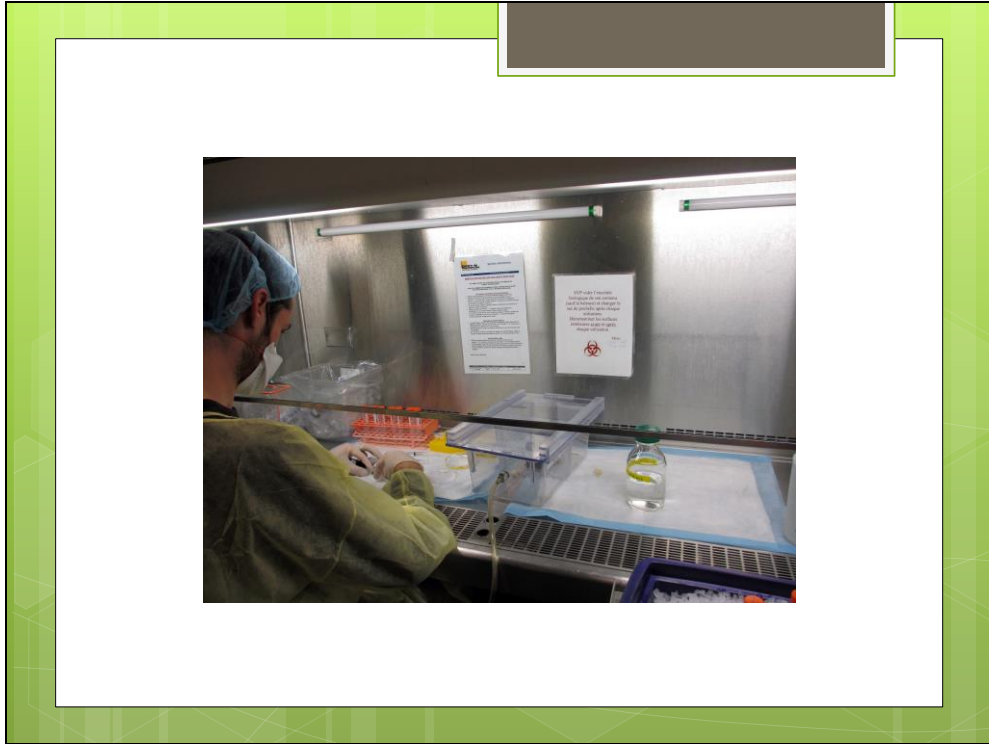


True or false: Where you place things inside the cabinet doesn't matter, as long as it's on the other side of the glass.

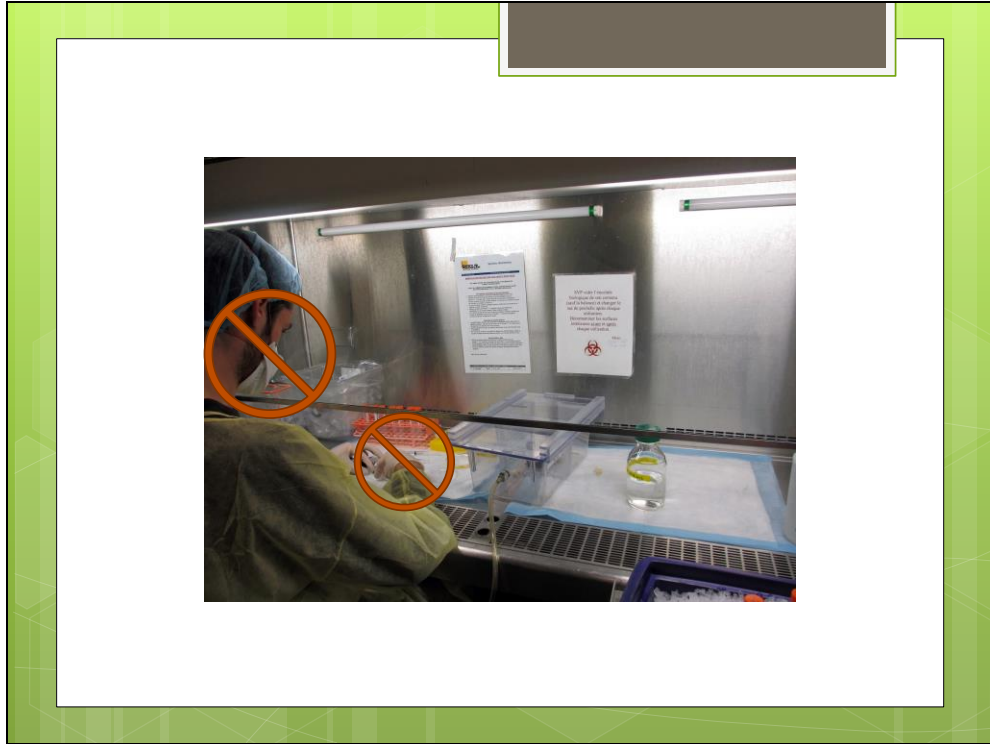


False! For the biological safety cabinet to work properly, you have to keep the ventilation grid at the front clear and avoid overcrowding at the back. That way, air can circulate properly and keep you protected from what you're handling.

Also, you have to keep clean equipment separate from dirty equipment.



Some health and safety requirements are being ignored here. Can you see what the problems are?



A respirator only works on a clean shaven face because it has to form a seal. If you have a beard, the respirator becomes useless and maybe even dangerous.

Also, the skin between the gown and the gloves is exposed. If the sleeves of your gown are too short, use Tyvek sleeves to make sure you are fully covered.

Handling

- Do not leave the needles tip exposed
- If necessary, use the one-handed method to recap the needle
- Replace glass tubes with plastic tubes
- After administration of the infectious material, use gauze to absorb any excess that leaks out



When handling an animal, you must be careful not to become infected yourself, either from your instruments or the animal. Proper restraint and work methods must be used to minimize the risk of bites and accidental injection. To minimize the risk of being bitten, you can use forceps to handle the mice or wear kevlar gloves in addition to nitrile or vinyl gloves.

You should never recap a needle. That is even more important in a CL2 environment. Dirty needles must be disposed of in a designated plastic container immediately after use. If it is absolutely necessary to recap the needle, use the one-handed method.

It is better to use a specially designed safety syringe. The needle is retractable to prevent needlestick between use and disposal.

After injecting the animal with the infectious material, you should wipe the injection site with a gauze to absorb any excess that leaks out.

Handling

Question :

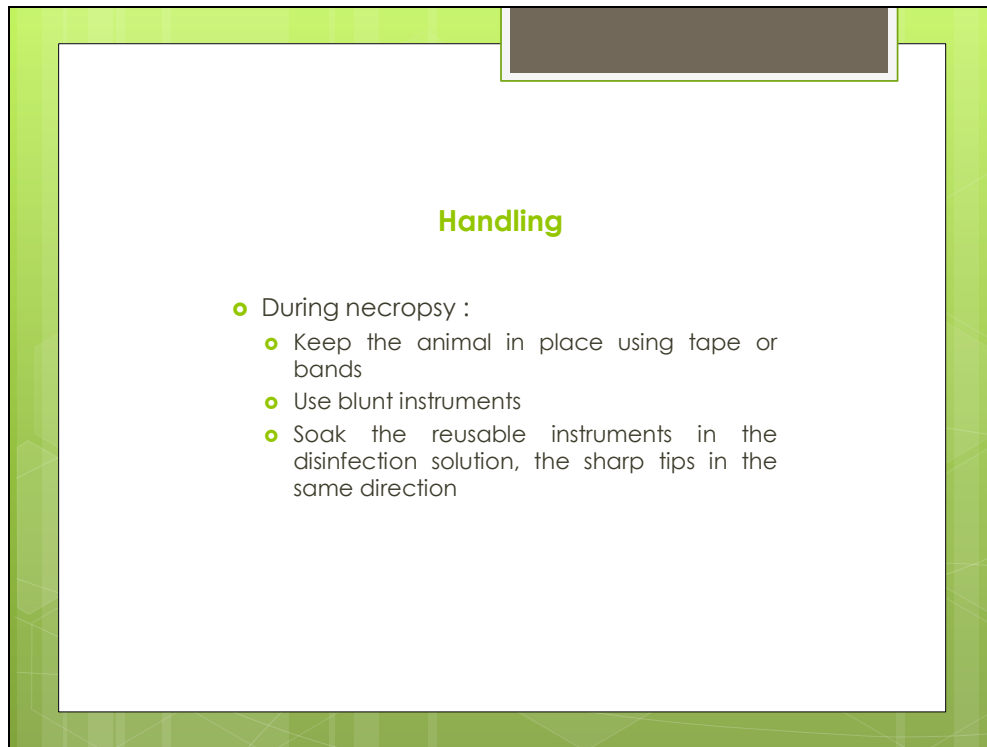
When you need to perform a basic restraint on an infected mouse, what is the best technique to use ?

- a) grid of the hood
- b) cage cover
- c) mezzanine
- d) feeder
- e) the cage



An infected mouse must not come into contact with anything other than the inside of its cage and your gloves.

You can use the feeder to perform a basic restraint, but try to avoid this if because it may get urine and feces into the food. The plastic tub, the grid next to the food, or the restraint in the cage are safe alternatives that won't contaminate the workspace.



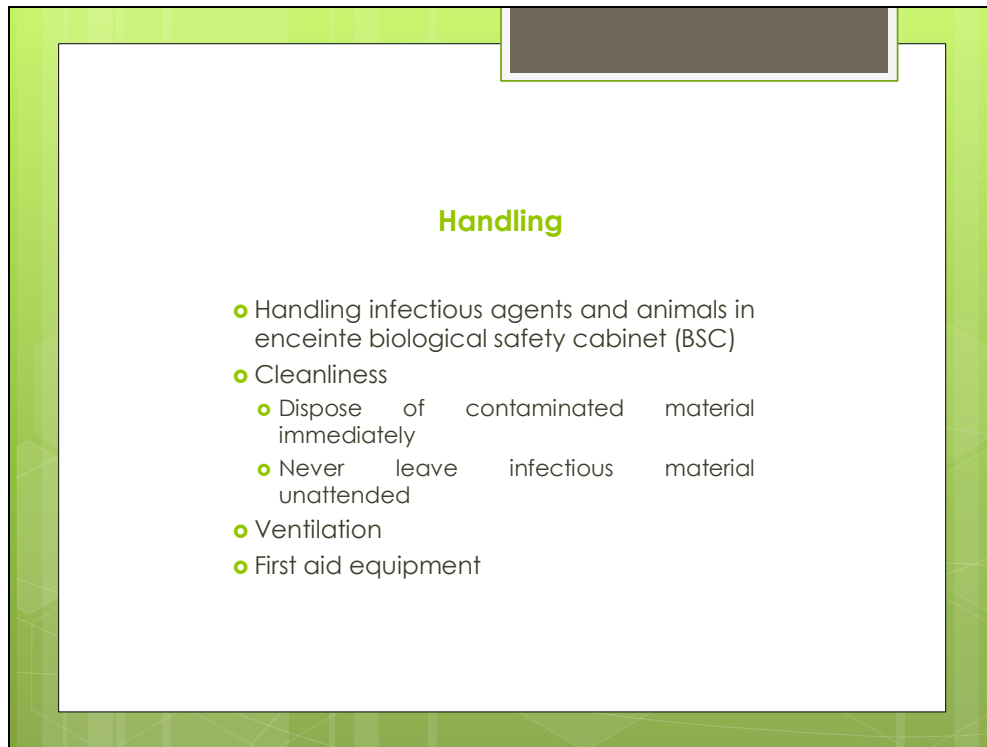
During autopsies on contaminated animals, be careful not to infect yourself with the sharp instruments you are using. Avoid using pointed instruments as much as possible. If you must, make sure you always put them in the same place, with the pointed ends facing in the same direction.

Instead of pins, use tape or bands to hold the animal in place on the work surface.

Disposable retractable scalpels can also be used to lower the risk of injury.

When disposable instruments are used, they must be disposed of in the designated solid biohazard containers immediately after use.

When reusable instruments are used, they must be soaked in the disinfecting solution in the BSC after use. Get in the habit of always pointing the sharp end in the same direction.



For maximum safety, always handle pathogens and infected animals in a BSC. Avoid creating aerosols. Things like handling litter, centrifugation, cleaning, and disinfecting can expose you to pathogens.

It is important to keep workspaces clean. A cluttered workspace is harder to disinfect and more likely to cause accidents and spills. Dirty equipment must be disposed of immediately after use. Don't bring in hard to disinfect items like books. If it won't be used for handling a pathogen or animal, leave it outside the CL2 room.

Always close doors behind you to maintain proper ventilation.

Know where the first aid stations are located (e.g., eye wash stations, emergency showers, first aid kits) before handling pathogens.



It is very important to always clean your work area before and after handling.

All material and equipment entering a CL2 environment must be disinfected before being brought out.

Floors and walls must be disinfected regularly to keep the work area clean and safe to use. Lab staff is responsible for this maintenance.

Decontamination

Question :

What is **abnormal** in the next picture ?





This person is not wearing gloves!

Decontamination

Basic principle: Material and equipment must be **disinfected** prior to disposal or reuse.

- All equipment must be **disinfected** before exiting CL2. This includes the **tools of external** workers.
- Provide only necessary equipment and avoid equipment that is difficult to disinfect (ex.: paper).

Material and equipment used for CL2 must be disinfected after every use and, if possible, sterilized in the autoclave before being reused.

Non-reusable equipment and waste can be incinerated by a specialized company.

Reusable equipment that is heat-sensitive or too large to fit in the autoclave must be decontaminated using other methods. In most cases, that means chemical disinfection.

The disinfectant used depends on factors like pathogen susceptibility, material resistance, and handler safety, among other things. Refer to the Public Health Agency of Canada pathogen safety data sheets. **If disinfection requires products other than those usually used by the lab, the research team must inform lab management and provide the right disinfectant for the infectious material.**

It may be useful to have a toolkit at the lab for workers from outside to use so they don't contaminate their own tools.



Decontamination

- Non-anatomical waste
 - Identified biohazard
 - Do not mix with carcasses
 - Sterilized in the autoclave by lab staff and discarded in domestic waste OR collected by a company specialized for incineration
- Carcasses
 - Identified biohazard
 - Collected by a company specialized for incineration

All animal waste (e.g., tissues, organs, carcasses, litter, blood and blood products, blood saturated items, bodily fluids) are considered biomedical waste.

Contaminated waste must be put through the autoclave or collected by a company specialized in this matter. Carcasses must not be put in with the other waste. They are also picked up by a specialized company and incinerated. In the meantime, waste and carcasses must be placed in hermetically sealed containers marked "biohazard."

Research teams are required to dispose of their waste and animal carcasses in the designated areas. The lab staff will then ensure it is processed properly.



Here are some different types of bags and containers with the biohazard logo you will see in the lab. Regardless of size or color, they all feature the biohazard logo.

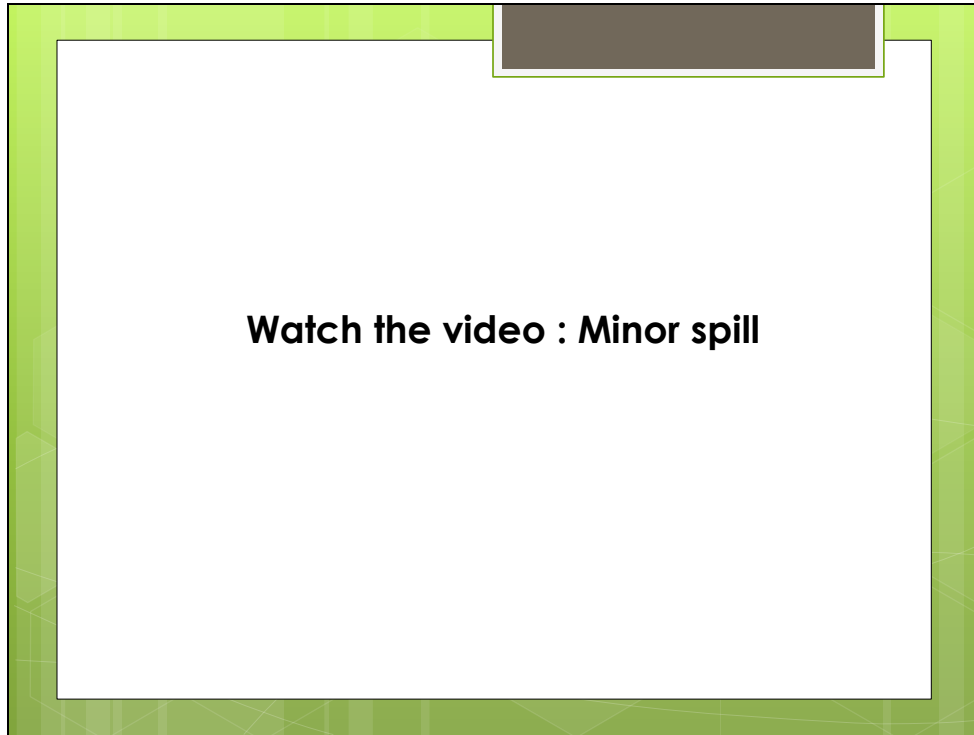
Colours are usually used to designate types of waste in hospital environments:

Red = human

Red or orange = animal

Yellow = non-anatomical waste (e.g., lab samples, bacterial cultures) and sharps

The research centre may process its own waste separately from hospital waste (e.g., carcasses picked up by Stericycle). In that case, the biohazard logo is what matters.

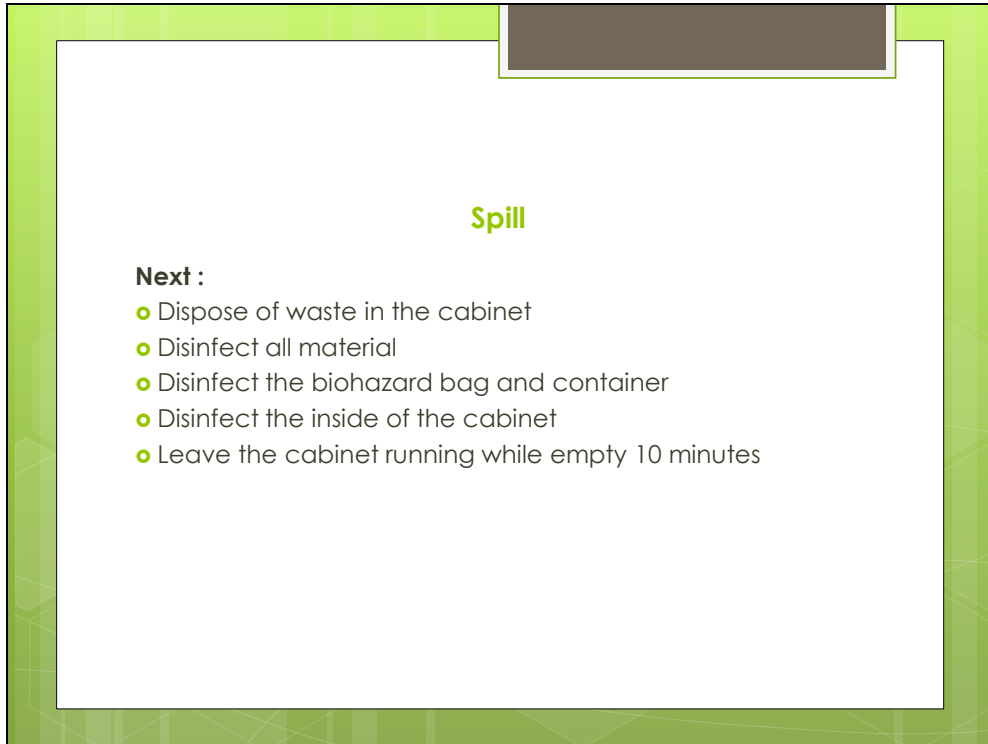


If a minor spill occurs inside the cabinet, you can clean it up yourself.

- Leave the BSC on.
- Move slowly to avoid compromising the effectiveness of the cabinet.
- Remove your second pair of gloves and place them in the cabinet, then remove any other potentially contaminated PPE. Put on fresh PPE before you continue. Be very careful to avoid contaminating yourself!

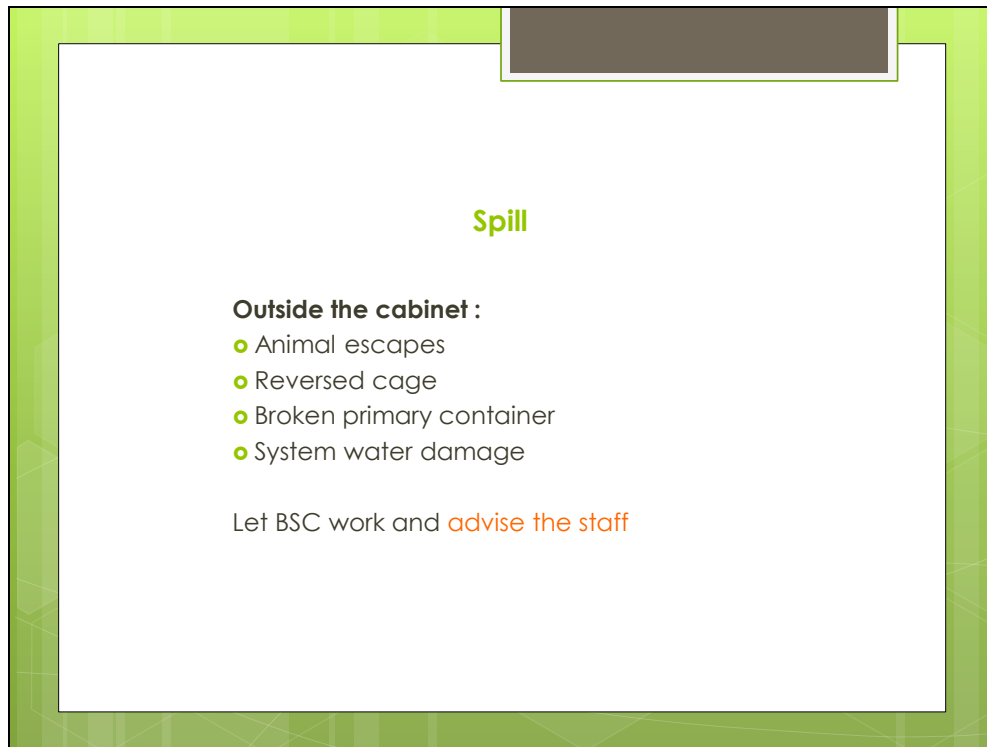
(If you are wearing only one pair of gloves, remove them carefully, making sure not to contaminate yourself, and put on a new pair **before** removing any other potentially contaminated PPE.)

- Lay paper towels on the spill and spray thoroughly with disinfectant, starting on the outside and working toward the middle to control any spills.
- Leave it for 30 minutes.



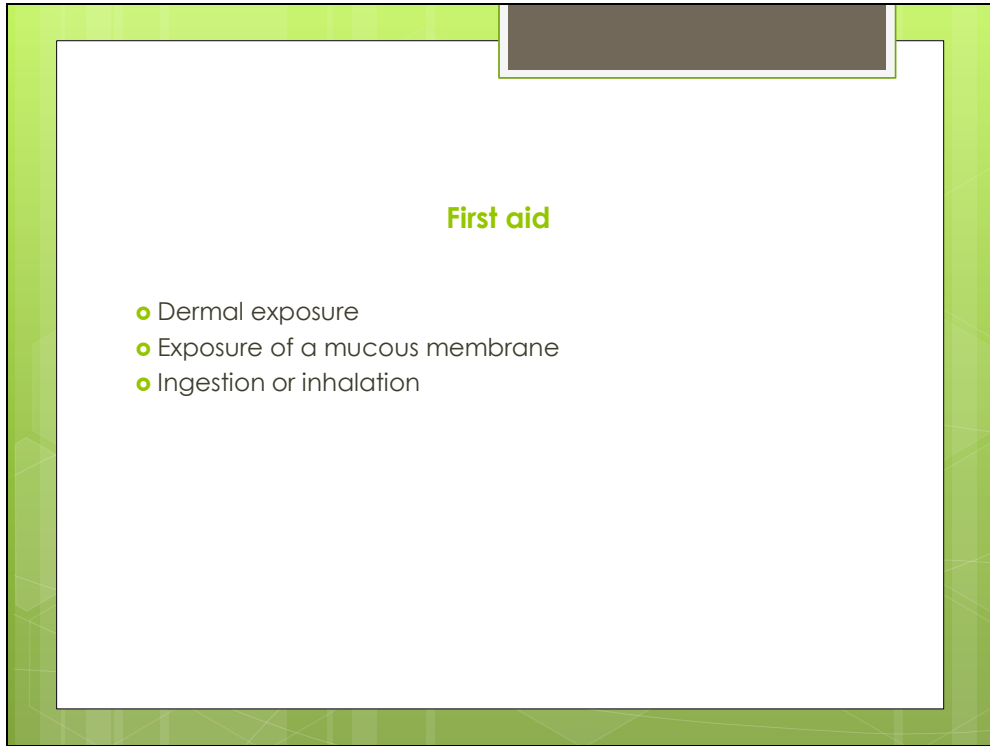
Next, clean up the cabinet as usual:

- Throw out all trash, including the paper towels placed on the spill, in a biohazard bag or container placed in the cabinet.
- Disinfect and remove everything inside the cabinet. If it can go in the autoclave, put it through.
- Disinfect the biohazard bag and/or container.
- Disinfect the inside of the cabinet and leave it running while empty for 10 minutes.



If a primary container of infectious material breaks inside a cabinet or cage or spills onto the floor, or if a rodent escapes onto the floor, leave the BSC on, leave the room as usual, and notify the staff. Technicians and employees will handle the cleanup.

The BSC can be used to filter suspended particles from the air in the room if necessary. The staff will clean up the spill safely (using a procedure similar to spills in the cabinet) and disinfect the floor and any other surfaces.



Any exposure to risk, whether by puncture, needlestick, mucous membrane contact (i.e., mouth, nose, eyes), ingestion, or inhalation requires immediate first aid.

First aid

In all cases :

- Go to emergency with Pathogen Safety Data Sheets (PSDSs)
- Ask to see an infectious disease specialist
- Appearance of symptoms = consultation
- Fill an incident report
- Return the report to supervisor

First aid is very important in cases of exposure to infectious substances or infected animals. In the event of exposure, remove your PPE and exit the room as normal. Proceed immediately to treat the infected area according to established rinsing and cleaning procedures.

Any kind of exposure to a pathogen requires a visit to the emergency room. If possible, bring the pathogen data sheet with you and ask to see an infectious disease specialist. Inform your immediate supervisor before leaving the facility. If you can't get in touch with them right away, inform them as soon as possible. An incident report will have to be filled out after the medical consultation.

If fever, infection, redness in the exposed area, or other symptoms appear in the days following the incident, see a doctor immediately. If possible, bring the pathogen data sheet with you.

If you have any questions, please sent them to formation@vrr.ulaval.ca or take notes and bring them with you during your practical training.

