

Biosafety manual for FuGU library, Biomedicum 1.
BSL-2 level laboratory

Version 2.0 (22.04.2020)

BEFORE ENTERING THE LAB:

No eating (e.g. chewing gum) or drinking is allowed.

Do not touch anything in the virus lab without gloves. Use double gloves (change outer gloves when necessary).

Using syringe needles, Pasteur pipettes and other sharps are not allowed.

No primary human cells are allowed, unless tested negative for HIV. No mycoplasma positive cells are allowed.

1. Personal health

Working in BSL-2 is not allowed with wounded or rashy hands. Persons with HIV are not allowed to work with lentiviruses.

2. Use personal protective clothing (PPC)

Put on gloves and lab coat before you enter the room. Use PPC designated only for working in BSL-2 lab. Add a second layer of outer gloves and oversleeves or use gloves with long wrist part as outer gloves. Safety glasses are optional. Only clothes and footwear that leave no bare skin are acceptable (no flip-flops, sandals).

Discard the outer gloves immediately to the waste bag inside the laminar in case of a spill and when you finish your work.

BEFORE STARTING WORK IN BSL-2 LAB

3. Waste collection:

- **Solid waste** is collected to small waste bags placed inside the laminar. After finishing work, close the bag with tape and put it to special waste container. Empty tip boxes are discarded to special waste. When the special waste bag is full, close with tape (double bags separately), mark it with QR code (TOY label) and leave in virus room.

Orange waste bag is only for waste not contaminated with viruses. Empty tip boxes can be discarded also to orange waste after they have been thoroughly decontaminated with ethanol and UV-radiation.

When the orange waste bag is full, close with tape and leave it outside virus room for the cleaning personnel to collect.

- **Liquid waste** is disposed with suction or collected into 500 ml plastic bottle containing 1 virkon tablet. When using suction, clean it with virkon and ethanol before and after use. Mark bottle with waste type (lentivirus, no virus), date and initials.

When the suction bottle is full, change it according to instructions. For sterile work, put a tip without a filter at the end of the aspiration controller.

4. Working outside the laminar

Material from the virus lab should not be taken out of the room before decontaminating with 1% virkon. As much as possible, work with disposable material. You can aliquot your reagents and medium before bringing them to virus lab.

WORKING IN BSL-2

Live cells cannot be taken out of virus lab unless RCV test shows negative results. Fixed or lysed cells can be taken out of BSL-2 (approved methods: common fixatives e.g. formalin, PFA; common lyse reagents, e.g. RIPA, SDS).

Dishes or plates containing recombinant viruses are not allowed to be opened outside of the laminar.

Any material to be placed into the laminar should be cleaned with 1% virkon and ethanol.

Reusable material should be cleaned with 1% virkon and ethanol before taking them out of the laminar. Change outer gloves, when contaminated.

Use filter tips. Mark your medium and cell culture plates clearly with your initials, date.

Spills inside the laminar

If there is no risk of spreading outside the laminar, spray the area with 1% virkon and ethanol, discard wiping cloth to special waste.

If there is a risk of virus spreading outside the laminar or aerosols were produced, clean the contaminated area, remove outer gloves, wipe the bottoms of your shoes if necessary and leave the room for 30 minutes. Put a sign to the door for other users and. Report the accident to the responsible person for safety.

Spills outside the laminar

Wipe the spills and the bottoms of your shoes if necessary with 1% Virkon and EtOH to avoid virus spreading even more. Use safety goggles if large amounts of viruses are spilled when cleaning the spills.

If there are any risks that the virus could spread outside the room or if it's known to produce aerosols, clean the contaminated area, remove outer gloves, wipe the bottoms of your shoes, if necessary and leave the room for 30 minutes.

Put a sign to the door for other users and for immediate emergency assistance, contact safety persons.

Clean the contaminated area and disinfect the surfaces with 1% Virkon solution. Dispose the waste as class 2 GMO waste.

Report the accident to the responsible person for safety.

Spills on clothing, skin, eyes.

If you spill virus to the lab coat or personal clothing take it off (if possible) and put it to an autoclave bag.

If you spill virus on your skin, dry with paper towel, wash the area well with soap, rinse with water (eye rinsing bottles are at the tap). If you spill virus on your eyes, rinse with abundant water, if necessary go to an eye clinic. After visiting the clinic, report the accident to the responsible person(s) for safety.

AFTER WORKING IN BSL-2

Clean suction with 1% virkon and ethanol and switch off. When collecting liquid waste in bottle, close it, shake well, incubate 24 h under laminar and discard the liquid to the sink in fume hood.

Clean the laminar with 1% virkon and then ethanol wipe off with paper towel.