

# BVC BIOSAFETY MANUAL

## For Room C505b

### Containment level 2 laboratory

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Biomedicum Virus Core



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# 1. INTRODUCTION

These rules and procedures are designed to allow the laboratory to be used with minimum of inconvenience and maximum of safety. However, no set of rules can substitute for careful working procedures, use of common sense and consideration of others.

Before you start your work here, please note the following

- All Biological Agents and Genetic Modification experiments must be filed and sanctioned by Board for Gene Technology/Geenitekniikan lautakunta ([www.geenitekniikanlautakunta.fi](http://www.geenitekniikanlautakunta.fi)). Filing GMM forms and obtaining approval is the responsibility of principal investigators (lab heads).
- The risk evaluation (Riskinarviointi) made for the Board for Gene Technology should include all methods and genetically modified organisms used in BSL2 work.

Furthermore,

- Our vectors and cell lines are not to be distributed outside of the Biomedicum Virus Core facility without the accepted MTA from the original source. Submitting and processing MTA's in completion is the responsibility of the principal investigator.

Before you can work in biosafety level 2 room:

- You must possess basic skills in normal cell culture techniques.
- Register as a BVC user and go through the biosafety training.

The procedures here are based on the regulations and recommendations of Board for Gene Technology/Geenitekniikan lautakunta and of CDC & NIH Biosafety guidelines: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition.

In Helsinki, November 7<sup>th</sup>, 2023

BVC management

## 2. GENERAL RULES

1. Room C505b is containment for biosafety level 2 biological agents.
2. No person will be allowed to work in this room unless they:
  - Have received adequate training in safe microbiological, virological or cell culture techniques. Generally, the applicant's knowledge on basic cell culture techniques is assessed during the registration interview and if satisfactory, this person is trained on the basics of safe microbiological/virological working methods. The completion of registration requires demonstrable understanding of the contents of this manual.
  - Has been registered BVC user or as STEMM user for this room.
3. The laboratory will be kept locked at all times. A current list of names of designated C505b workers will be kept as an electronic file by BVC personnel. The users can be traced investigating the Flexim lock usage if any misuse of the facility is suspected.
4. The laboratory doors must be kept closed during the virus work. Maximum number of people simultaneously working in the tissue culture room is 3.
5. Recombinant virus production is confined to Room C505b. However, transduced cell lines that have been tested for the absence of replication competent virus (RCV), can be transferred outside of the lab. If no such tests are available, the safety of the virus preparation for the environment has to be guaranteed by other means.
  - Fixed and lysed cells are allowed to be brought to BSL1 level laboratory without RCV test.
  - Approve fixing methods: Common fixatives e.g. formalin and PFA.
  - Approved lysing methods: Common lysing reagents that dissociate proteins e.g. RIPA and SDS.
6. Dishes/plastic ware that contain recombinant viruses are not allowed to be opened outside of the laminar.
7. No eating (even chewing gum) or drinking is allowed in the laboratory.
8. Don't use your phone inside the facility.
9. BVC staff is regularly checking and maintaining the incubators but customers are required to add more sterile water to the trays when they get empty and keep an eye of the CO<sub>2</sub> levels. The guide how to check the pressure of the bottle is found from the facility.
10. Hypodermic syringe needles, glass pasteur pipettes or sharps must not be used when working with infectious substances unless the BVC management has

been convinced that no alternative procedure is available. Disposal must only be via a “sharpsafe”.

11. Double gloves, dedicated BVC lab coat and if appropriate, sleeve covers, mask and safety goggles must be always worn. Discard the outer gloves to the small waste bag inside the laminar immediately in case of a spillage or when you finish you work.
12. Recordkeeping. This is your own responsibility and required by GTLK (Board for Gene Technology). For each experiment, record date, organisms used, viral titers and if the transduced cell lines are to be transferred outside of the BVC laboratory storages, data showing the negative RCV test.
13. Infectious solid and liquid waste are collected in certain way to separate containers. See below section 4. TISSUE CULTURE PROCEDURES IN BVC
14. Cell culture plates or trays holding the plates and reagent containers must all contain the following information: Date, your initials and your lab, cell line, virus type.
15. Every customer will be added to BVC emailing list. The list is used to inform customers about important issues and changes in schedules.
16. Working with HIV constructs is not allowed with wounded hand or with rash.
17. If a customer is using other psedotype viruses than VSVg, they need to contact the BVC personnel before starting their experiments.
18. Non-tested primary human materials (tissue, cells, cell lines, blood) are not allowed at any circumstances to BVC laminar hood or BVC incubator. Primary derived cultures are only allowed in STEMM laminar and STEMM incubator dedicated for such materials.
  - Wild-type HI-virus from patient samples can act as a helper virus to rescue the integrated vector into new viral particles to spread transduction beyond the original target cell (Sakuma et al, Biochem.J.2012)
19. Customers can use the ultracentrifuge in 5<sup>th</sup> floor for concentrating lentiviral particles. Please note that BSL1 substances are also used in the same room. Before starting, check the instructions from BVC webpage. The same guide has been printed next to the ultracentrifuge. The centrifuge rotor can be borrowed from the Klefström lab. Before this you need to reserve the rotor and ultracentrifuge separately with a calendar.

### 3. SPILLS AND EMERGENCIES

The principal investigator (PI) and/or lab personnel are responsible for initiating clean-up and disinfection in the event of a biohazard spill in a laboratory. Once the material has been contained, absorbed, and removed, housekeeping/facilities management should be contacted to finalise the clean-up and disinfection of the area. The PI is responsible for ensuring that all corrective actions and emergency procedures are followed in accordance with applicable University procedures and regulations. Every major spill must be immediately reported to a Biomedicum Virus Core management person.

A lab user who has caused an emergency situation is responsible for reporting the incident to the Board for Gene Technology within one day. Emergencies and dangerous situations can occur if there is a possibility that GMO spreads outside of the biosafety level 2 laboratory or if there is a considerable threat for health or environment. Always when emergencies happen, please contact first BVC manager Elina Hurskainen or core director Juha Klefström to assess the situation.

Spills inside the laminar:

- If there are no risks that virus could spread outside the laminar, just wipe the spillage with 1 % Virkon and 70 % ethanol. Change your gloves after they have been in contact with the viral matter.
- If there is any risk that the virus could spread outside the laminar 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 notify BVC personnel as soon as possible via phone, email or by visiting face to face in our office B508b. BVC will inform other users via our mailing list.

Spills outside the laminar:

- Wipe the spills and the bottoms of your shoes if necessary with Virkon and ethanol to avoid virus spreading. Use safety goggles if large amounts of viruses are spilled when cleaning the spills.
- If there is any risk that the virus could spread outside the laminar 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 notify BVC personnel as soon as possible via phone, email or by visiting face to face in our office B508b. BVC will inform other users via our mailing list.

### Spills on clothes/body:

- If you spill virus on the lab coat, remove it and place it into an autoclave bag. Close the bag and inform BVC personnel as soon as possible.
- If you get spills on your personal clothing, remove them if possible, spray the contaminated spot with Virkon and let it dry for few minutes. Don't leave the room at this point. After the Virkon has affected for few minutes, spray the contaminated area with ethanol. Wash the clothes as soon as possible.
- If you get spills on your bare skin or they absorb through your clothes, wash the spot with soap and water and disinfect it with 70 % EtOH. If you notice any cuts or rash in your skin, contact Occupational healthcare for further examinations. Inform the BVC management.
  - NOTE! The current BVC facility unfortunately does not have a sink or running water! During emergencies use the water in the facility and don't mind spilling it on the floor.

### Emergency numbers:

Juha Klefström cell phone 044-377 3876

BVC office phone 02941 25494 (office hours)

Ellisiv Nyhamar cell phone 0415792954

Please note! These numbers are for serious emergencies only, not to inform about reagents running low!

## 4. TISSUE CULTURE PROCEDURES IN BVC

### BEFORE ENTERING THE LAB

- Absolutely no coffee cups, food items, chewing gum etc are allowed in the facility.
- Notebooks, laptops etc. should be left in the front room. You can leave your keys or phone in your pocket but these should not be used inside the facility.
- Please note that if you take anything inside the BSL2 facility, these should not be taken out to a BSL1 anymore!
- Dress appropriately
  - BVC provided lab coat
  - DOUBLE gloves (change outer gloves when necessary)
  - Sleeve covers
  - Shoe covers (no outside shoes allowed!)
  - Safety glasses (highly recommended for lentivirus work)

### BEFORE STARTING TO WORK

- Collect all necessary cell culture material next to you (pipette tips, 50 ml falcons, pipettes, petri dishes, Virkon, waste bottles etc) to avoid unnecessary traffic in BSL2 room.
- Prepare the laminar hood: Wipe the hood with 1 % Virkon and 70% ethanol.
- Prepare the liquid waste container and solid waste bag.

### BVC WASTE RULES

#### SOLID waste:

- Collect all non-viral and non-hood-related solid waste outside to the waste box (orange for energy waste and black for mixed waste).
- Solid waste that has been contacting viruses or kept inside the laminar hood are collected to the small waste bag inside the hood.
  - Collect all used pipettes, tips and other waste that has been in contact with cells or viruses into this bag (NO SHARPS).
- After you are done seal the small bag with tape and place it inside the larger waste container.
- GMO solid waste container:
  - Double bags
  - When full seal the inner AND outer bags separately with tape, attach a QR-code on the side and leave the sealed bag inside the facility.

#### LIQUID waste:

- Collect all liquid virus waste to the 500 ml plastic bottles containing 1 tablet of Virkon (1 %). Virkon affects already in minutes but the waste is discarded by the staff earliest after 24 h incubation.
- Close all 500 ml plastic bottles containing 1 tablet of Virkon and your liquid waste. On the side of the bottle write the date, your initials/lab and virus type on a piece of tape and place it on the bottle.
- Leave used liquid waste bottle in the cardboard box under the water bath.

GLASS/SHARPS: You will not generate this type of waste. The facility is sharp safe!

#### AFTER FINISHING YOUR WORK AND BEFORE LEAVING THE LAB

- Ensure that all your cell culture plates or trays holding the plates contain the following information: Date, your initials and your lab, cell line, virus type.
- Close all plastic bags properly with tape. Place all non-contaminated material back to shelves.
- Wipe the laminar hood with 1 % Virkon followed by 70% ethanol.
- Take care of your waste.
- If the large waste bags are full, seal bot bags properly with tape. Attach a QR bar code on the side of the bag.
- Check that the facility is tidy and in order for the next user.
- Leave your gloves inside the facility. Leave your jacket and shoe covers in the front room.
- Mark your hood hours.
- Before returning to your lab, wash your hands in the nearest sink.

These procedures follow the recommendations and regulations by Geenitekniikan lautakunta ([www.geenitekniikanlautakunta.fi](http://www.geenitekniikanlautakunta.fi)) and NIH/CDC publication: Biosafety in Microbiological and Biomedical Laboratories (<http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm>).



## 5. BVC SERVICES

All the items listed below are included to the hourly fee for customers working in Biomedicum Virus Core.

- General facility supervision and support
- Partial personnel support
- Hood and facility use
- Basic facility supplies and equipment
- Registered customers are able to use EVOS® FL Imaging System which can be reserved through the Online Hood Hour Reservation Calendar (more information: <http://www.helsinki.fi/fugu/Products/BVC.shtml>)
- Decontamination and disposal
- Cell culture medias (DMEM), serum (FBS), penicillin/streptomycin - solution, L-glutamine, trypsin-EDTA, PBS, polybrene
- Cell culture plastics etc
  - 10 cm, 15 cm, 6-well, 12-well, 24-well, 96-well plates
  - Cell culture flasks
  - 5 ml, 10 ml and 25 ml pipettes
  - Pipettes and filter tips
  - Gloves (XS, S, M, L)
  - Syringes, filters and micro centrifuge tubes, 15 and 50 ml tubes

Please note! You may use your own cell culture medium, reagents, plastic etc in BVC but you would still be charged according to the basic service cost rate. Remember to mark your personal items!

Please visit our website for more information.

Following services can be ordered from Biomedicum Virus Core by sending an email to [bvc-support@helsinki.fi](mailto:bvc-support@helsinki.fi). These services are not included in the BVC user fee and will be charged separately.

- RCV and p24 tests for lentiviruses
  - RCV (replication competent virus)
  - p24 (the capsid protein titer test)
  - The tests will be performed every two weeks on Wednesday
    - Test dates can be found from the BVC Online Hood Hour Reservation Calendar
- Cell lines: 293FT, Phoenix Amphotrophic, Phoenix Ecotrophic, HeLa
- Lentiviruses, different scales (MINI, MIDI, concentrated 180 ul and 380 ul)
- Retroviruses

For further information, please visit our website or contact our customer service!

**GOOD LUCK WITH YOUR EXPERIMENTS!**

Remember to cite and mention us in your acknowledgements!