ML-II level, viral lab safety for room H3.318A

Access

• During the activities, doors of the laboratory must be closed.

• working in the laboratory is permitted only to those who receive permission for this from the

staff. You must be GMO approved and receive an introduction before starting your work from Jan Theys. A short introduction on viral work and procedures for this lab is given by Arjan Groot. You can be provided with key card access after your introduction.

Logbook

• A logbook is present in laboratory. If performing viral work a sheet must be filled in after your experiment. The logbook also has to contain your GMO overview our GMO number is IG10-012. When you belong to another group, you have to bring a copy of your GMO permit.

Hygiene procedures

• Before the activities begin, a sufficient quantity of disinfectant must be readily available.

•In the morning time open the night panel and remove any disinfected materials (pipettes) from the “inactivation barrel” and put them in a taped “biohazard” marked transparent plastic bag in the green waste container. Fill the “inactivation barrel” with freshly made bleach solution and turn on the UV for 20 minutes.

• The tabletop/flow hood on which the activities are being carried out must be disinfected after the activities are completed. In any case, disinfection must take place on a daily basis. Disinfection must be carried out immediately following any spills. Use bleach, 3 tablets in 5 L of water. Clean with water afterwards.

• The laboratory must be kept tidy and clean at all times, **all** waste needs to go into the green bin with yellow lid. After being sealed and disinfected all green bins will be collected and decontaminated under hospital regulations.

Personal hygiene

• When working in the laboratory, do not place anything in your mouth, therefore do not eat,

drink or smoke. Food, coffee cups, and other eating and drinking implements are not allowed in the

workspace.

• Avoid touching your face and hair with your hands; avoid manipulating contact lenses.

• For activities such as blowing your nose, use disposable tissues instead of cloth handkerchiefs.

• While working in the ML-II lab, do not wear rings, bracelets or wristwatches.

• Wash your hands always after handling potentially contaminated material and before leaving the laboratory. Washing with warm water and soap is recommended using your elbows opening the tap and using the soap dispenser.

Clothing

• Wearing a lab coat and gloves is mandatory. It is not allowed to wear these clothes outside the laboratory. When performing viral work, use the designated lab coats with a biohazard sticker for viral work. After use these coats should be autoclaved and washed daily.

• The lab coat must be buttoned when worn. The sleeves must entirely cover street clothes.

• The laboratory clothing must be changed frequently. The frequency of changing/washing/repairing

clothing can be in accordance with the normal building procedures, except when clothing is

contaminated by spills. The laboratory clothing must be autoclaved before sending to the laundry.

•When working with live viruses it is mandatory use **double** layered gloves and sleeve protectors in the viral safety cabinet. We use purple colored gloves throughout the lab and additional green ones when working with live virus. When using the microscope or incubator remove one green glove to handle equipment, so only with purple gloves you are allowed to touch equipment.

• If there are spills of potentially contaminated material on laboratory clothing, this clothing must be

autoclaved in a taped “biohazard” marked transparent plastic bag.

Work procedures

 • When working with viruses only **one** person can use viral safety cabinet at the time.

• **Never** use the vacuum system when performing viral work.

• During all procedures the occurrence of aerosols should be prevented as much as possible.

• All aerosol-producing activities must be carried out in the biological safety cabinet.

• If people worked with live viruses in the designated biological safety cabinet there must be a 30 minutes intermission before the next person can start working. This is to ensure that all possible virus containing aerosols are gone, that could potentially cross contaminate materials. Check and use the parking disc to indicate the time the safety cabinet was last used.

Centrifuging

• Use the centrifuge with the sealed bucket rotor when spinning down cells. When high speed centrifuges are being used for viral concentration purposes clean the reusable tubes with 70% ethanol/1% SDS twice and rinse them with water before putting them in a seal bag for autoclaving. **Don not** leave them in 70% ethanol/1% SDS solution as this will degrade the plastic and makes the tubes prone to breaking.

• Centrifuge tubes must be made of unbreakable material and must be closed in a leak-proof fashion

during centrifuging.

• Inspect the tubes for cracks and other imperfections before use and make sure the centrifuge still

closes properly.

• The centrifuge tubes should never be filled so that the liquid can contact the lid during centrifuging.

Therefore, they may only be filled to 4/5 of their volume; if angle rotors are used, they may only be

filled to 2/3 of the volume.

• If leakage occurs while centrifuging, the buckets have to be opened in the safety cabinet to prevent the spreading of aerosols and the tubes and the rotor must be disinfected (both internally and externally).

• The centrifuge must be regularly cleaned (e.g. inside of lid).

• Filling and closing the centrifuge tubes must take place in the biological safety cabinet.

• The use of hermetically sealed buckets (which can be autoclaved with their contents if leakage

occurs) is mandatory.

• The tubes must be inspected for leakage after centrifuging.

Pipette technique

• Using the mouth for drawing liquids into the pipette is forbidden.

• To prevent the formation of aerosols, it is also forbidden to blow out pipettes, unless direcly in the bleach solution in the “inactivation barrel”.

• The outflow of the pipette must be directed along a surface to prevent dripping.

• Pipettes must be plugged with a filter. Our sterile plastic pipettes have this filter.

• Only use disposable plastic pipettes. Glass pipettes are not allowed.

• Disposable pipettes and/or pipette tips that have been in contact with viral supernatants should be placed in the “inactivation barrel” with bleach (2 tablets in 2.5 L of tap water) in the flow cabinet after use. 30 minutes after you finish working the pipettes should be collected in a taped “biohazard” marked transparent plastic bag and be deposited in the green waste container and the bleach solution should be replaced by fresh bleach solution.

Injection needles and syringes

• The use of injection needles is forbidden.

• Syringes used for viral taps should be placed with their filter in “inactivation barrel” and be treated like contaminated pipettes.

• The use of syringes must take place inside the biological safety cabinet.

Biological waste

• The potentially contaminated material must be discarded into the green containers in the lab.

• Any virus contaminated pipettes must be after inactivation put in a taped “biohazard” marked transparent plastic bag in the green waste container.

• All used flasks, dishes, tubes, etc. should be carefully discarded in sealed in a “biohazard” marked transparent plastic bag in the green waste container.

• Gloves and sleeve protectors can be turned inside out and discarded in the green waste container.

• Non-heat resistant material should be decontaminated with 70% ethanol/1% SDS.

Decontamination following spills of potentially contaminated material

• First of all, wash your hands (and face if necessary) and change into clean laboratory clothing.

• Put on fresh double gloves

• Remove as many obstacles as possible from the contaminated area.

• Cover the contaminated area with tissues soaked in disinfectant (bleach 3 tablets on 5 L).

Wait at least 10 minutes. Then remove the tissues. Clean the bleach with water soaked tissues.

• After this, wipe up the material with tissues collect tissues in a “biohazard” marked transparent plastic bag and seal with tape

• Put the bag with waste material in the green container.

• Clean the surface with soap and water.

• After these activities, wash your hands thoroughly (take off your gloves).

• All waste material including towels, mops and mop water must be discarded in the green container.

• If it was a big spill notify the person responsible for the lab.

Cleaning workspace

• Regularly the laboratory must be cleaned. During this process, the floor and the work surfaces are mopped or wiped with warm water and soap.

• During cleaning activities, a laboratory coat and gloves must be worn.

Transport of material Internal

• The material must be packed in a hermetically sealed, leak-proof, unbreakable outer container that

is externally decontaminated before transport.

• The packaging must be provided with a biohazard sticker.

Work protocols for working in biological safety cabinets

 • Read and combine with “Hygiene procedures”

• Before starting the experiment, collect all the materials that are necessary.

• Walk beside the biological safety cabinet as little as possible and open the laboratory doors as little

as possible.

• Remove the night panel from the work opening and activate the biological safety cabinet.

• Ten minutes after activating the biological safety cabinet, wipe down the workbench and the edges

of the airflow with 70% ethanol/1% SDS 1%.

• Place the required materials in the workspace of the biological safety cabinet after wiping them

down with 70% ethanol/1% SDS.

• Check the operation of the biological safety cabinet. Disturb the airflow as little as possible while

carrying out activities in the biological safety cabinet (use slow arm movements and don not place to many boxes, pipets etc. in the cabinet).

• Always keep the exhaust slots at the front and the back of the worktable open.

• Disinfect the work surface of the cabinet after the activities are completed.

• After completing the activities, allow the exhaust fan of the biological safety cabinet to operate for

at least 20 minutes.

• Following any spills, leave the cabinet on overnight. Clean the contaminated surfaces.

• After cleaning, allow the exhaust fan to run for another 20 minutes.

• If the exhaust system of the biological safety cabinet stops working (for example, during a power

outage), the activities must be stopped immediately, any opened tubes must be closed, and the work opening must be closed with the night panel.

Incubator

• All dishes with cells producing viruses or being transduced should be stored in the incubator marked

“Viral Work” in special leak proof boxes to prevent viral spills in this incubator

• After being in culture for at least two weeks or being passaged at least three times after the last transduction cells can be considered virus free and be grown in the other incubators