



Karolinska
Institutet

Virus lab work guidelines

A list of rules and guidelines (Hanteringinstruktioner) will be available in the cupboard (D0411a). An Introduction for each new user is required before initiating any work in the room. This introduction will be given by the person in charge of the room or delegated to an appropriate replacement (virusfacility@ki.se).

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Good healthcare work environment and microbiological practice

1. Avoid rings, bracelets, untied hair, and other personal items which can impede good hygiene and contribute to the spread of infection interfering with sterile work.
2. No eating, drinking, applying cosmetics, using tobacco products or handling foodstuff within the working area.
3. Wear a dedicated BSL-2 cell culture lab coat (yellow/white striped, behind knotted) in the room but not outside the working area. Lab coats are washed/autoclavable and collected every Friday.
 - Clean lab coats can be picked up from the room for clean lab glassware (C0421).
 - Dirty lab coats should be placed in the waste room (D0431).
 - If you have a contaminated lab coat that needs to be autoclaved (decontaminated) before being washed, place it in an autoclave bag and label it with the autoclave label available in the quarter waste rooms (D0431).
4. Gloves are mandatory when working, even outside the laminar flow hood. Double glove when you are working in the hood.
5. If necessary, use a visor or such if there is a risk of body fluids splashing.
6. All surfaces are to be kept clean and tidy. You are responsible for leaving everything clean when you finish your work.
7. Always label everything with your name (initials), group name (initials) full date (2023-06-10) and contents. Unlabeled items will be discarded. If the material is hazardous, it should be clearly marked.
8. Sharp objects (i.e., needles, glass pipettes) should be disposed of accordingly using the yellow sharps container.
9. Use only distilled water in the equipment (incubators and water bath) and add 0.1% Hirschmann rea-stabil to the water (1 ml/liter water) to prevent the formation of bacteria, algae, and mold.
10. Avoid the formation and spread of aerosols, spillage, and spatter.
11. Large-scale spills and accidents should be reported immediately to your group leader, Jens Hjerling-Leffler (Scientific director: jens.hjerling-leffler@ki.se, 08-524 869 74) and to the VirusTech Core lab manager (virusfacility@ki.se), telephone: 08 524 873 87).
12. Have routines for dealing with unwanted events such as risk assessments and/or standard operation procedures.
13. On leaving the lab you are responsible for shutting off all equipment and turning off the lights.

DISCLAIMER: YOU ARE RESPONSIBLE FOR WHAT HAPPENS IN THE ROOM WHILE YOU ARE INSIDE IT.

Before entering the virus lab, the antechamber (D0411a)

1. Wash your hands.
2. Tie up your hair and put on a hairnet then remove rings, bracelets or other personal items which interfere with sterile practice.
3. Cover your shoes with blue shoe covers. If you prefer, bring a clean set of shoes to wear inside the cell culture room.
4. Put on the yellow/white striped lab coats. You can find them in room C0421. Lab coats are washable and autoclavable and should not be used for longer than 5 working days. On Fridays, all coats will be collected and sent for washing/autoclaving.
5. Put on one pair of gloves (EcoShield nitrile 250, green).
6. Do not enter if someone has the incubators open.
7. Before entering the room make sure the sluice's door is closed
8. No more than three people are allowed at the same time in this room.

Working in the Biological Safety Cabinet Class 2 (BSC-2)

Basics of the laminar flow hood

1. The laminar flow unit in the lab is a BSC class 2 which means it protects both the specimen and the operator.
2. Wear gloves when working in the hood.
3. Avoid moving your hands in and out of the hood. Use slow, smooth movements.
4. The hood should be appropriately disinfected before and after use.
5. Any spillage should be immediately cleaned up.
6. Set up BSC-2 with a clean portion, a work area, and a contaminated area. Do not move contaminated items over clean items.
7. Do not block the front grill and rear grill with items that may provide a contamination route.
8. The hood functions better when there are fewer things in the cabinet: every object disturbs the flow in a diameter 2,5 times its size and this area is not assured to be sterile.
9. Depending on the kind of work you are doing, additional care must be taken.

Prepare to work in the BSC-2 hood.

1. Create a checklist of work materials that you need to handle within the BSC-2.
2. Open the protective glass of the BSC-2 hood.
3. Switch ON the hood (ON/OFF button), light and ventilation.
 - Please see below "The virus lab equipment (page 7)" for detailed instructions
4. Wait 5 minutes for proper airflow before starting working. The flow rate should be NORMAL (green LED on).
5. Decontaminate the back side, side windows, sash (front window), and metal surfaces

by wiping them down with 70% ethanol at the beginning of each experiment.

6. Put a new autoclavable bag in the holder.
7. Place a waste bottle (size: 500 ml) with one Virkon tablet and 100 ml of tap water inside the hood to collect liquids and pipette tips.
8. Decontaminate your items (filter tip boxes, pipettes, pipette controller, etc.) with 70% ethanol-soaked paper and load them into the BSC-2.
9. Keep 1% Virkon solution inside the hood to be used immediately in case of spillage. Virkon is only active for 7 days after preparation (should be pink when it's active). Prepare fresh Virkon every week (preferably on Mondays). Make sure to write down the date the Virkon solution was made.

Start virus work in the BSC-2 hood.

1. Put on an outer pair of gloves (SHIELDskin nitrile 300, orange). This outer pair should cover the cuffs of the lab coat.
2. Always use two pairs of gloves when working with viruses (inner pair: green, outer pair: orange). If you spill anything on the external pair (orange), remove it, place it in the autoclave bag and take a new pair of orange gloves.
3. The outer orange pair of gloves always stay inside the BSC-2 hood. For example, if you have forgotten something outside the hood take off the orange gloves, discard them in the yellow autoclave bag, put on a fresh pair of orange gloves and take what you have forgotten and continue working.
4. Put the solid waste in the yellow autoclave bag (serological pipettes, plates, etc.).
5. Liquids and pipette tips (they can puncture the autoclave bag) must be pipetted in a 500 ml solid waste container (1 Virkon tablet + 100 ml of water) and properly sealed before disposing them in the yellow autoclave bag.
6. Any spillage inside the hood should be contained with a 1% Virkon solution. Pour 1% Virkon solution on the spillage, place paper towels on it to soak up the liquid and dispose paper towels in the yellow autoclave bag (here the Virkon can act). Clean the area again with 1% Virkon solution and remove residual Virkon with plenty of 70% EtOH and discard the paper towels into the yellow autoclave bag.
7. Please also check underneath the working area for any spillages.

Finish working in the BSC-2 hood.

1. Close your liquid waste container and toss it in the yellow autoclave bag.
2. Take off your outer layer of gloves (orange) and put them in the autoclavable bag, close the bag.
3. 1% Virkon and 70% EtOH decontamination
 - a. Decontaminate your items (filter tip boxes, pipettes, pipette operator, etc.). Spray them with 1% Virkon and let them stay in the hood for **no less than 10 minutes** to guarantee efficient disinfection.
 - b. In parallel completely spray the interior of the hood with 1% Virkon. Spray the metal surfaces, back side, side windows, the inside of the front window and the armrest. Contact time **no less than 10 minutes to guarantee an efficient disinfection.**
 - c. Also spray the yellow autoclave bag with 1% Virkon. Contact time is **10 minutes.**

4. After the 10-minutes Virkon-mandatory contact time.
 - a. Toss the yellow autoclave bag in the yellow infectious waste container.
 - b. Items: Remove residual 1% Virkon with 70% ethanol-soaked paper towels, wipe them dry and remove the items from the hood. Paper towels can go into the yellow infectious waste container.
 - c. Wipe off residual 1% Virkon solution from all sprayed hood surfaces. Paper towels can go into the yellow infectious waste container.
5. Then wipe all Virkon treated hood surfaces (metal surfaces, backside, side windows, inside the front window and arm rest) with plenty of 70% ethanol, to remove all residual Virkon. Paper towels can go into the yellow infectious waste container.
6. Close the sash of the hood.
7. Switch off the hood.

Before leaving the virus room to the antechamber (D0411a)

1. Turn off all the equipment when you are finished. Check the microscopes, centrifuges, and hoods.
2. If the yellow infectious waste container is full, close it tightly, fill in your information in the "**smittförande ämne**" label and take it to the quarter waste room (D0431). Always bring a clean new container.
3. All surfaces are to be kept clean and tidy. You are responsible for leaving everything clean when you finish your work. Remember to place all your reagents and plastic ware in the proper fridge/freezer, closet/drawer or take them with you.
4. Enter the sluice and close the door behind you. Never leave both doors open (virus room and antechamber) at the same time.

Before leaving the virus lab antechamber

1. Leave your lab coat on the hanger by the door in the antechamber.
2. Trash the used blue shoe covers in the yellow waste container.
3. Remove hair cover (yellow waste container).
4. Remove your inner layer of gloves (green) (yellow waste container).
5. Wash your hands.
6. Turn off the lights and close the door behind you.

The virus lab equipment

- Take care of the equipment.
- Report if there are any issues with it.
- Always turn off the equipment when you finish using it.

BSC-2 hoods

ESCO Class II Airstream AC2 G3

Turning on the BSC -2

1. Raise the sash to the indicated normal operational height (READY state). The lamp will turn on when this height is reached.
2. Turn on the fan by pressing the FAN button. This will start the warmup procedure (default: 3 minutes). All buttons are disabled during warm-up period.
3. After default 3 min you can start working.

Turning off the BSC-2

1. Turn off the fan by pressing the FAN button.
2. Lower the sash to the fully closed position.

Labome Mars Silence Biosafety Cabinet class 2

Turning on the BSC-2

1. Raise the sash to the indicated normal operational height (arrow button up).
2. Turn on the fan by pressing the FAN button (green). There will be an alarming sound till the fan reaches its operational speed. You can silence this alarm, but it will automatically stop after a while.
3. Turn on the light (blue).
4. Wait another 5 min before starting to work.
5. The BSC is ready for work.

Turning off the BSC-2

1. Turn off the fan by pressing the FAN button (green).
2. Lower the sash to the fully closed position (arrow button down).
3. Turn off the light (blue).

Centrifuges

Beckman Coulter Avanti J-15R

- Suited for 15 ml, 50 ml, 500 ml centrifuge tubes and plates (96, 48, 24, 12, 6-well).
- Check maximum centrifugation speeds.
 1. Tubes: Max 4750 rpm
 2. Plates: Max 4450 rpm
- When centrifuging virus-containing liquids always use bucket covers.

- After use, please leave the lid open so that any water condensate can evaporate.

Thermo Scientific Sorvall ST 16

- Suited for 15 ml, 50 ml centrifuge tubes.
- When centrifuging virus-containing liquids always use bucket covers.
- After use, please leave the lid open so that any water condensate can evaporate.

Thermo Scientific Sorvall Legend Micro 17R

- Suited for 1.5 ml and 2 ml microcentrifuge tubes.

Microscopes

Motic AE31

- Inverted microscope for cell culture vessels.
- Brightfield with a halogen lamp.
- HXP metal halide lamp for fluorescence.
- HXP lamp is connected to a 60 min timer with 15 min intervals. Press 2 times to turn on the lamp for 30 minutes. This is important for the longevity of the lamp.
- GFP and RFP/mCherry filter sets.
- Microscopes should be left clean.
- Lenses are only to be cleaned with Kimwipes.
- It is good practice to disinfect the stage after using microscopes.
- Switch off the microscope once you are finished (Halogen lamp).

Zeiss Axiovert 40 CFL

- Inverted microscope for cell culture vessels.
- Brightfield with a halogen lamp (potentiometer knob has a loose contact and is fixed to one position, please do not touch it).
- Mercury lamp HBO 50 for fluorescence.
- Mercury lamp is connected to a 60 min timer with 15 min intervals. Press 2 times to turn on the lamp for 30 minutes. This is important for the longevity of the lamp.
- GFP and RFP/mCherry filter sets.
- Microscopes should be left clean.
- Lenses are only to be cleaned with Kimwipes.
- It is good practice to disinfect the stage after using microscopes.
- Switch off the microscope once you are finished (Halogen lamp).

Incubators (Incubator A and B)

- Keep the incubators open for the minimum time possible.
- Always label your flasks and plates with your name and full date.
- Always check the water level and color (blueish from Rea-Stabil, left cupboard D0411). If the water tray is at low levels or empty fill up with sterilized distilled water (available in room C0421), add 1 ml of Rea-Stabil per liter water (1:1000 dilution).
- If you spill contaminated liquids in the incubator, pour 1% Virkon solution on the contaminated area, put paper towels on it and let it act for at least 10 minutes. Discard paper towels in a yellow autoclave bag. Then clean the area with 70% ethanol-soaked paper towels and discard them into the yellow autoclave bag. Close the bag and toss it into the yellow infectious waste container.
- Incubators will be deep cleaned every third month by the VirusTech Core facility.
- The other incubators belong to CRISPR Functional genomics and MolNeuro. These incubators have restricted access. Do not store your cells there, if you are not affiliated with them.

Water Bath

- The water is replaced by lab Armor beads, which drastically reduces the risk of contamination. It may take a bit longer to warm up your liquids.
- The beads temperature is set to 37°C please do not change it.
- Do not turn off the water bath, it can stay on.

In case of an accident

Spillage on gloves:

1. Remove your outer pair of gloves (orange), put them in the autoclave bag and take a new pair.

Spillage on the laminar flow hood surface:

1. Spillage on the laminar flow hood working area:
2. Please also check underneath the working area.
3. Pour 1% Virkon solution on the spill (no- spraying on spills! Spray can cause aerosols!)
4. Put paper towels on the spill.
5. Let it soak up all the liquid.
6. The Virkon continues to decontaminate in the wet tissue.
7. Therefore, you can discard the paper towels into the yellow autoclave bag.
8. Clean the area again with 1% Virkon solution.
9. Then remove residual Virkon with plenty of 70% Ethanol and discard the paper towels into the yellow autoclave bag.
10. Discard your orange gloves in the yellow autoclave bag.
11. Put on new orange gloves and continue working.

Spillage on the lab coat:

1. Remove the lab coat and put it in an autoclave bag (clear plastic bag, can be found in room D0411a).
2. Seal the autoclave bag and label it: "to be autoclaved" label.
3. Send the lab coat for autoclaving. Place the labelled bag in room D0431.
4. When autoclaved it can be unpacked and sent for washing (Lilac washing bag in quarter waste room D0431).

Spillage on the floor of the virus lab:

1. Exit the room and wait 15 min to let any possible aerosols to settle. If you leave the room area, make sure to put a note on the door warning others not to enter!
2. After 15 min re-enter the room.
3. Put on a new pair of orange gloves.
4. Pour 1% Virkon solution on the spill (no spraying) then add Vermiculite (in room D0411, White bucket) on the spill (or paper towel if the spill is not too big).
5. Let Virkon work for no less than 10 minutes.
6. Discard vermiculite or paper towels into a yellow autoclave bag.
7. Clean floor with 1% Virkon and then with plenty 70% ethanol using paper towels, discard them into the yellow autoclave bag.
8. Discard your orange gloves into the yellow autoclave bag.

9. Seal the bag and discard it in the yellow waste container.
10. Report the spillage to facility manager.

Personal exposure to contaminated equipment/material

On intact or wounded skin, upon injury with a contaminated object.

1. Rinse your skin thoroughly (10 min) in the sink and disinfect it with disinfectant. Clean and bandage the wound if there is one.
2. Report the incident to your supervisor, safety representative and/or VirusTech Core personnel and preferably report the incident together. In case of serious incidents, the manager should always be contacted.
3. Report your incident here: <https://staff.ki.se/reporting-incidents>.
4. State the location or situation so that the matter can be followed up and create a basis for development and improvement of the work environment.
5. Seek medical attention if necessary.

Contact on the eyes:

1. Rinse your eyes thoroughly with the eye wash near the sink in room D0411a.
2. Report the incident to your supervisor, the coordinator, facility manager and seek medical attention.

Name	Responsibility area	Phone number	E-mail
Manager	VirusTech core facility manager	08 524 873 87	virusfacility@ki.se
Akos Vegvari	Biosafety Delegate	08-524 877 07	akos.vegvari@ki.se
Carina Bengtson	KI Biosafety Coordinator	08-524 862 89	carina.bengtsson@ki.se
Karolinska Solna Infectious disease unit B2	Infections	Professor Anna Färnert 08-517 752 85 Administrator Sahra Bunner 08-524 815 56	Karolinska Universitetssjukhuset Solna Infektionskliniken, NB6:02 171 76 Stockholm

1% Virkon solution

- Prepare 1% Virkon in spray bottle fresh every week.
- 1 tablet of Virkon (5 g) in 500 ml of liquid gives a 1% Virkon solution.
- 1% Virkon solution lasts for about 7 days.
- Virkon is only active when it has a pink color.

Waste management

Yellow infectious waste container

1. When the container is full.
2. Close it tightly.
3. Fill in your information on the label (biologiskt avfall, Smittförande ämne).
 - i. Sjukhus/motsv: KI Biomedicum
 - ii. Avd/motsv: VirusTech
 - iii. Name: Your name
 - iv. Date: JJJJ-MM-DD
4. Paste the label on the yellow infectious waste container.
5. Bring it to the quarter waste room D0431.
6. Bring clean new containers and store them under the sink in room D0411a.

Cardboard boxes

- Empty glove boxes for example.
- Can be recycled.
- Discard them in room D0431.

Empty plastic bottles

- Such as empty media, PBS bottles, etc.
- Can be stored in yellow bin labelled "Empty bottles".
- If full you can discard some of them in room D0431.