

Standard operasjonsprosedyre: Håndtering av dyr med virale vektorer eller GMM

SOP nr: 12-14

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## HANDLING ANIMALS WITH VIRAL VECTORS OR GMOs

### 1.0 PURPOSE

- 1.1 To ensure that everyone working with biological vectors or gene modified organisms (GMOs) fully understands the inherent risks.
- 1.2 To ensure that experiments are carried out in the correct way and that suitable measures are in place in order to reduce the risk of infectious pathogens spreading to the Section of Comparative Medicine (KPM) via biological vectors or GMOs.
- 1.3 To provide guidelines to employees, users and the laundry on how to handle animals and equipment that has been in contact with biological vectors or GMOs and on the correct use of PPE (personal protection equipment).

### 2.0 DIVISION OF RESPONSIBILITY

- 2.1 All SOPs can be found at our [homepage](#). You should familiarize with all SOPs relevant to your project.
- 2.2 Everyone who handles animals at KPM, IMB, must at all times be familiar with the hygiene rules described in SOP 2-01 "Attire in MDU".
- 2.3 Everyone who works with biological vectors or GMOs in the MDU, Conventional and KPM must at all times follow the rules set out in this SOP (SOP 12-14) and be familiar with SOP 14-01 "Internal and external communications relating to projects".
- 2.4 Injecting or working with biological vectors or GMOs inside the Barrier unit is forbidden.

#### **KPM is responsible for the following:**

- 2.5 The room manager is responsible for informing and demonstrating the routines for quarantine rooms (for example DU-008a) for KPM users before the start-up of projects. This includes a demonstration of how to handle cages with biological vectors/GMOs and also the use of the class 2 safety cabinet, lab coats and other PPE. In addition, the room manager will explain to users that the cages must not be sent to the laundry, but will be taken care of by the room manager.
- 2.6 The room manager is responsible for maintaining quarantine measures for the animals and for making sure that their cages are labelled in the correct way by the user. In the case of minor lapses, such as the unsatisfactory labelling of the cages, these will be raised with the user directly. If more serious deviations occur, such as failing to adhere to quarantine rules, these will be reported to the PMSK.



- 2.7 The room manager is responsible for changing the cages of animals injected with biological vectors or GMOs, both at the end of the quarantine period and when normal cage changing routines are followed. The cages must be changed according to the protocol described in point 3.10 below.
- 2.8 The room manager is responsible for making sure that chlorine is available throughout the whole quarantine period in locations where animals with viral vectors are to be changed and handled.
- 2.9 The HSE coordinator is responsible for making sure that chlorine waste is handled in the correct way.
- 2.10 When new biological vectors/GMOs are to be introduced that are not listed in point 4.5 in the table below, the HSE coordinator and the PMSK are responsible for deciding whether the quarantine period should be extended or whether to do different procedure with the changing of cages and daily monitoring of animals. In this case, the HSE coordinator will continually update the table to include the new biological vectors or GMOs.
- 2.11 The PMSK and the section manager are responsible for assessing the risks involved in the use of biological vectors or GMOs and the possible consequences for MDU, conventional and KPMe. The HSE coordinator can play an advisory role by reviewing and explaining the safety data sheets (SDS) relating to biological vectors and GMOs.
- 2.12 If the SDS for a specific viral vector/GMO is not available, the PMSK, the HSE coordinator and the users must arrange a meeting to discuss the risks surrounding its introduction to the section and the measures that should be implemented.

**The KPM user is responsible for the following:**

- 2.13 The KPM user is responsible for submitting the SDS and the completed form «HSE declaration and risk assessment for animal experiments», and must wait for approval on this before starting an experiment.
- 2.14 In the case of self-made viral vectors/GMOs, a risk assessment of the preparation must also be submitted to the HSE coordinator before it is brought into KPM. If necessary, a meeting between the KPM user, the HSE coordinator and the PMSK should be arranged.
- 2.15 In the case of any changes in FOTS, the KPM user is responsible for informing the PMSK of this and must wait for approval before starting the experiment.
- 2.16 The KPM user is responsible for following the correct procedure for cells, biological vectors or GMOs before they are imported to MDU, conventional or KPMe.
- 2.17 The KPM user is responsible for informing the room manager of the start-up time for the experiment and the time interval involved. The email about the start-up must be sent to [komparativ@basalmed.uio.no](mailto:komparativ@basalmed.uio.no) and the user will subsequently be contacted by the room manager.
- 2.18 The KPM user is responsible for following KPM rules governing the handling of biological vectors/GMOs and according to the relevant biosafety level (BLS). The normal procedure must always be followed, including the labelling/marketing of cages, notes in Science Linker and the use of PPE, class 2 safety cabinets and the correct disinfectant etc.
- 2.19 KPM users must clearly notify the room manager if they wish to change the cages themselves, in which case the room manager will go through the cage changing routine with the KPM user (see point 3.10). At the end of the quarantine period, including the first cage change, no special rules apply as to who changes the cages.

- 2.20 KPM users who use safety cabinets and laboratories outside MDU, conventional and KPMe are responsible for ensuring that chlorine or another suitable disinfectant are available in locations housing animals and cages.
- 2.21 In cases where this SOP 12-14 is not suitable for a particular experiment, the KPM user is responsible for creating his/her own SOP on the handling of animals with viral vectors/GMOs and for ensuring that the new SOP is readily available in the rooms where such animals are housed.
- 2.22 The KPM user is responsible for labelling the cages with a note informing that the SDS is available in the room and including other information relevant to the experiment.

### 3.0 PROCEDURE

- 3.1 The KPM user must follow the supplier's recommendations when preparing biological vectors/GMOs. If other procedures are to be used, the supplier must be contacted and must approve the new procedure. You are **not** allowed to carry out this procedure in KPM laboratories or animal housing rooms. Only approved laboratories in the MDU, Conventional or KPMe can be used for work with vectors/GMOs. The laboratory in question must be entered on the FOTS application.
- 3.2 The KPM user must be aware of the risk involved in the development of living organisms and must inform KPM of the extent of the risk at least two weeks before the start-up of the experiment. This information must be provided by submitting the SDS from the supplier and by filling out an HSE declaration. If no SDS is available, the user must draw up a risk assessment and arrange a meeting to discuss the risks involved.
- 3.3 Biological vectors/GMOs classified as BSL-1 or BSL-2 are permitted in the MDU and Conventional unit. In KPMe, only those classified as BSL-1 are allowed. **Importing biological vectors or GMOs classified as BSL 3 or BSL 4 into the Section of Comparative Medicine is forbidden.**
- 3.4 **The handling of BSL-1 biological vectors or GMOs (applies to both KPMe, conventional and MDU):**
- The injection of biological vectors/GMOs into animals must be carried out on a class 2 safety cabinet and in an approved laboratory (see appendix 2 for an overview of laboratories in the MDU).
  - The safety cabinet must be turned on at least 4 minutes before it is used.
  - A small yellow container for needles must always be at hand when giving injections on the safety cabinet, and needles must be placed in this container immediately after use.
  - Two pairs of gloves must be used and these must be disposed of as hazardous waste in the yellow container after use.
  - All disposable equipment that has come in contact with the preparation must be disinfected with a suitable agent before being disposed of as hazardous waste. Remember to use the correct PPE for the disinfectant concerned.
  - After injection, the animals are placed in a clean cage and are taken back to the animal housing room.

- The cages must be given a label showing the type of biological vector/GMO, the date of injection and the date for the end of the quarantine period.
- The cages must be taped along the edges between the lid and the cage to show that the animals are in quarantine, and the tape must be marked with stripes to indicate that the cage must not be opened.
- In rooms where an SDS is available, this should also be indicated on the cage labels.
- During the quarantine period, the cages must not be opened unless strictly necessary and then only when the cage(s) in question are placed on a class 2 safety cabinet (see point 3.5).
- At the end of the quarantine period (72 hours), the animals must be moved to a clean cage, as described in point 3.10.
- After the end of the quarantine period and the cage change, the cage(s) can be handled in the same way as other cages in the room but the note with information about the biological vectors/GMOs must remain on the cage(s).

3.5 If it is necessary to open the cages during the quarantine period, this must be carried out on a class 2 safety cabinet (see illustration 1):

- The safety cabinet must be turned on at least 4 minutes before use.
- All transport of cages must be carried out using a trolley.
- A lab coat and two pairs of gloves must be used, and the inner, long pair must well pulled up over the sleeves of the lab coat.
- After the inspection has been completed, the surface of the safety cabinet, the trolley and the outer pair of gloves must be disinfected with a suitable agent. Then throw both pairs of gloves into a yellow container and wash your hands with soap.

3.6 After euthanising animals, or if dead animals are found, the cages must be opened and the cadavers removed on a class 2 safety cabinet:

- Follow the hygiene and clothing instructions, as described in point 3.5 above.
- Have a cadaver bag at hand and place the cadaver in it.
- Tie up the bag on the cabinet and complete the cleaning procedure, as described in point 3.5.
- Apply chlorine to the cadaver bag and place it in the freezer.

3.7 When inspecting animals in quarantine, their cages must not be opened unless strictly necessary. They can be taken off the rack and inspected without opening the lid. Gloves must be discarded immediately after handling these cages and must not be used to touch other surfaces.

3.8 Surfaces that have been in contact with the cages must be washed with soap and thoroughly disinfected with a suitable disinfectant. We recommend Klorin (chlorine), as this has been proven to neutralise the vast majority of viral vectors. Chlorine must be diluted at a ratio of 1:3. This applies to all surfaces where cages or animals have been, such as a safety cabinet, LAF bench or trolley. Two pairs of gloves and protective glasses must be used and both pairs of gloves must be discarded once cleaning has been completed.

## 3.9

**Animals with BSL-2 biological vectors/GMOs can only be housed in the tox room in the MDU:**

- Injections of BSL-2 must be given on a class 2 safety cabinet in room DU-008.
- All mice injected with biological vectors/GMOs classified as BSL 2 must be housed in a separate quarantine room (for example DU-008a) for the entire lifespan of the mice.
- Animals with biological vectors/GMOs described as BSL 2 to BSL 1 (BSL2/BSL1) in the SDS must be put in quarantine in the tox room for the number of prescribed hours and then be moved to a normal housing room (see overview in table 4.5). The cages must be changed before moving the animals, as described in point 3.10 below.
- The mandatory clothing for use in quarantine rooms and for injecting BSL-2 biological vectors/GMOs is shown below (see under References point 7.2 below):



Lab coat



Two pairs of gloves



Protective glasses



P3 face mask/full mask

- **NB! In the tox room DU-008a there is a fume hood for chemicals but no class 2 safety cabinet. Animals housed in DU-008a and injected with biological vectors/GMOs must be handled on a class 2 safety cabinet in room DU-008, see the room plan (appendix 2).**

**3.10 How to handle animals that have been in quarantine and are to be given new cages (both BSL 1 and BSL 2):**

- Ask the laundry whether the autoclave will be available for use. If it is unavailable, cage changing must be postponed.
- Turn on the class 2 safety cabinet at least 4 minutes before use.
- Fetch a paper bag, autoclave tape and a lab coat, if necessary, from the storeroom in the MDU. There is room for 6 cages of category 500 in each bag.
- Prepare the disinfectant, for example a solution of 100dl Klorin (chlorine) to 300dl of water. Use PPE when handling Klorin.
- Fetch as many trolleys as you need. **Trolley no 1 should be used for dirty cages and trolley no 2 for clean cages.**
- Put on a lab coat and two pairs of gloves – the inner, long pair must be pulled well up over the sleeves of the lab coat.

- Take trolley **no 1** to the animals, place the cages containing mice on the trolley and move them to the safety cabinet.
- Open the paper bag and put it in an easily accessible place on the lower level of trolley **no 1**.
- Place a yellow hazardous waste container near at hand for discarding dirty gloves and paper.
- Put on clean outer gloves, fetch one clean cage from trolley **no 2** and position the cage on one side of the safety cabinet (now the clean zone).
- Fetch one cage from trolley **no 1** and position it on the other side of the cabinet (now the dirty zone).
- Open the dirty cage, discard the outer pair of gloves and put on a new pair of gloves. Move the mouse from the dirty to the clean cage.
- Discard the outer gloves once again and put on a new pair, close the clean cage (now containing the mouse) and place the cage on trolley **no 2**.
- The water bottle in dirty cages must be emptied. **NB:** do not empty more than one bottle into each cage – the bedding should only be damp (not soaking wet) and the bottles must be empty before autoclaving on the dry program.
- Close the dirty cage, open the filter of the cage and place the bottle and the cage in the paper bag.
- Before touching a new cage, you must always change your gloves. Repeat the above process until all the cages are changed and put the new cages on trolley **no 2**.
- When cage changing has been completed, or the bag is full, seal the bag carefully with tape.
- Turn off the safety cabinet and clean it with soap. Wait until the cabinet is dry before you disinfect it with Klorin (chlorine). To avoid wear and tear on the cabinet, the cabinet should be rinsed with water after 30 minutes.
- Discard the outer pair of gloves and close the hazardous waste container. Discard the inner pair of gloves and wash your hands before putting on new inner gloves.
- Apply a little Klorin to trolley **no 1** and push it to the autoclave. Inform the laundry that the autoclave can be turned on.
- Mark the yellow container with “biological vectors or GMOs”. Discard the outer gloves as ordinary waste.
- Empty any remaining Klorin solution or other disinfectant in a suitable and marked container.
- Take off the lab coat and place it in a bag. The bag must not be opened until the lab coat can be put in the basket outside the cloakroom and must **not** be taken into the animal rooms.
- The animals can now be taken into the animal housing room.
- If you use a full face mask when working with viral vectors, you cannot use it for any other task unless it is first disinfected with Klorin, which must be allowed to work for 30 minutes after application. The mask must then be rinsed thoroughly with water and new filters must be inserted. The old filters must be discarded as hazardous waste.

**INSTRUCTIONS FOR THE LAUNDRY:**

- Turn on the autoclave, which must be maintained at a temperature of 121°C for 1 hour.
- When the autoclave program has finished, take the cages to the dirty side of the laundry.

- Empty the contents of the cages and clean them in the normal way.
- Discard the paper bag, which cannot be reused.

**INSTRUCTIONS FOR KPMe (Only BSL-1):**

- Follow the same procedure as described above.
- These cages must be handled and changed as the last task of the day.
- Since there is no BSL-2 cabinet in KPMe, a P3 mask with an extra filter must be used, as well as a lab coat and two pairs of gloves.
- Users must use their own laboratories for injections and cages must be changed inside the cage room after the end of the quarantine period.
- Clean the cabinet thoroughly with soap and let it dry. Then disinfect it with Klorin (chlorine).
- When they are ready, the cages must be transported in a paper bag to the equipment room at the end of the corridor, near KPM's offices. Remember to apply Klorin solution to the bag before leaving the room.
- Change clothes immediately after handling the cages.

**Conventional Unit (Only BSL-1)**

- Follow same procedure as above for MDU.
- Make sure to use a BSL-2 bench in one of the laboratories.
- The cages from Conventional unit will always be autoclaved to return to MDU. When you are done with changing the cage just place the cage inside the Autoclave room with a note saying "virale vectors".
- Make sure to clean up the BSL-2 bench when done with the cage changing, and disinfect with Klorin.
- Change clothes after handling cages containing virale vectors. It is recommended to change the cages at the end of the day.

## 4.0 HEALTH, SAFETY AND THE ENVIRONMENT (HSE)

- 4.1 It may be necessary to draw up routines for some biological vectors or GMOs that differ from the routines described in this SOP 12-14. Any deviation from this SOP must be clearly marked on the cages and the SDS/new SOP must be kept in the housing room where new routines are available.
- 4.2 The changing of cages involving biological vectors must be carried out on a class 2 safety cabinet (see appendix 1 and 2) to minimise the risk of exposure to the surroundings and to persons handling the cages. If this is not possible, an alternative solution must be agreed.
- 4.3 After handling cages with biological vectors/GMOs, a suitable disinfectant must be used to disinfect/neutralise them. KPM recommends the use of Klorin (1% calcium hypochlorite) as this has been shown to be the most effective against the majority of biological vectors. Protective glasses and gloves must always be worn when handling chlorine solution. Ethanol is not at all effective and should

**not** therefore be used. If using Virkon, we recommend Virkon S and check on the data safety sheet, whether it is fit for the purpose.

4.4 Disposable items, such as gloves and blue paper, that have been in contact with animals injected with viral vectors must be discarded in a yellow container which must be properly closed after each use. This applies however full/empty the container is and for the entire period that the animals are in quarantine. Therefore, consider how big a container you need for your task.

4.5 **Table of vectors and biosafety levels**, see under References points 7.3, 7.4 and 7.7 below. The following are examples, and the table should be added to when necessary:

Vectors	Biosafety level	Measures
<b>Adeno-related virus vectors:</b>		
Adeno-Associate Virus (AAV) without helper virus	BSL 1	Place in quarantine for 72 hours in normal animal rooms.
Adenoviral Vector	BSL 2	Must be kept in the tox room until termination.
Adeno-Associate Virus (AAV) with helper virus	BSL 2	
Adeno-Associate Virus (AAV) without helper virus	BSL2/BSL1	Place in quarantine for 72 hours and then move to normal animal housing rooms.

<b>Herpesviridae vectors:</b>		
Epstein Barr Virus vectors (EBV)	BSL 2	Must be kept in the tox room until termination.
Herpes Simplex Viral Vectors (HSV)	BSL 2	

<b>Lentviral vectors</b>		
Non-human viruses	BSL 2	Must be kept in the tox room until termination.
Human viruses	BSL 2+	BSL 2+ cannot be used on animals at KPM, IMB.





<b>Poxvirus-based vectors (Vaccinia):</b>		
Nyvax, Trovac, Alvac	BSL 1	Place in quarantine for 72 hours in normal animal rooms.
MVA, WR, NYCBOH, Copenhagen, Temple of heaven, Liser	BSL 2+	BSL 2+ cannot be used on animals at KPM, IMB.

<b>Retrovirus vectors:</b>		
Foamyvial vector Non-integrating	BSL 1	Place in quarantine for 72 hours in normal animal rooms .

<b>Other viral vectors:</b>		
Baculovirus vectors	BSL 1	Place in quarantine for 72 hours in normal animal rooms .
Sendai viral vectors	BSL 2	Must be kept in the tox room until termination.
Sinbis viral vectors	BSL 2	

## 4.6

Klorin	CAS nr	Piktogram	Faresetninger	Sikkerhetssetninger
Natriumhypokloritt løsning	7681-52-9		H290; Kan være etsende for metaller.	P101 Dersom det er nødvendig med legehjelp, ha produktets beholder eller etikett for hånden.
Natriumhydroksid	1310-73-27727-21-1		H314; Gir alvorlig etseskader på hud og øyne. H400; Meget giftig for liv i vann.	P102 Oppbevares utilgjengelig for barn. P234 Oppbevares bare i originalemballasjen. P260 Ikke innånd gass/tåke/damp/aerosoler. P280 Benytt vernehansker/ verneklær/ øyevern/ ansiktsskjerm.

			<p>H411; Giftig, med langtids virkning for liv i vann.</p> <p>EUH206 Må ikke brukes med sammen med andre produkter. Kan frigjøre farlige gasser (klor).</p>	<p>P301+P330+P331 VED SVELGING: Skyll munnen. IKKE fremkall brekning.</p> <p>P301+P310 VED SVELGING: Kontakt umiddelbart et GIFTINFORMASJONSSENTER/ en lege.</p> <p>P303+P361+P353 VED HUDKONTAKT (eller håret): Tilsølte klær må fjernes straks. Skyll eller dusj huden med vann.</p> <p>P305+P351+P338 VED KONTAKT MED ØYNENE: Skyll forsiktig med vann i flere minutter. Fjern eventuelle kontaktlinser dersom dette enkelt lar seg gjøre. Fortsett skyllingen.</p> <p>P310 Kontakt umiddelbart et GIFTINFORMASJONSSENTER/ en lege.</p> <p>P405 Oppbevares innelåst.</p> <p>P501 Innhold/holder leveres til kommunalt mottak for farlig avfall</p>
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**4.7 Klorin (chlorine) risk assessment:**

H-settings regarding direct contact:

H314 causes severe chemical burns to the skin and eyes, category 1.

H318 causes severe harm to the eyes, category 1.

Probability	5	1*5	2*5	3*5	4*5	5*5
	4	1*4	2*4	3*4	4*4	5*4
	3	1*3	2*3	3*3	4*3	5*3
	2	1*2	2*2	3*2	4*2	5*2
	1	1*1	2*1	3*1	4*1	5*1
		1	2	3	4	5
	Consequence					

Unwanted incident	Protective measures	K*S (Consequence * probability)
Exposure to Klorin (chlorine/bleach) while cleaning soiled surfaces	Mandatory protective equipment: lab coat, full face mask with filter and two pairs of gloves. Do not mix Klorin with other substances. Must only be used in well ventilated areas.	4 * 1

The consequence index for Klorin is 4 due to the high degree of toxicity.

Klorin (bleach) must be diluted at a ratio of 1:3. If protective glasses, nitril gloves and a lab coat are worn, the probability will be reduced to 1 since the risk of exposure will be extremely small.

- 4.8 Empty Klorin containers can be delivered to the recycling unit. Any left-over Klorin or Klorin solution must be delivered to the recycling unit in its original packaging or other suitable packaging. Klorin must **never** be emptied out into the sink because it is extremely harmful, with long-lasting toxicity for aquatic life.
- 4.9 Klorin must only be used in dedicated laboratories: in the MDU, these laboratories are DU-008 and DU-014. In these rooms, a container for diluting Klorin (see appendix 4) and a waste container for Klorin must always be available. Lab coats will only be available whilst the experiment is in progress.
- 4.10 The can containing concentrated Klorin must be stored in the chemicals cupboard. Klorin solution in the dilution bottle (see appendix 4) can only be kept for a maximum of 3 days and must also be stored in the chemicals cupboard during this period. The dilution ratio of 1:3 is written on the bottle. Any left-over solution more than 3 days old must be disposed of in a marked waste container after use.

## 5.0 EQUIPMENT AND MAINTENANCE

- 5.1 Soapy water
- 5.2 Chlorine or other disinfectant
- 5.3 Gloves
- 5.4 Paper towels
- 5.5 Face masks
- 5.6 Lab coats
- 5.7 SDS (safety data sheet) for the biological vectors or GMOs
- 5.8 Class 2 safety cabinet

## 6.0 HISTORY OF EDITING

- 6.1 SOP written 04.07.2022 by H. Tandberg /K. Zelewska
- 6.2 Revised and added information regarding conventional unit 24.11.2023 (Helene Tandberg)

## 7.0 REFERENCES

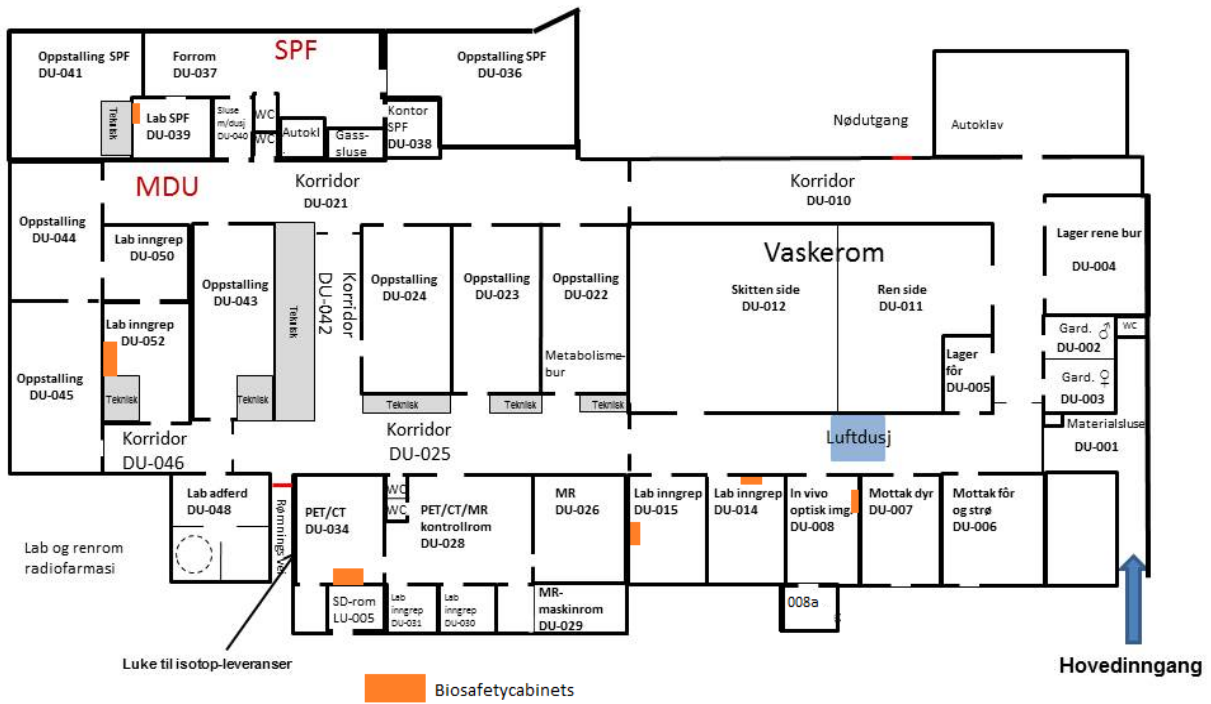
- 7.1 [National library of medicine](#) (link accessed 04.07.2022).
- 7.2 Information copied from SOP 12-09 “Daily inspection of animals in DU008a (the tox room)”
- 7.3 Form for viral vectors [Dartmouth](#) (link accessed 04.07.2022)
- 7.4 Form 2 for viral vectors from [Washington university](#) (link accessed 04.07.2022)
- 7.5 Information on procedures related to viral vectors from [Tel Aviv university](#) (link accessed 04.07.2022)
- 7.6 General knowledge of AAV from [the European Commission document](#) (link accessed 04.07.2022)
- 7.7 “Guidelines for research involving viral vectors” written by the University of Kentucky, Department of Biological Safety, printed and read May 2022.

### Appendix 1. Class 2 safety cabinet



**Appendix 2: Plan of the Section for Comparative Medicine at IMB showing the location of biosafety cabinets**

Planskisse ny dyreavdeling ved IMB



**Appendix 3: Biohazard symbol:**



**Appendix 4: Dilution bottle**



**Appendix 5: Klorin (chlorine)**



**The difference in handling quarantine between BSL 1, BSL 1/2 and BSL 2 for viral vectors and GMMs:**

