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Direction des services vétérinaires

Standard operative procedure

Title: Measures to be taken: specific pathogen free rodent animal facility – breeding colonies	Number: CQ-1
Scope: This is a directive from the Direction des services vétérinaires (DSV) for users and employees of the animal facilities of the Université Laval (campus and affiliated research centres).	
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Purpose: Describe the procedures to be followed in order to maintain a high status of hygiene in the rodent breeding colonies.	Version 2 – EN

### General information

- Many pathogens have known effects on the overall health status of rodents and cause significant clinical signs.
- Pathogens causing no clinical signs can also have deleterious effects on the immune system of the animal, the breeding success, etc. Their effects must be taken into consideration.
- Eradication of pathogens known to have an influence on the validity of experimental results and therefore on the rational use of animals is paramount.
- The introduction of unwanted microorganisms is usually linked to the following factors: animals, equipment, biologicals and employees/users of animal facility. It is therefore imperative to put in place good practices to reduce the risk of contamination.
- All the elements should be considered in the implementation of work practices. These measures are mandatory for all specific pathogen free rodent breeding colonies.
- All material used (cages, bedding, nesting material, feed, water bottles, enrichment material) must be autoclaved and be stored and handled in ways to prevent contamination.
- All handling of animals or unwrapped sterilized material must be done under biosafety cabinet or changing station.

- The number of people with access to the breeding rooms must be limited.
- In areas where both breeding and experimental rooms are present, personnel must be careful to prevent cross contamination, and additional measures must be taken, if needed.
- Mandatory PPE (personal protective equipment) that must be donned before entering a breeding room is:
  - Scrubs;
  - N95 respirator;
  - Hair bonnet;
  - Room dedicated gown (mandatory replacement after sentinels manipulation or after the work day);
  - Gloves;
  - Tyvek sleeves if risk of wrist skin exposure;
  - Dedicated animal facility shoes with footbath/sticky mat (e.g. Dycem®) or room dedicated shoe covers;
  - OR
  - Dedicated animal facility shoes, excluding access to break rooms, locker rooms, dirty loading dock and wash area.

## **Procedures**

### **Animals in breeding colonies**

- Accept only animals with health status that respects the SOP CQ-2, Health status of rodent animal facilities.
- House animals in positive pressure ventilated microisolators in order to minimize the entry of pathogens.
- In order to reduce the risk of contaminating them, minimize direct manipulation of animals except when needed (e.g. health exams, treatments). Manipulate all mice with tunnels or forceps soaked in hydrogen peroxide. Manipulate rats with their home cage tunnel.

### **Food and water**

- Purchase irradiated or autoclavable food.
- Sterilize the autoclavable food using steam (autoclave) according to the supplier's directives in appropriate containers. Store food to be consumed in those sterilized containers and always open them under biosafety cabinet or changing station.
- Transfer irradiated food in sterilized sealed containers (e.g. cages) under biosafety cabinet or changing station. Store the food in the same manner.

- Offer animals water filtered and treated by reverse osmosis or sterilized using steam (autoclave).
- Offer only irradiated food supplements from certified suppliers (e.g. ClearH<sub>2</sub>O, Bio-Serv) or that can be autoclaved (e.g. sunflower seeds). When a container is required, ensure it is also sterile.

### **Cages, bedding and cage changes**

- Prepare cage bottoms with bedding and nesting material. Cage parts (bottom, wire bar lid, filter top) can be:
  - Placed in autoclavable linen bags;
  - Placed on a rack covered with a closed autoclavable slipcover;
  - Bottoms piled up with lid on top placed on a rack covered with a closed autoclavable slipcover;
  - Assembled (complete cage) and closed as to prevent any contamination (ex. with autoclave tape indicator);
  - Assembled (complete cage) and placed on a rack covered with a closed autoclavable slipcover.

Note: Clearance under the hood glass must be considered when bottoms are piled up in a bag or under a slipcover.

- Prepare water bottles in closed cages as to prevent any contamination (e.g. with autoclave tape indicator), in metal racks placed in autoclavable linen bags, or in metal racks and closed with suitable sipper caps.
- Prepare other material (nesting material, enrichment devices, etc.) in closed cages or sterilisation wraps. Filled cages can be:
  - Placed in autoclavable linen bags;
  - Closed as to prevent any contamination (e.g. with autoclave tape indicator);
  - Placed on a rack covered with a closed autoclavable slipcover.
- Sterilize material with appropriate autoclave cycle.
- Spray a solution of hydrogen peroxide on the material before bringing it in the breeding room.
- Spray or wipe the interior of the hood with hydrogen peroxide before use as per local SOP. Let set with contact time provided by the company.
- Prepare the hood's work surface by placing a cloth soaked with hydrogen peroxide or by spraying a large amount of disinfectant on the surface.

Note: If a cloth is not used, ensure that the work surface is always wet, spraying more disinfectant as needed.

- At all times, spray or dip gloved hands with hydrogen peroxide before handling sterile equipment or animals (use as needed). Spray them also after animal manipulation.
- It is recommended to spray or dip gloved hands in hydrogen peroxide whenever they come out of the hood.
- If it wasn't stored under a slipcover until use, spray the sterile equipment before introducing it into the hood. Unpack the equipment and avoid touching its content to minimize the risk of contamination.

Note: Although not mandatory, it is recommended to disinfect equipment stored in a sterile cover. However, if there is reason to believe that the material under the cover may have been contaminated (e.g. handling error, cover left open for several hours, rack in use for more than 2 weeks), disinfection or re-sterilization of material is required.

- Remove the dirty cage from the ventilated rack. It is recommended to wipe all its surfaces except the filter with a cloth soaked with hydrogen peroxide.
- Wipe the spot left vacant on the ventilated rack if dirty. If applicable, test and wipe the automatic water valve with another hydrogen peroxide soaked cloth. Change the valve if defective or dirty.
- Place the dirty cage in the hood. Remove the filter top. Using tunnels or forceps previously soaked in hydrogen peroxide, transfer the mice one by one in the clean cage. Transfer rats using their cage tunnel. Do a quick health check during the transfer. If a tunnel is used, ensure ventral check.
- Note the births, deaths or health problems on appropriate cage card and/or in the database.
- Transfer part of the nest in the clean cage. Transfer enrichment material or replace if dirty, broken or consumed. Add food as needed. Change the water bottle if needed. Place the filter top and return the clean cage to its previous location on the ventilated rack (particularly important if the water valve is on the rack).
- Note: If cage location is changed, replace or disinfect the valve.
- If sentinels are present on the rack, take a sample of soiled litter before removing the dirty cage from the hood, following the SOP CQ-3 Sentinel health monitoring program – rodents.
- Put the dirty cage on a cart.
- Keep the work surface clean. Always keep the cloths soaked with hydrogen peroxide and change them at the end of the day or before if they become soiled.
- Finish with the sentinels' cages in order to mix the soiled litter samples collected in the sentinels' new cages.

- Empty and clean the hood. Cover the cart and dirty cages. It is recommended to spray it with hydrogen peroxide before leaving the room.

**Animal contamination**

An animal is considered contaminated if the cage is opened NOT under a functioning hood, if the hood is malfunctioning during handling, if there is a water leak on the rack and there is reason to believe the water may have entered the cage of an inferior shelf, if an animal contaminate itself inside the hood or if it exits the hood.

- When an animal is contaminated, place it in a clean cage and notify the facility manager and the research team. The animal should be transferred to experimental housing rooms or euthanized. Consult a veterinarian if the animal must be kept in breeding room.
- If the animal escapes into the hood and that it has not been contaminated (e.g. has not touched the air grille, the gown or the undisinfected exterior of the cage), put it back in its cage.

**References**

CALAM, *CALAM Standards of Veterinary Care*, 2007.

CCPA, *Guide to the Care and Use of Experimental Animals*, 1993.

IRCM, PNF SA 001.01.

FELASA: Working Group on Health Monitoring of Rodent and Rabbit Colonies, Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units, *Lab Anim* 2002 36: 20.

SOP updates		
Version 2	Novembre 15 2021	Inclusion of mandatory PPE in general information. Clarification of animal handling methods. Inclusion of alternative acceptable methods for material sterilization and storage. Addition of numerous precisions in procedures. Clarification of animal contamination.