



SOP 3100

Date: June 2020

Title: Animal Husbandry Procedures

1.0 Purpose

This SOP describes the procedures for cage prep and cage changing. Additionally, this SOP also describes daily husbandry procedures for each of the species housed in the VRC animal facility.

2.0 Procedures

2.1. Scheduled Cage Changes

- 2.1.1. Cages are changed as scheduled by the Facility Manager. Cages and equipment that become soiled or wet will be “spot” changed and/or cleaned regardless of the scheduled cleaning frequency. If feed and/or water levels become low, prior to the next schedule cage change, they are to be topped off with autoclaved water and irradiated feed.
- 2.1.2. Micro-isolator cage components and water bottles are inspected thoroughly to ensure that the integrity of the component(s) is in good condition, prior to being used. If the integrity of the component is in question, they will be set aside for the facility manager to inspect. Any damaged materials will be discarded after the facility manager has deemed it necessary for disposal.
- 2.1.3. Mouse cages, feed, and water bottles will be changed out at least 1 time per week.
- 2.1.4. Guinea pig cages, feed, and water bottles will be changed at least 1 time per week.
 - 2.1.4.1. Guinea pig cages will be thoroughly monitored after their scheduled cage changing and should be actively spotted changed when appearing overly soiled. Feed and water levels will be checked daily and topped off with autoclaved water and irradiated feed, ensuring that the feed hopper is ½ full and both bottles maintain more than 50% water capacity.
- 2.1.5. Cage changing procedures are designed to prevent transmission of disease from one cage to another. Sterile microisolators that are opened only within a change hood. Animals that may be shedding infectious agents (ABSL2) are opened only within a biological safety cabinet.
- 2.1.6. The exterior of a microisolator is considered contaminated with rodent pathogens and therefore dirty. The interior contents of a microisolator, including the water bottle, wire bar lid, and animals are considered clean. Interior contents are always to be handled with gloved hands or forceps that are wet with disinfectant.

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2.1.7. During cage changing, animals are to be handled gently, and proper restraint techniques are used. Noise should be kept to a minimum to reduce animal anxiety. Voices should be kept low; loud singing and shouting is not permitted. Music devices may not be operated in an animal room unless headphones are used, and the volume is set to a reasonable level (see SOP 1100). Equipment should be handled quietly.

2.2. Microisolator Set-up

2.2.1. A microisolator set-up is defined as a solid-bottom plastic cage, filtered cage top, wire-bar with feeder trough, enrichment toy, bedding, and feed.

2.2.2. Microisolator set-ups (cages) are assembled after all the components have been washed and sanitized. Any components that are dirty, come in contact with the floor, or any other contaminated surface, will be sent to dirtyside cagewash and rewashed before they are used.

2.2.3. Assembly of mouse microisolator set-ups occur in the cleanside cagewash. Bedding, feed, and an enrichment toy are placed in each microisolator set-up. The set-up is completed by placing the filter top on the cage. The set-ups are transferred to a bulk truck and are set aside for sterilization in the autoclave.

2.2.4. A steam sterilization integrator is used for each load of cages sterilized in the autoclave. The cages are removed as soon as possible from the autoclave to prevent the breakdown of the feed and/or equipment. The steam sterilization integrator is checked after each cycle to ensure sterilization has been achieved (see SOP 4300).

2.2.5. All sterilized cages will be marked with a green colored autoclaved tag to identify that they have been sterilized and ready for use. Cages should be used within 1 week of sterilization.

2.2.6. Assembly of GP microisolator set-ups occur in the cleanside cagewash. Irradiated Bedding, sterilized feed hopper, and steriled enrichment toy are placed in each microisolator set-up. The set-up is completed by placing the filter top on the cage. The set-ups are transferred to a bulk truck and are then ready for use. The caretaker staff provides irradiate feed to the feed hoppers during cage changing in the animal room.

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2.3. Daily Husbandry Requirements

- 2.3.1.** Animals are observed twice daily to assess their health status (except on weekends and holidays when they are only checked once per day). Abnormal observations are recorded on the Animal Health Observations form.
- 2.3.2.** Feed and water are checked twice daily; completed no later than 2 hours after the start of the workday and again in the afternoon between 1:30 pm and the close of business (except on weekends and holidays when they are only checked once per day).
 - 2.3.2.1.** Mouse Water bottles and feed hoppers should be at least ½ full. Feeders and water bottles are refilled under the change hood or biological safety cabinet, as needed. GP feed hoppers and water bottles can be refilled cage side.
- 2.3.3.** The cage is checked to ensure that the correct number of animals are in each cage, as it is indicated on the cage card. If any discrepancies are found the immediate supervisor or facility manager will be notified.
- 2.3.4.** In any cage in which there is a sick animal, an Animal Health Monitoring Card (red card) is filled out and placed in the cage-card holder, in front of the cage card. The red card should be completed in its entirety and contain a description of the symptoms or signs that are found.
 - 2.3.4.1.** The new red card and cage information is recorded on the Animal Health Observations form, the red triage flag located on the outside of the animal room is turned out to alert the Technician that there is an animal health problem that requires attention.
 - 2.3.4.2.** Cages with red cards should be monitored carefully, during daily health checks and cage changing, to ensure that the health of the animal(s) is stable. If the animal(s) appears to have deteriorated, the Technician is notified again to re-evaluate the case. If a Technician is not available, the Facility Manager is notified.
- 2.3.5.** Each cage is checked for overcrowding. In accordance with the Guide, and the size of our mouse microisolator, no cage should have more than 5 adult mice. If cages are overcrowded, animals must be separated to ensure the number of animals per cage does not exceed the maximum number.

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- 2.3.6.** A maximum of 2 guinea pigs are housed in a cage at a time. Guinea pigs are regularly weighed (every 2-weeks) to ensure they are within the recommended guidelines to prevent overcrowding and general health monitoring.
- 2.3.7.** If an animal is found dead, the animal is removed from the cage and placed in a biohazardous plastic bag. Animals from cages marked with a biohazard are double bagged. All the appropriate cage card information is written on the exterior of the bag and the bag placed in the carcass freezer located in the vivarium corridor.
- 2.3.7.1.** The death and the number of animals left in the cage are recorded on the cage card. The death is recorded on the Record of Mortalities sheet and the Animal Health Observation form.
- 2.3.7.2.** The death is then reported to the technician who then informs the investigator, facility management, and veterinarian. The time and date the investigator is informed is noted on the Record of Mortalities sheet.
- 2.3.7.3.** If the nature of the death is sudden or unexpected, the veterinarian may decide whether the animal should be submitted to pathology and whether an incident report should be filed with the ACUC.
- 2.3.7.4.** If a guinea pig is found dead or moribund, the Facility Veterinarian and scientific staff will be notified immediately to determine the course of action to be taken. Guinea pigs should be submitted to pathology to determine cause of the illness or death.
- 2.3.8.** Soiled or wet cages are changed immediately.
- 2.3.8.1.** If the animals appear to have any adverse health observations (i.e. lethargic, cold to touch, abnormal gait, etc.) or found dead due to the wet cage, a veterinary technician will be notified immediately, and measures will be taken to determine how the water leakage occurred.
- 2.3.9.** A Room Husbandry Log is in every animal room. The log is filled out and signed off on a daily basis.
- 2.3.10.** Temperature and humidity are read from the room thermometer/hygrometer. Readings are taken no later than 2 hours after the start of the workday and again in the afternoon before the close of business. Readings are documented on the room husbandry log.

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2.3.10.1. Rooms are maintained at 72° +/- 5 degrees and between 30-70% humidity. Any temperature or humidity readings which are not within acceptable limits are reported to facility management immediately.

2.3.11. Changing hoods and carts are cleaned daily with a disinfectant. Unless they are in use, change hoods should not be cluttered with supplies or contaminated with dirty debris.

2.3.12. Animal rooms are swept and mopped at least once a day, or as required. Floors are mopped using the facility approved disinfectant, which located in the janitorial closet

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2.3.13. Doorjambes, vents, and all flat surfaces are cleaned at least weekly with the facility approved disinfectant.

2.4. Aseptic Cage Changing (Mouse Cages)

2.4.1. Mouse microisolators are changed using aseptic techniques. Upon entering the animal room, the caretaker must don the proper personal protective equipment.

2.4.2. The following materials are needed in the room for cage changing:

2.4.2.1. Autoclaved Microisolator set-ups

2.4.2.2. Facility approved disinfectant spray bottle.

2.4.2.3. Underpads

2.4.2.4. Autoclaved water bottles or carboy. Autoclaved water should remain covered with an underpad when outside of the hood.

2.4.3. Gloves are sprayed with a disinfectant each time hands enter the hood and before touching a clean cage following contact with a dirty cage. The gloves are changed between each side of each rack.

2.4.4. All surfaces inside the hood are sprayed thoroughly with a disinfectant and allowed to sit for 15 minutes with the blower turned off prior to beginning work inside the hood.

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2.4.5. All mouse cage changes must be performed under the Class II Type B1 Biological Safety Cabinet (hood). Caging and supplies are placed within easy reach but **are not to be stored inside of the hood**. Two underpads are thoroughly wet with a disinfectant and are positioned inside the hood not to obscure airflow; the underpads should remain wet at all times.

2.4.6. Sentinel cages and animals exposed to biohazards are changed last.

2.4.7. Mouse cages are changed according to the following steps:

2.4.7.1. A clean microisolator (cage to which animals will be transferred) is sprayed into the hood and placed on the left side of the hood. The dirty microisolator (cage to be changed) is sprayed into the hood and placed on the right side of the hood. The clean sentinel cage is sprayed into the hood and placed on the right side of the hood to the right of the dirty microisolator.

2.4.7.2. The clean cage lid of the clean microisolator is removed and placed beside the cage.

2.4.7.3. A water bottle is placed into the clean microisolator. **The water bottle must be sprayed with disinfectant before entering the hood.**

2.4.7.4. The filter lid of the dirty microisolator is removed and placed beside the cage.

2.4.7.5. The wire-bar lids are opened sufficiently to allow the transfer of the animals. During transfer, the animals are examined for abnormalities and the number and sex of the animals in the cage is compared with what is written on the cage card. Abnormalities are recorded on the appropriate husbandry forms and reported to the veterinary staff or management (see SOP 3400).

2.4.7.6. **Ensure that ALL animals have been transferred to the clean cage.**

2.4.7.7. The wire-bar lid on the clean microisolator is closed and the cage lid is replaced. The cage card is transferred from the dirty cage to the clean cage. The clean microisolator is **sprayed with disinfectant before being** placed back on the ventilated rack in its former position. The shelf is wiped with disinfectant.

2.4.7.8. A small scoop of bedding is taken from the dirty cage and placed into the sentinel cage.

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2.4.7.9. The feed from the dirty microisolator is dumped into the dirty cage. The water bottle is removed and placed into a “dirty” water bottle basket. The cage lid of the dirty cage is replaced, and the dirty cage is **sprayed with a disinfectant before being** removed from the hood and placed onto a “dirty” bulk truck or cart.

2.4.7.9.1. When the dirty bulk trucks are full, they will be sprayed thoroughly with a disinfectant and allowed to sit on the rack in the animal room for 15 minutes. The rack will then be wheeled down to dirty side cage wash for disposal.

2.4.7.10. Gloves are sprayed before touching the clean cage again.

2.5. Special Husbandry Requirements for Mice

2.5.1. All caretakers performing mouse cage changes will work in pairs unless otherwise directed by the facility manager. This is to ensure all animals are accounted for when transferring from one cage to another.

2.5.2. Mice are housed up to 5 per cage. All mice should be the same sex, age and strain unless otherwise directed by investigator or Animal Study Protocol.

2.5.3. Each cage will have environmental enrichment such as enrichment toys, and/or nesting material.

2.5.4. Male mice can become aggressive, and some strains are more aggressive than others. If fighting occurs, the animals must be separated. An injured animal should be housed alone, and a red card should be placed on the microisolator (see SOP 3400).

2.5.5. Group housed mice frequently barber to show dominance. Barbering is a normal occurrence and is evident with the less dominant animals having hair loss, especially around the head and facial area. If barbering goes beyond hair loss and results in skin lesions, the injured animal should be housed alone, and a red card should be placed on the microisolator.

2.5.6. Unless otherwise directed by the Facility Manager or Facility Veterinarian, mice are fed ad libitum. Mice receive PMI NIH Rat and Mouse Autoclavable 5% fat Diet (NIH-31 formula) or Irradiated NIH-41 Mouse Diet.

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- 2.5.7. Wet microisolators are changed promptly. Animals in a wet cage should be closely observed during cage change for any adverse effects of being in a wet cage and reported accordingly.
- 2.5.8. Some rodents undergoing experimental procedures and some weanling, mutant, transgenic, and knockout rodents are unable to eat from wire bar lid feeders. For those rodents, biscuits and feed dust (fines) should be placed on cage floors in plastic receptacles or petri dishes. If it is necessary to wet the biscuits or fines, the cages must be changed daily.
- 2.5.9. For breeding females, it is best to avoid disturbing the cage when the female about to deliver or recently delivered a litter. If cage conditions allow, the microisolators are not changed until 3 days post-partum.
- 2.5.10. The teeth of mice grow throughout life therefore teeth are checked for malocclusion if a mouse appears to be very thin and appears to not be eating. If malocclusion is observed, it is reported to the veterinary staff or management (see SOP 3400).

2.6. Aseptic Cage Changing (Guinea Pig Cages)

- 2.6.1. Guinea Pig microisolators are changed in a clean environment, using aseptic techniques. Upon entering the animal room, the caretaker must don the proper personal protective equipment.
- 2.6.2. Guinea Pig microisolators are changed at least once per week. Cages which become heavily soiled after cage change will be spot changed as required. Guinea Pig cages do not have to be changed in biological safety cabinet, unless the Animal Study Proposal indicates otherwise.
- 2.6.3. The following materials are needed in the room for cage changing:
 - 2.6.3.1. Sanitized or autoclaved microisolator cage set-ups
 - 2.6.3.1.1. Sanitized microisolator cage set-ups: cage bottom, lined with a molded cage liner, and autoclaved enrichment toys and J-feeders, and sanitized cage top.
 - 2.6.3.1.2. Autoclaved microisolator cage set-ups: Sanitized cage bottom and top, enrichment toy and J-feeder, set-up and then autoclaved as an autoclaved microisolator cage set-up.

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- 2.6.3.2. Facility approved disinfectant spray bottle.
- 2.6.3.3. Underpads
- 2.6.3.4. Autoclaved water bottles or carboys.
- 2.6.3.5. Irradiated feed (PMI Certified PicoLab Guinea Pig Diet)
- 2.6.4. Gloves are sprayed with the disinfectant before touching a clean cage and after being in contact with a dirty cage. The gloves should be changed between each side of each rack.
- 2.6.5. Guinea Pig Cages are changed according to the following steps:
 - 2.6.5.1. A clean microisolator (cage the animals will be transferred) is sprayed and placed on the left side of the changing cart. The dirty microisolator (cage to be changed) is sprayed with the disinfectant and placed on the right side of the changing cart.
 - 2.6.5.2. The cage lid of the clean microisolator is removed and placed beside the clean cage.
 - 2.6.5.3. The J-Feeder is placed upright with-in the cage and fill with irradiated feed.
 - 2.6.5.4. The water bottles and cage lid of the dirty microisolator is removed and placed on the beside with the dirty cage.
 - 2.6.5.5. Animals are transferred into the clean cage. During transfer, the animals are examined for abnormalities and the number and sex of the animals in the cage are compared with what is written on the cage card. Any abnormalities are recorded on the appropriate husbandry forms and reported to the veterinary staff or management (see SOP 3400).
 - 2.6.5.6. The clean microisolator filter lid is replaced. The autoclaved water is placed into the external bottle holder on the lid. The cage card holder is transferred from the dirty cage to the clean microisolator.
 - 2.6.5.7. The rack shelf is wiped with disinfectant prior to returning the clean cage. The clean microisolator is then **sprayed with a disinfectant** and returned to the ventilated rack in its former position.

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2.6.5.8. The feed from the dirty microisolator is dumped into the dirty cage. The water bottle is removed and placed into a “dirty” water bottle basket. The cage lid of the dirty cage is replaced, and the dirty cage is **sprayed with a disinfectant before being** placed onto a “dirty” bulk truck or cart.

2.6.5.8.1. When the dirty bulk trucks are full, they will be sprayed thoroughly with a disinfectant and allowed to sit on the rack in the animal room for 15 minutes. The rack will then be wheeled down to dirty side cage wash for disposal.

2.6.5.9. Gloves are sprayed before touching a clean cage again.

2.7. Special Husbandry Requirements for Guinea Pigs

2.7.1. Guinea Pigs are pair housed (or singly housed when medically or scientifically required) in guinea pig microisolator cages. They should always be housed with another guinea pig of the same sex, unless an approved protocol indicates otherwise.

2.7.2. Each cage will have environmental enrichment, unless removed as per request by the Veterinarian or Investigator.

2.7.3. Unless otherwise directed by the Facility Manager or Facility Veterinarian, guinea pigs will be fed ad libitum with a diet of Irradiated Feed fortified with Vitamin C (PMI Certified PicoLab Guinea Pig Diet). The feed will be changed once weekly, during the schedule cage changing and topped off as required.

2.7.4. Fresh oranges, apples, kale, and/or cabbage may be given up to three times per week. Supplements should be given in small quantities as determined by the vet and should not exceed the daily diet. Fresh items must be washed thoroughly to avoid pesticide residues and possible contaminations.

2.7.5. Guinea pigs play with the sipper tubes on the water bottles. To ensure that there is an adequate water supply, two water bottles used for each microisolator and will be topped off with autoclaved water, as required.

2.7.6. Wet microisolators are changed promptly. Animals in a wet cage should be closely observed during cage change for any adverse effects of being in a wet cage and reported accordingly.

2.7.7. Feed is delivered in stainless-steel “J” feeders. Enough feed is placed in each



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feeder to last until the next cage change. Leftover feed is discarded when the microisolator is changed.

- 2.7.8.** Guinea pigs are checked regularly for foot lesions and for neck lesions caused by feeders. Toenails grow quickly and require routine trimming to prevent nail breakage.

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