

STANDARD OPERATING PROCEDURES
DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

SOP#: 455.3

Date Issued: 10/20

Date Revised: 3/24

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TITLE: **Naked Mole Rat (*Heterocephalus glaber*) Husbandry**
SCOPE: Animal Care Personnel
RESPONSIBILITY: Facility Manager, Technical Staff, Veterinary Staff
PURPOSE: To Outline the Proper Procedures for Receiving, Physical Examination, Evaluating Health Status, Handling, Care and Husbandry Practices Related to Naked Mole Rats

I. PURPOSE

1. To ensure the highest quality of animal health and preclude the development of spontaneous diseases or disorders, which could compromise the integrity of studies and the interpretation of results.
2. To describe the care and health assessment for the USDA covered species: the naked mole rat (NMR).
3. To ensure personnel handling NMRs perform their duties in a manner that complies with all current governing laws, regulations, and guidelines.

II. RESPONSIBILITY

1. The Veterinarians oversee all aspects of animal health and are assisted by all program staff.
2. Facility Managers ensure implementation of all procedures.

III. GENERAL CHARACTERISTICS

1. NMRs are hairless, which makes them unable to regulate their body temperature outside a very narrow range.
2. They have poor vision, but are extremely sensitive to changes in the environment such as smell, vibration and noise.
3. NMRs are eusocial, live in colonies, and **must not** be mixed or combined with other colonies, as they will become extremely aggressive. Each colony is composed of 1 breeding female (the queen) and 1-3 breeding males. All remaining animals are non-breeding workers.
4. Their life span has been reported to be up to 32 years, are highly resistant to infectious diseases and cancers, and do not display the expected age-related degenerative changes seen in other rodent species.
5. They have a high tolerance for increased carbon dioxide (CO₂) levels, so CO₂ euthanasia is inappropriate in this species.
6. NMRs are very docile and rarely bite when handled, but care must be taken to change gloves between handling individuals from different colonies.

7. **Note:** The *Guide* recommends a cage sanitation interval of at least weekly for most species, but this would not be appropriate for NMRs due to their sensitivity to changes in scent. Absence of scent cues or introduction of foreign scents can lead to fighting and pup cannibalism, so limiting the number of cages changed within a multi-cage colony can help ameliorate these concerns. Additionally, this species has adapted to higher levels of gases such as ammonia (NH₃) and carbon dioxide (CO₂), so do not require the higher frequency of cage changes or increased ventilation needed in other more common laboratory rodents, (i.e., mouse and rat).

IV. PROCEDURES

1. Receipt

- a. Individual cage cards are prepared for each colony and include the following information:
 1. Investigator
 2. IACUC #
 3. Species
 4. Sex
 5. DOB (or age on arrival if unavailable)
 6. Body Weight
 7. Arrival Date
 8. Source
 9. USDA assigned animal number (e.g. Fiscal Year- N- # of animal)
- b. Animal Medical Records are required for all animals, and can be maintained as either individual medical records or as colony records: (**see SOP #012** entitled, ***Animal Medical Records***)
- c. Shipping crates are inspected for signs of damage and shipment accuracy.
- d. Technician will notify the Facility Manager regarding any damage to crate or contents.
- e. Facility Manager will notify the Fiscal and Business Specialist of damaged or rejected shipments, or orders improperly filled (i.e., weight, age, number requested).
- f. Shipping containers are lightly wiped with Oxivir Tb™ or Peroxigard and taken to the housing room. Upon opening, an environmental swab is taken and pooled feces from the shipping container collected for diagnostics.
- g. Animals are examined for any signs of illness or abnormality. The clinical veterinarian will be immediately notified of health concerns or deaths that occurred in transit.
- h. The number of new animals and new cages are recorded on the ***Per Diem Sheet*** and ***Progress Notes*** located in the ***Room Logbook***. Notation in the ***Progress Notes*** will include the vendor source and a statement reflecting general appearance on arrival.

2. Husbandry

- a. Housing
 1. NMRs are housed in static cages on an open-rack, metal shelving unit and the room maintained within the following parameters:
 - a. Room temperature 79° F- 85° F
 - b. Humidity 40%-60%

- c. Lighting: 24 hrs of red lights. Do not use white lights in housing room.
 2. Noise/Vibrations: **NMRs are extremely sensitive to vibration and noise** and disturbances can cause them to become stressed and lead to a panic response.
 3. Open and close doors **slowly and quietly** so they do not slam shut.
 4. Enrichment: Paper towels, cotton nestlets, and Enviro-Dry placed as needed in cages for nesting materials
 5. Bedding: Approximately ¼ inch of bedding per cage compartment or soft pelleted paper bedding-<https://www.envigo.com/7084-pelleted-paper-contact-bedding>.
- b. Cage Changing
1. Caging systems are generally composed of
 - a. Cage/tunnel system
 - b. Separate nesting chamber
 - c. Separate urination/defecation (toilet) chamber
 2. NMRs are generally docile when being handled, but bite proof gloves worn under nitrile gloves may be needed.
 3. Care must be taken when using cleaning supplies or handling with gloves, as they are highly scent-driven. A complete cage change-out can lead to increased fighting and/or mortality, so dirty bedding and fecal transfer are required to prevent detrimental effects.
 4. The caging system, except for the toilet chamber should not be cleaned unless necessary since they depend on odors in the absence of good eyesight. **(See NMR Room Sheet CMDC 271)**
 5. The following should be completed **daily** and noted on the **Room Status Sheet**.
 - a. Remove and throw away old food left inside each compartment.
 - b. Inspect and remove fecal pellets or mold from all compartments, including the toilet chamber.
 - c. Carefully inspect the bedding for animals/food that may be buried/deceased.
 - d. Ensure temperature and humidity requirements are within set limits and document on **Room Status Sheet**
 - e. Assess animal health and notify veterinary staff if any issues
 - f. Count and record any new births. If any are noted, place a cage card on the cage to indicate that the colony is not to be disturbed for 48 hours.
 6. The following should be completed **Weekly** (or as needed) and recorded on the **Room Status Sheet**.
 - a. Dump and wipe soiled cages using hot water with a disposable towel as needed and on a rotating basis with a **minimum frequency of once per 7 days** for each cage in the system.
 - b. The Toilet Chamber should **NEVER** be dumped and wiped clean on the same day as other cages in the system.
 - c. Depending on the number of cages in the colony, no more than 1-2 cages should have complete change-outs with a sanitized cage every 2-3 weeks (please see Naked Mole Rat Change Out Schedule maintained in the room, as it changes with changes in size and number of each colony).

- d. Remove a sample cup of dirty bedding (without fecal pellets) from the Toilet Chamber and distribute in any replaced or dumped cages in the system to maintain the scent of the colony.
- e. **Toilet compartment change must be delayed by one week to help the NMR reestablish the colony scent.** Replace the Toilet Chamber with a sanitized cage (cage-washer at 180°F or higher) the following week from all other cages.

3. Feeding

- a. The feeding schedule is laminated and posted in the room and inside of the room book.
- b. Clean vegetables and fruit thoroughly with hot water and inspect carefully before chopping into ~1/2" pieces. If there are any rotten or moldy parts, throw the entire piece away. **Carefully check the sweet potatoes, as they are a major food source for the NMRs.** Potatoes could contain mold in the pest hole, which is covered by dark skin or soil.
- c. The amount of food given depends upon the size of the colony. (Each animal eats roughly four (1/2") inch pieces of food daily).
 - 1. Pairs receive 4-6 small pieces of sweet potatoes.
 - 2. Large colonies should receive 4-6 large handfuls of cut food (excluding sweet potatoes).
- d. Food should be placed in an identified food cage compartment and not in the nest or toilet area, though the colony may move it to their nesting cage.
- e. **2919 Rodent chow is soaked in water and mashed into a thick consistency to be given daily to each colony, promoting nutritional support in addition to fresh food.**
- f. **DO NOT PROVIDE WATER. NMRs obtain water requirements from vegetables and can develop diabetes if water is provided ad libitum.**
- g. Please see the following chart for feeding schedule:

Day of the Week	Food (Daily)	Additional Comments
Monday	Sweet Potatoes & 2919 Rodent Chow (add water and mix to thick paste) Provide 1-2 petri dishes full of mash depending on colony size.	
Tuesday	Sweet Potatoes & Raisins	Put food in at least 2 cage compartments if there are more than 10 animals in the colony (Applies to daily feeding)
Wednesday	Sweet Potatoes & Celery	
Thursday	Sweet Potatoes & Grapes	Grapes have to be sliced in half or animals won't eat
Friday	Sweet Potatoes & Carrots	
Saturday	Sweet Potatoes & Apples	
Sunday	Sweet Potatoes & frozen peas/corn defrosted on Friday	Alternate peas and corn weekly

- h. Additional food items that can be used: apples, fresh beans, turnips, carrots, and cucumbers. Peas or frozen mixed vegetables can be substituted as needed.
- i. Avoid chow or supplements with high calcium level.

4. Health Surveillance

- a. Each housing room is assigned to an Animal Care Technician. Daily observations of the Animal Care Technician are recorded on the **Room Status Sheet** within the **Room Log Book**.
- b. On weekdays, health surveillance will occur twice per day, prior to 9:30 AM, and again after ~1 pm.
- c. On weekends and holidays, the initial health surveillance should take place upon the arrival of the Animal Care Technician, and again just prior to completing their assigned shift.
- d. Observations and communications must be noted on the **Room Status Sheet** and the Facility Manager's **Animal Health and Environmental Concern Form**.
- e. Each animal/cage is observed to evaluate:
 1. Food intake
 2. Urine and fecal output
 3. Assurance of fresh vegetables and fruit are present
 4. General appearance (presence of lesions/injuries, new pups, deceased animals, disposition)
- f. **Emergencies must be immediately reported to the veterinarian, which includes but is not limited to:**
 1. Active bleeding
 2. Dull, lethargic, or unresponsive
 3. Ataxia, seizure, other neurologic signs
 4. Open mouth breathing, pale or blue mucous membranes
 5. Increase or excessive vocalization
 6. Limb paresis or paralysis
 7. Any evidence of trauma (fight wounds can be around the mouth with scabs usually apparent on the dorsal abdomen)
- g. Colony medical records must include, at a minimum, weekly entries made by the research staff in the **Progress Notes**, which at least summarize the following for the colony:
 1. An impression of overall condition
 2. Food intake and voidings
 3. Any clinical abnormalities or complications
 4. Any treatments administered in response to observed abnormalities
 5. Any experimental procedures
- h. At least monthly, each animal's microchip is scanned and the animal is weighed, along with receiving a light swab of olive oil to restore moisture to the skin. Weights are recorded and maintained in the room and in the Box Storage. The technician will send this document to the veterinarian each month to monitor any trends in weight-loss, in which case they may decide to weigh and monitor the animal more frequently.

Whenever health status/observations for an individual animal differ from colony animal's health status/observations, an entry specific to that animal must be made in the colony records. Refer to **SOP #006** entitled, **Animal Health and Environmental Surveillance** for specific procedures of reporting and recording health concerns.

5. Room Duties

- a. Upon completion of cage changing, feed and daily health surveillance, the following tasks are to be performed: (Room duties described below are minimal

requirements, additional duties and frequencies are at the Facility Manager's discretion).

1. **Daily** - complete **Room Status Sheet** by recording:
 - a. Minimum and maximum **room temperature** and **humidity** (measurements out of the acceptable range are described under the **Health and Environmental Concern** column of the **Room Status sheet** and reported to the Facility Manager via the **Health and Environmental Concern Form**) **immediately**
 - b. Feed is available, fresh, and clean.
 - c. Husbandry duties (e.g., caging/equipment changes/procedures performed).
 - d. Housekeeping duties (e.g., room duties/procedures performed)
 - e. Significant health or environmental concerns.
 - f. Time of observation and the initials of technician.
2. Circumstances may require the temporary use of portable space heaters to maintain ambient temperature in a naked mole rat housing room within acceptable ranges. When portable space heaters are used in vivariums, such equipment should be capable of being remotely monitored and adjusted whenever possible. **Placement of such equipment should be sufficiently distant from all animal primary enclosures that temperature extremes inside the cages is not possible.** Whenever such equipment is used, the frequency of ambient temperature monitoring and recording must be in accordance with this SOP and ensure environmental conditions remain within acceptable ranges.
3. Assure no investigator's supplies or trash is left in the room.
4. **Check and replenish supplies** as needed.
5. Wipe down counter/sink areas with Oxivir Tb™
6. Assure any new arrivals are recorded on **Per Diem Sheet**.
- b. Floors are to be **swept and mopped** Monday, Wednesday, Friday or when soiled.
- c. **Weekly room duties** include:
 1. Wipe down door and doorframe with Oxivir Tb™
 2. Physically count animals within the room
 3. Review paperwork
- d. **Semi-monthly room duties** include:
 1. Sanitize all room cleaning equipment (e.g., not kept in the room, broom, dustpan, etc.).
 2. Inspect hospital barriers to ensure no holes or damage in the barricade.
- e. **Monthly room duties** include:
 1. Wash walls with Oxivir Tb™ as needed.
 2. Sanitize cage weights by rinsing with hot water and spraying down with Oxivir Tb™
 3. Inspect/change filter in the humidifier as needed.

6. Euthanasia Procedures

- a. Because NMRs thrive in hypoxic environments (low Oxygen) with high Carbon Dioxide levels, CO₂ is not a sufficient method of euthanasia as with other rodents.
- b. NMRs will instead be overdosed with 5% isoflurane gas until respiration has ceased.

- c. Once respiration has visibly stopped, death will be verified by cervical dislocation or decapitation, and the carcass will be available for 48 hours.

7. Hospital Ward Chambers: Pregnant Queens

- a. When a **Queen** is visibly pregnant (can be verified by observation and weight gain) she will be set up into a Hospital Ward consisting of 3 cages (Nesting Chamber, Toilet Chamber, Food Chamber) along with 2 drones.
- b. Drones will be swapped out weekly *or as needed* depending on stability of pup count. If the Queen is cannibalizing any pups, do not swap out drones until there is a stable count so as to decrease stress. All information will be recorded at the cage card level and in the Hospital Ward Document on the online drive (e.g. BOX).
- c. Daily husbandry practices will be performed until litter drops. Mix dirty bedding from colony into the hospital ward, and vice versa for scent distribution.
- d. Hospital Wards with newborn pups will not be disturbed for 48 hours unless to replenish soiled food/mash.
- e. **No scheduled husbandry will be performed on hospital ward cages and their associated colony for at least 3 weeks** to ensure the least amount of disturbance in order to decrease pup mortality and cannibalism. Clean bedding can be added as needed, and dirty bedding can still be removed where needed.
- f. After pups are of size (typically 2-3 months of age), the Technician may reintroduce them to the colony on a weekday morning, and will observe periodically throughout the day to ensure no fighting or aggression is seen amongst the colony.

8. Hospital Ward Chambers- Illness and Injury Isolation

- a. When a colony member is seen ill or injured, they will be placed into a Hospital Ward in the same fashion as the Pregnant Queen: 3 chambers and 2 drones that will be swapped weekly. Dirty bedding will continue to be mixed back and forth between Hospital Ward and the normal colony housing cages.
- b. Once the individual has healed properly, they can be reintroduced to the colony. The technician doing the reintroduction will observe the colony throughout the day to ensure no fighting or bullying is taking place.

Approved:

Date: