STANDARD OPERATING PROCEDURE #612
WORKING WITH IMMUNODEFICIENT MICE

1. PURPOSE

This Standard Operating Procedure (SOP) describes the procedures for working with immunodeficient mice.

2. CONSIDERATIONS

Immunodeficient mouse models, such as the severe combined immunodeficient (SCID) and nude mice, are commonly used as models for immunology, infectious disease, cancer, stem cell biology, and other research. Immunodeficient mouse strains are susceptible to a wide range of infectious agents, including pathogenic and opportunistic viruses and bacteria.

Among the pathogens that can affect immunodeficient mice, *Corynebacterium bovis* is a common pathogen found in immunodeficient mouse colonies; it causes hyperkeratotic dermatitis, also known as “scaly skin disease”. The infection affects immunodeficient mice with nude mice being the most susceptible. *C. bovis* is commonly present in the environment (countertops, biological safety cabinets, hands, pens, outside of cages, etc.) and is transmissible by direct contact with infected animals or fomites. *C. bovis* may also be carried by immunocompetent mice. Immunodeficient animals purchased from approved vendors arrive disease-free and may contract the infection in the animal facility.

Clinical signs appear shortly after infection and include scaly dermatitis (yellow/white flakes on the skin), pruritus, weight loss and dehydration. In SCID mice, areas of alopecia and ocular inflammation may also be present. The dermatitis can be transient but once infected, animals will continue to shed *C. bovis* despite having no clinical signs of the disease. Diagnosis can be made by microbiologic culture of the skin or oral cavity of infected animals or by PCR. Veterinary Care staff must promptly be notified of any mice with clinical signs of hyperkeratotic dermatitis.

Currently, there is no effective treatment for the infection. Only depopulation and aggressive environmental decontamination can eradicate the organism. Adequate training of animal users is key to prevent contamination and spread of *C. bovis.*

Infected animals are not suitable for research use due to their poor condition. Hyperkeratotic dermatitis can hinder immunologic studies and may also be associated with decreased transplantable tumor take. In addition, since infected animals remain carriers of *C. bovis* for life, they act as reservoirs of infection for the colony.

3. RESPONSIBILITY

Principal investigator (PI) and their research staff, veterinary care staff.

4. MATERIALS

4.1. Accel® disinfectant spray

4.2. Personal protective equipment (PPE):

- 4.2.1. Gloves
- 4.2.2. Mask
- 4.2.3. Bonnet
- 4.2.4. Disposable gown
- 4.2.5. Disposable sleeve covers

5. PROCEDURES

5.1. Since immunocompetent mice can be carriers of *C. bovis*, always plan to enter the immunodeficient mouse room first during the work day.

5.2. It is preferable to work with different groups of immunodeficient mice on different days to prevent possible cross-contamination. If different groups must be handled on the same day, manipulate mice in the following order:

5.2.1. Non-infected nude mice

5.2.2. Non-infected furred mice

5.2.3. Imaged mice

5.2.4. Animals suspected of being infected

5.3. Use dedicated materials and equipment (scales, pens, calipers, instruments) with immunodeficient mice. Equipment should be sterilized by autoclaving or gas sterilization prior to use. If sterilization is not possible, items should be thoroughly disinfected by spraying with Accel®. Items that cannot be sterilized or disinfected (i.e., paper, notebooks) should never be placed inside the biological safety cabinet (BSC) and are to be considered contaminated.

5.4. Personal protective equipment (PPE):

5.4.1. All PPE is single use only and should be disposed of when leaving the room.

5.4.2. PPE should be worn at all times when in the immunodeficient mouse room, regardless of whether animals will be handled.

5.4.3. Gloves:

5.4.3.1. Gloves should be inspected for tears frequently and changed as needed. Stretch gloves over the cuff of the disposable gown to cover any exposed skin

5.4.4. Mask:

5.4.4.1. Masks must cover the mouth and nose at all times

5.4.5. Bonnet

5.4.6. Disposable gown

5.4.7. Disposable sleeve covers:

5.4.7.2. Sleeve covers must extend over the gloves by at least one inch.

5.5. Working in the BSC:

5.5.1. The BSC in the immunodeficient mouse room must always be left on.

5.5.2. Use the BSC within the housing room at all times; do not handle immunodeficient mice in the shared BSC in the anteroom.

5.5.3. Clean the BSC before and after each use by spraying all inside surfaces of the BSC, including the sash, liberally with Accel®. Wipe clean.

5.5.4. Place cages away from the front grill of the BSC.

5.5.5. When handling mice, make sure the BSC is empty of all other equipment or materials.

5.6. Handling mice:

5.6.1. Avoid handling mice whenever possible; animals can be observed through the cage. Consider using handling forceps when possible.

5.6.2. Only open one cage of mice at a time.

5.6.3. Before opening the cage, spray the sides of the cage and lid with Accel®. Care must be taken not to wet the filter.

5.6.4. Place the filter-top lid face up next to the cage.

5.6.5. Spray hands and wrists liberally with Accel® prior to touching the inside of the cage. Gloves must remain wet with Accel® at all times during handling of animals.

5.6.6. Spray hands with Accel® after touching cage lids/sides, cage cards, pens, calipers, etc.

5.7. Breeding immunodeficient mice:

5.7.1. It is preferable to order experimental animals directly from a commercial supplier’s pathogen-free colony.

5.7.2. When animals must be bred, breeders must be ordered directly from a commercial supplier.

5.7.3. Note that some strains of nude mice need to be maintained using specific breeding strategies. In certain strains, homozygous female nude mice do not lactate adequately. Therefore heterozygous females are bred to homozygous males. Approximately 50% of the pups born will be homozygous. Contact the supplier to determine optimal breeding strategies for immunodeficient mice.

5.7.4. Breeding animals should be replaced after 6 months with animals ordered from a commercial supplier.

6. REFERENCES

6.1. Burr HN, Wolf FR, Lipman NS. “Corynebacterium bovis: Epizootiologic Features and Environmental Contamination in an Enzootically Infected Rodent Room”. J Am Assoc Lab Anim Sci. Mar 2012; 51(2): 189–198.

6.2. Burr HN, Lipman NS, White JR, Zheng J, Wolf FR. “Strategies to prevent, treat, and provoke Corynebacterium-associated hyperkeratosis in athymic nude mice”. J Am Assoc Lab Anim Sci. 2011 May; 50(3):378-88.

6.3. Charles River technical sheet - “Corynebacterium bovis”: http://www.criver.com/files/pdfs/infectious-agents/rm_ld_r_corynebacterium_bovis

6.4. University of Missouri Comparative Medicine Program and IDEXX-RADIL - Diseases of Research Animals (DORA) website: http://dora.missouri.edu/mouse/corynebacterium-bovis-hyperkeratitis-associated-coryneform/

6.5. Foreman O, Kavirayani AM, Griffey SM, Reader R, Shultz LD. “Opportunistic bacterial infections in breeding colonies of the NSG mouse strain”. Vet Pathol. 2011 Mar;48(2):495-9.

SOP REVISION HISTORY

DATE	PREVIOUS VERSION	NEW VERSION
2017.01.19	5.7 (NO TEXT)	5.7. Breeding immunodeficient mice: 5.7.1. It is preferable to order experimental animals directly from a commercial supplier’s pathogen-free colony. 5.7.2. When animals must be bred, breeders must be ordered directly from a commercial supplier. 5.7.3. Note that some strains of nude mice need to be maintained using specific breeding strategies. In certain strains, homozygous female nude mice do not lactate adequately. Therefore heterozygous females are bred to homozygous males. Approximately 50% of the pups born will be homozygous. Contact the supplier to determine optimal breeding strategies for immunodeficient mice. 5.7.4. Breeding animals should be replaced after 6 months with animals ordered from a commercial supplier.