RUTGERS UNIVERSITY Office for Research

CMR STANDARD OPERATING PROCEDURE

TITLE: Handling Gnotobiotic Inoculated Cages		
SOP Category: Gnotobiotic	**CMR SOP #: 4.01**	**Page: 1 of 4**
Effective Date: 7/11/23	**Approval: LaTisha V. Moody, DVM, DACLAM**	
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SCOPE:

This document describes the procedures to be followed when handling BSL-2 biohazard cages in gnotobiotic animals. Rutgers Environmental Health & Safety (REHS) reviews IACUC protocols and identifies hazards such as radioactive material, chemical hazards, and biohazards. To ensure safe handling of animals and disposal of bedding and carcasses this document outlines notification requirements and points of contact. This SOP applies to all Gnotobiotic Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Gnotobiotic Research Staff members (RS) at the Rutgers University facilities.

OBJECTIVE:

The objective is to outline the proper procedures for safely conducting husbandry of germ-free (GF) animals that are colonized with BSL-2 biohazardous agents including pathobionts to reduce the risk of exposure to research and animal care staff.

TERMINOLOGY:

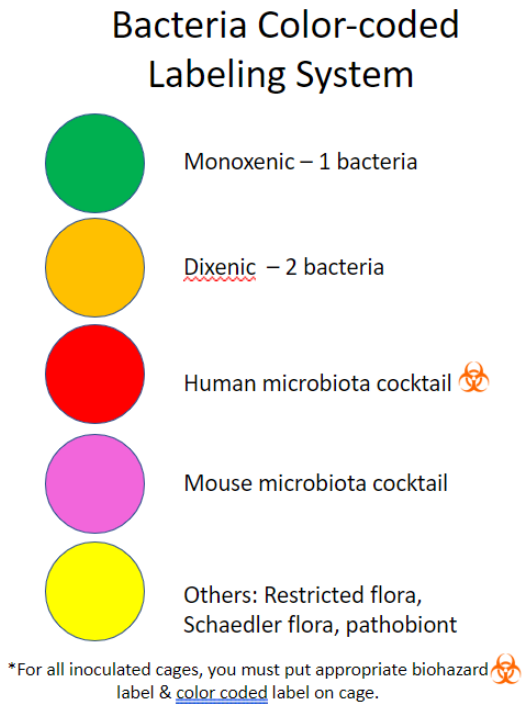
- **Germ-free (axenic)** - An animal that is free of all foreign life forms apart from itself, including bacteria, viruses, fungi, protozoa, and other saprophytic or parasitic life forms.
- **Gnotobiotic** – animal with ‘known’ or completely defined microorganisms.
- **Monoxenic or dixenic** – gnotobiotes that have been associated with one (*monoxenic*) or two (*dixenic*) species of organisms.
- **Pathobiont** – a microorganism that has the potential to cause dysregulated inflammation and disease under certain environmental conditions.
- **Restricted microbiota** - A gnotobiotic animal that has been associated with a defined microbiota, such as altered Schaedler flora (ASF), and that has been

removed from the isolator into a maximum barrier room where it becomes colonized by additional organisms.

- **Specific Pathogen Free** (SPF) - Animals free from a specified list of pathogens and potential pathogens or opportunists, but otherwise having an undefined microbiota.

PROCEDURES:

1. REHS generates hazard signage at time of IACUC protocol review. In addition, REHS sends an email to the PI, with instructions to notify CMR staff prior to hazard usage.
2. At least 1 week prior to administration of the biohazard or chemical agent, the Principal Investigator (PI) or research staff notifies husbandry supervisor, gnotobiotic facility manager and gnotobiotic technician of the duration, location, and number of cages.
3. Gnotobiotic facility manager & supervisor designates specific housing requirements and provides cage hazard labeling and signage to research staff. Administration of hazards may commence only after requirements are met.
4. All experimental biohazard in GF mice are housed in double HEPA filtered Individual Ventilated Cages (IVC) sealed positive pressure (SPP) caging (Allentown). In the event a gnotobiotic researcher requires the use of SPF mice for their gnotobiotic study, the mice will be screened for pathogens such as pinworms, fur mites and *C. bovis*.
5. PI or research staff must post the hazard signage on the room door or in room binder for the duration of hazard. Hazard signage lists procedures for the use of hazards. When hazard is no longer in use hazard signage can be removed or flipped over.
6. PI or research staff must email the Gnotobiotic facility manager for inoculum, chemical or biohazard labels. The PI must affix cage hazard labeling to the cage card once the animals in the cage have been exposed to the hazard without obstructing the RFID barcode. Date of dosing must be included on cage hazard label. Follow hazard signage for proper procedures.
    a. Examples of bacterial **biohazard inoculum** includes human microbiota and *Helicobacter.*
    b. Examples of a **chemical biohazard** include 5-Ethynyl-2'-deoxyuridine (EdU).
7. Color coded non-biohazard stickers will also be affixed to the cage with the date of dosing and bacterial inoculum.
    a. Examples of **non-biohazard inoculum** includes *Lactobacillus sp.*, *Segmented Filamentous Bacteria* (SFB), and *Bifidobacterium sp*.



8. If cage becomes non-hazardous during the study as indicated on the signage, hazard sticker must be defaced with lab dot and dated when non-hazardous.
9. If euthanasia is requested for an animal with a cage hazard label, refer to REHS signage for disposal and handling procedures.
10. When returning soiled cages to vivarium, PI or research staff must label cages with appropriate hazard label for proper disposal. The cage must be placed in a biohazard bag provided in the room and placed on a cart off the floor.
11. Please refer to SOP *#4.04 Gnotobiotic Facility Operation Allentown SPP Caging for* changing cages. There are a few differences stated below.
12. When changing biohazard or inoculated cages, the biosafety cabinet and gloves must be re-sterilized between each inoculum to ensure there is no cross-contamination. The feed and water cannot be used between different biohazards/inoculums.
13. There is no sterility testing performed for biohazard or inoculated cages including GF control animals.
14. When changing biohazard cages inside the BSC, the soiled cages are placed in a biohazard bag. The water bottles and food are emptied into the cage bottom and the in-cage HEPA filter is placed on the cage floor. The cage **cannot** be sealed/latched in the biohazard bag for autoclaving purposes. Therefore, the cages are placed in a biohazard bag inside the biosafety cabinet after finishing with cage changes. The biohazard bag is tied in a single knot and placed outside of the room on a cart.
15. If the research lab euthanizes the animals from the biohazard cage and the cage card is no longer needed, remove the RFID cage card, card holder, and other

cards from the cage. Place the items on the cart outside of the room. Place the RFID card into the red drop box so that it can be deactivated and notify the gnotobiotic staff. Place the carcass in a biohazard bag and place it in to the gnotobiotic carcass refrigerator for disposal.

16. The cages are autoclaved by the gnotobiotic core in the MSB biohazard autoclave on cage cycle #1 and then brought to the dirty side of cage wash and can be treated as a normal dirty cage for cleaning.