RUTGERS UNIVERSITY Office for Research

CMR STANDARD OPERATING PROCEDURE

TITLE: Germfree NKP Shipper Cages to Transport GF Mice		
SOP Category: Gnotobiotic	**CMR SOP #: 4.02**	**Page: 1 of 2**
Effective Date: 9/26/2023	**Approval: LaTisha Moody, DVM, DACLAM**	
Revisions:7/1/21 6/1/23 8/10/23		

SCOPE:

This document describes the procedures to be followed when transporting germ-free mice across the Rutgers campuses. This SOP applies to all Gnotobiotic Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) utilizing the gnotobiotic core at Rutgers University.

OBJECTIVE:

The objective is to transport germ-free (GF) mice and maintain germ-free status.

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.

- **NKP Isotech Cage (NKP)** – an autoclavable germ-free transport cage

Important Tips:

- Seal the cage in sterile conditions!
- Once the filter lid is securely closed you can transport the animals – making sure that the lid is not covered with anything that will obstruct airflow during transport.
- Make sure to provide sterile wet food/mash/autoclaved rodent in transport cage/container.
- The NKP cage filter in the metal lid should be changed after 20 x autoclave cycles or sooner if the filter paper gets wet or damaged. The *base is polycarbonate, do not autoclave higher than 132 °C.*

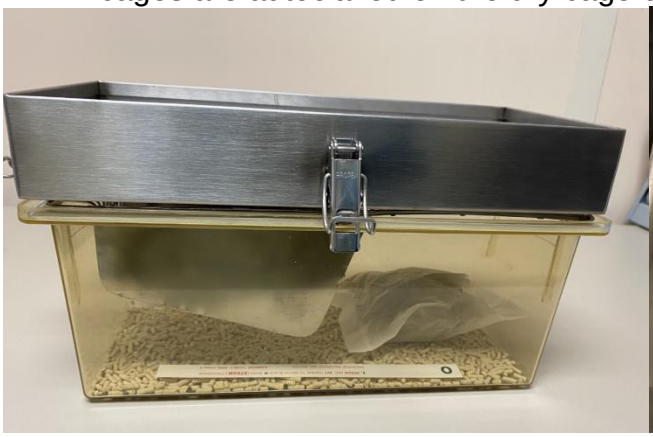
List of supplies Needed			
Clidox 1:3:1 (sterilant)	Sterile gown/sleeves	Sterile wrapped NPK cage with 50g Biofresh bedding	Pen and sharpie

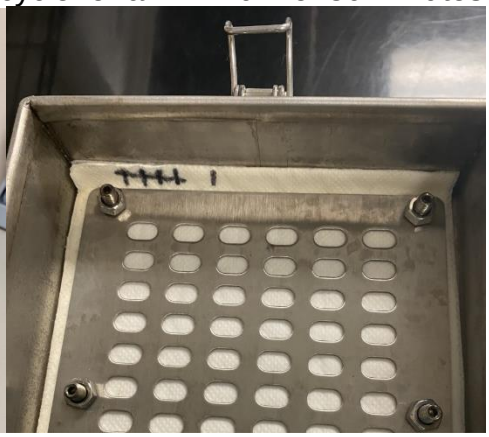
Sterile water to clean BSC surface	Sterile gloves	A chemical respirator if needed (*must be fit tested prior to use)	Autoclaved food

PROCEDURES:

Transfer GF Mice using the NKP Cage from Flexible-film isolator (method requires 1 person)

1. On the previous days before transferring, make a tick mark on the filter media using a sharpie then wrap and autoclave the cage bottom containing 1 scoop of 50g Biofresh bedding, 100 grams (about 3.53 oz) of food, wire bar and the metal lid with filter according to the *SOP #4.18 Prepping and Sterilizing Supplies*. The cages are autoclaved on the dry cage cycle for a minimum of 30 minutes.





2. Put a 1L sterile water bottle on the unsterilized BSC surface. Next unwrap the autoclaved NKP cage under the biosafety cabinet (BSC) using the drape as a sterile surface and open the sterile gloves. Sterile sleeves are optional. Put on sterile gloves and move the lid and wire bar onto the sterile drape.

3. Make sure both the inner port cap and outer port cap is closed. Inside the isolator, place animals into autoclaved paper bags or autoclaved glass bottles. Open the inner port and place the container of animals inside the port. Seal the inner port cap with the rubber bands and then open the outer port cap.

4. Remove the animals from the port and quickly transfer the animals to the BSC in the sterile NKP cage. This *must* be performed within 10 minutes.

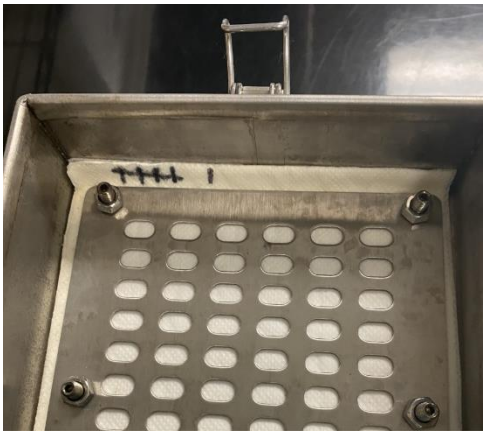
5. Without touching the sterile NKP cage bottom gently transfer the animals into the cage either from the paper bag or glass bottle.

6. Using non-sterile gloves, carefully open the sterile water bottle and pour enough water to moisten the sterile food inside the NKP cage.

7. Put on a new pair of sterile gloves and place the wire bar onto the cage. Replace the NKP cage lid and secure the latches on all sides. Be careful not to touch the inside of the sterile cage. Place the NKP cage in the designated pickup location with the printed acfc request and cage card.

8. Gnotobiotic staff member(s) should notify the facility supervisor that the cage is ready for transport. The same day of the transfer, email to reply to the acfc transfer request regarding the gnotobiotic technician time and any other fees associated with packing germfree mice. The receiving facility supervisor is responsible for closing out the acfc transfer requests.

Transfer GF Mice using the NKP Cage from Sealed Positive Pressure (SPP) IVC cages (method requires 1 person)



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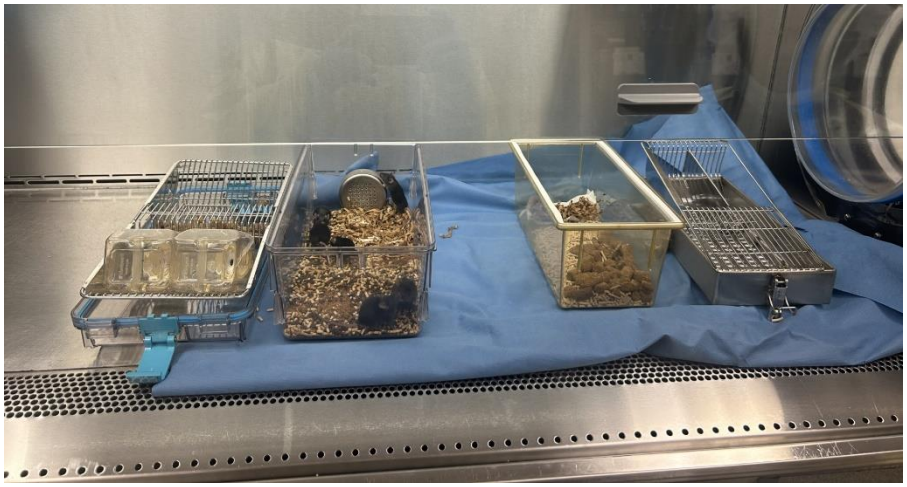
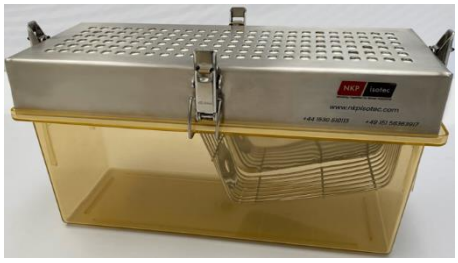
2. Unwrap the autoclaved NKP cage under the biosafety cabinet (BSC) using the drape as a sterile surface and open the sterile gloves. The sterile gloves are placed on the BSC surface next to the sterile drape. Put a 1L sterile water bottle on the unsterilized BSC surface. Sterile sleeves are optional.
3. Place SPP cage containing the GF mice on the left side of the sterile drape. Open the SPP cage lid without touching the inside sterile surface of cage and place on the left of the drape with sterile side of lid facing up.



4. Put on sterile gloves and move the lid and wire bar of NKP onto the sterile drape on the right side.
5. Transfer animals from SPP cage to NKP cage. Gather food in one corner of cage and wet food using the 1L water bottle. Replace your gloves with a new sterile pair and then close NKP cage. Using the latches of the metal lid, secure the metal lid to the cage bottom. Ensure lid is tightly sealed before removal from BSC.
6. The old RFID card will be deactivated (write void) if there are no animals remaining in the original SPP cage for transfer. The RFID card can be placed on the NKP cage to identify the animals during transfer. A new RFID card will be printed by the receiving supervisor.
7. Transfer cage can be transported in an approved transfer container (i.e. corrugated box with labels) and approved temperature-controlled vehicle to the South Campus. Mice can be maintained sterile inside the NPK Transfer cage for 72 hours.



Unpacking GF Mice from the NKP Isotec Transfer Cage at Receiving Institution at South Campus (method requires 2 person)

1. Prepare the BSC by performing a dirty scrub using the facility disinfectant to remove visible debris inside of the BSC that the GF mice will be unpacked in.
2. Sterilize surface by using 1:3:1 Clidox (spray liberally with Clidox and let contact time of 15 minutes, plus 5 minutes with blower turned on). Ensure all sterile housing is prepped before continuing with the next step of bringing in GF mice if placing into sterile caging. For additional sterile working surface, you may add a small sterile drape onto the bottom of the BSC once contact time is acquired.
    a. Outside of the BSC, place a Sealing Sticker for M1 Gnoto-Cage on top of the metal lid to cover the holes where the filter media is exposed.
    b. Important Note: work very quickly, once the sticker is placed over the filter top, the animals have no more than **30 minutes** of oxygen!!
8. The operator will put on their chemical respirator.
9. Liberally spray into the BSC the following items (with 1:3:1 Clidox): the NKP sealed transfer cage (seal with sticker before applying Clidox), swabs, pouch containing at least 4 microcentrifuge tubes for fecal collection, sterile water, and pouches containing sterile paper towels.
10. Place cage onto the left side of the BSC (the designated “dirty side”) and let it sit for a contact time of 15 minutes followed by 5 minutes with blower turned on. Make sure to monitor the animals the entire time.
11. Once contact time is finished, the sterile operator will don sterile gloves/sleeves or gloves/gown and carefully unlatch the metal lid from the cage bottom to open the cage.
12. The lid will be placed on the biosafety cabinet surface to the left of the NKP cage.
13. The sterile operator will moisten the swab using the sterile water and swab the mice and inside the NKP cage.
    a. For validation purposes only:
        i. Swab #1 – swab the BSC surface after contact time, wipe first with sterile paper towels to prevent contact with sterilant
        ii. Swab #2 – swab cage bottom and mice
        iii. Swab #3 – swab the edge of the metal filter-top lid inside the cage perimeter and culture in various media.
        iv. The sterile operator will collect feces from each mouse from the NKP transfer cage, then place mice into new sterile cage with food, water and bedding.
14. The new mice are added to the room census and the PI is notified.

Cleaning the NKP Transport Cage and Metal Lid

1. It is of utmost importance to clean the sterilant off the metal lid and cage *immediately* after use. The Sealing Sticker must be kept on the lid for cleaning to avoid getting the filter medium wet.
2. Bring sterile water and sterile paper towels into the biosafety cabinet and pour the water onto the paper towels.

3. Use the moistened paper towel to wipe off the sterilant on all sides of the cage and the top and sides of the metal lid.
4. Any extra 1:3:1 Clidox can be stored in a certified REHS container. A request made on myREHS can be made to pick up the chemical waste once container is full.
    a. Note: Once mixed, Clidox 1:3:1 must be used within 24 hours of mixing. This chemical is also light sensitive and will easily degrade when exposed to light.
5. The cage bottom and wirebar can be placed in the dirtyside cage wash for cleaning. The filtertop lid can be returned to the gnotobiotic core to hand wipe with hydrogen peroxide disinfectant wipes and reassemble cages.

Link to NKP video: https://www.youtube.com/watch?v=ESfvFIQfSV4&t=3s