RUTGERS UNIVERSITY Office for Research	RUAC STANDARD OPERATING PROCEDURE	
TITLE: DISINFECTANT IMMERSION – for specific rodent pathogen clean up		
SOP Category: Veterinary	**RUAC SOP #: 7.07**	**Page: 1 of 3**
Effective Date: 04/22/2025	**Approval:** David C Reimer DVM	
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SCOPE - This SOP applies to Veterinary Staff (VS) and Research Staff members (RS).

OBJECTIVE: To eradicate pathogens by disinfectant immersion and cross-fostering. Please refer to protocol 201800190 (Reimer) for further details.

SUPPLIES:

- Sterile gauze, coffee filter, or mesh strainer for iodine immersion
- Sterile beaker
- Iodex AR/18 (1.75% iodine) or Betadine solution (0.5% iodine) or other medical surface disinfectant as approved in the IACUC protocol
- Warm sterile distilled water
- Sterile gauze for transport
- Petri Dishes (Sterile cell culture dishes)
- Thermometer suitable for liquids

PROCEDURES

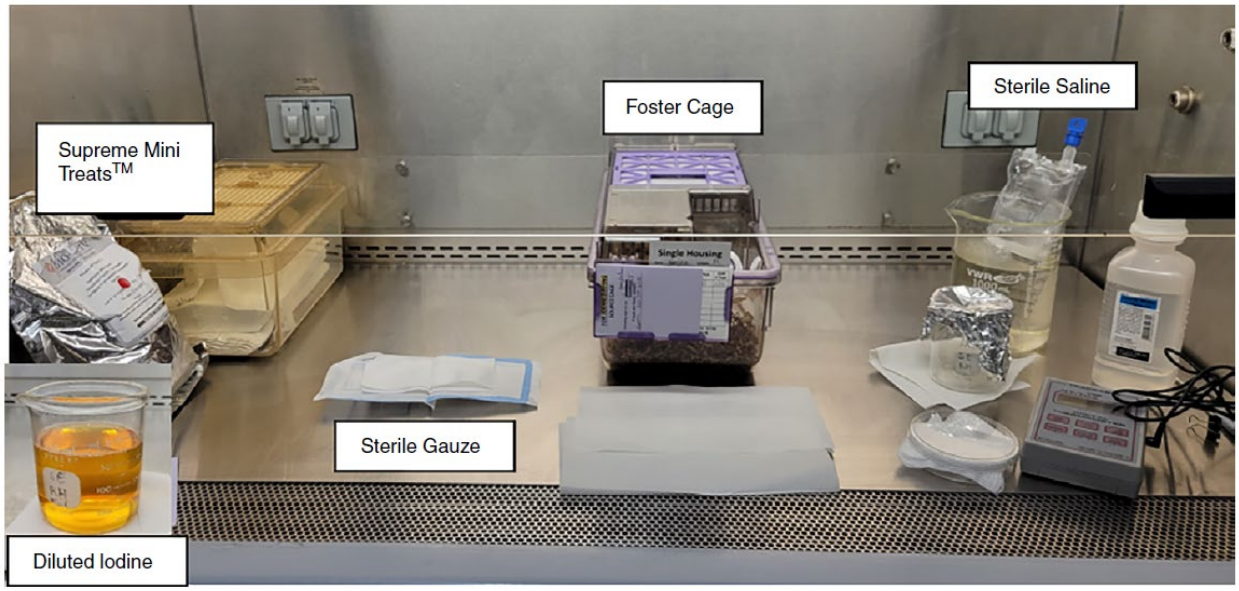
1. Set up at least two monogamous breeding pairs or a single trio breeding cages of **recipient** foster mice in the “clean” room.
2. Up to 1 day later, Set up a timed, monogamous breeding pair or trio breeding cage with **donor** mice in the source “dirty” room.
3. Check for pregnancy in both “clean” and “dirty” rooms starting on gestation days 12-14 when dams will be visibly pregnant. Separate known pregnant females into their own birthing cages.
4. Within 48 hours of birth, immerse donor offspring in a warm disinfectant solution for pathogen elimination. The steps needed for immersion take place in a biological safety cabinet and are as follows:
    a. Make a warm solution of disinfectant and place into a sterile beaker. The solution should be no warmer than 37°C (98.6°F). Disinfectant options listed below:
        i. 50 ppm iodine solution (3 mL Iodex AR/18 in 1 L water OR 10 mL Betadine solution in 1 L water)
        ii. up to 2% concentration chlorhexidine
        iii. 2-6% concentration hydrogen peroxide
        iv. 50 ppm hypochlorous acid
    b. Warm the solution by placing the containing beaker inside a larger beaker filled with pre-warmed water.
    c. Immersion procedure:

i. Prepare a sterile square of gauze, sterile coffee filter, or sterile mesh strainer in a sterile cell culture dish.
ii. Remove donor pups from their cage and place them on the sterile gauze, coffee filter, or strainer.
iii. Place the pups onto a sterile square of gauze in a sterile cell culture dish, and hand them off to "clean" personnel, who will transfer them into the "clean" room.
iv. Gather the corners of the gauze or filter together, or pick up the strainer
v. **After confirming the temperature of the solution, submerge the pups into the solution for up to 10 seconds**.
vi. Change gloves.
vii. Place pups onto a piece of dry, sterile gauze and gently pat dry.

d. Cross-fostering procedure:
i. Remove and euthanize all but 2 or 3 of the foster dam's neonates to prepare the foster dam to receive the donor offspring. The foster dam's litter should be at least 1-2 days older than the donor pups at this time point.
ii. Pick up the foster dam and encourage her to urinate and/or defecate into your hand.
iii. Gently rub dirty bedding, urine, feces, and nesting material of the foster mother onto the donor pups.
iv. Place the donor pups into the foster cage and observe the dam to ensure she is caring for the pups. This will consist of behavior such as picking up the pups and gathering them into her nest, grooming them, nursing them, etc.
v. Nutritional supplementation such as Supreme Mini Treats™ may be added to the cage.
vi. Observe the foster dam later that same day and then once daily for up to two days after cross fostering to ensure she is caring for the donor offspring.
vii. If fostering is ultimately unsuccessful and a different dam unavailable, euthanize the pups.
viii. It is important to change gloves and use disinfectant (MB-10 or Peroxigard) between procedural steps to limit contamination of "cleaner" areas with materials brought over from "dirtier" areas (such as the cell culture dish and other supplies).

5. At weaning, collected fresh feces from the pups and foster mother for submission for specific excluded pathogen PCR testing (e.g. *Helicobacter*).
6. With the receipt of NEGATIVE test results for the desired excluded pathogen, the cleaned mice are permitted to remain in the SPF colony room for line propagation.
7. With the receipt of POSITIVE test results for the desired excluded pathogen the cleaned mice are immediately removed from the clean room

and returned to a non-pathogen excluded room or humanely euthanized and the process repeated. The foster dam is euthanized.



Immersion setup

REFERENCES

Watson, J., Thompson, K. N., & Feldman, S. H. (2005). Successful Rederivation of Contaminated Immunocompetent Mice Using Neonatal Transfer with Iodine Immersion. *Comparative Medicine, 55*(5), 465-469.

Kehoe et.al. (2025) Effective Eradication of Mouse Norovirus and Helicobacter spp. in Laboratory Mice (Mus musculus) via Iodine Immersion and Cross-Fostering Technique. JAALAS