

HANDLING & USE OF IMMUNE DEFICIENT MICE IN-PERSON TRAINING

UNIVERSITY OF SOUTH FLORIDA
COMPARATIVE MEDICINE

Attendee Name: _____
Print Sign Date

Discuss/Review:

- ___ Complete AALAS Learning Library tutorial “**Handling and Use of Immune Deficient Mice**”
- ___ SOPs 11, 408, 413, 1015, 1127, The Guide, 9 CFR, The AWA & IACUC Principles
- ___ Mice have various T, B, NK cell, macrophage, dendritic cell, complement, and cytokine deficiencies
- ___ Mice are spontaneous mutants (SCID, NOD-SCID) or genetically-engineered (Rag-KO, NSG)
- ___ Immune deficient mice require careful handling and use. Use **immune deficient mice in isolation first**.

Entry Procedures:

- ___ Go directly to isolation, use **immune deficient mice in isolation first**, then other mice, and last, mice housed in ABSL-2 biocontainment
- ___ Whenever possible, in isolation housing and use, **work in pairs**.
- ___ Equipment (e.g., anesthesia machines, scales, calipers) and supplies **dedicated to isolation** (SOP 413)
- ___ Prior to entry, ensure that all reagents and **biologics** have been **characterized free of infectious agents** or are **sterile preparations**, **primary containers** are decontaminated by saturate spraying with Oxivir, and these are placed in nonporous **secondary containers** (e.g., Ziploc bag, conical tube, screw-top containers) that can be surface decontaminated by **saturate spraying with Oxivir** during isolation entry.
- ___ Appropriate sequence of donning PPE, disposable gown and shoe covers donned at the facility entrance, then **outside the isolation door don in sequence** (a) **bouffant**, (b) surgical **mask** that covers the nose, (c) push your thumb through the seam of the sleeve of the gown, (d) put on a pair of **gloves** ensuring cuffs overlap the gown, then **enter** the isolation room ensuring shoe covers make **contact** with the **antimicrobial adhesive mat** just inside the door. Before handling anything after isolation entry, **don in sequence** (e) **moisture proof sleeves** or apron, and (f) put on a **second pair of gloves** ensuring gloves overlap sleeves and no skin is exposed. Decontaminate sleeves and gloves by **saturate spraying with Oxivir** allow 5 minute contact time.
- ___ Whenever secondarily covered supplies and equipment of a substantive volume are delivered to isolation, a **two person transfer procedure** should be utilized with (a) the interior individual donning PPE in advance in accordance with entry procedures described below, (b) keeping the door **closed** while the exterior individual **saturate sprays the secondary container** of equipment/supplies with Oxivir, (c) the door is **opened**, and the sprayed equipment/supplies transferred to the **interior cart**, (d) the door is **closed**, and the interior cart and equipment/supplies **sprayed again** by the interior individual and allowed to sit for **5 minutes** inside isolation.

Use Procedures:

- ___ Each occupied **microisolator** in isolation housing **must remain in its labeled location** on the rack and must be placed in this same rack space **for the duration of study**.
- ___ Prepare the biosafety cabinet for handling and use of mice. Ensure the biosafety cabinet is on. **Saturate spray the biosafety cabinet interior with Oxivir** and allow it to sit for 5 minutes.
- ___ If using portable **equipment** within the biosafety cabinet, ensure these are saturate sprayed with Oxivir, including all surfaces that may come into contact with mice.
- ___ As a **work surface** for instruments, equipment, or supplies, Oxivir soaked paper towels or blue pads may be placed inside the hood.
- ___ If inhalational **anesthesia** is planned, saturate spray with Oxivir the induction chambers and rodent nosecone of the quad anesthesia unit and let it sit for 5 minutes.
- ___ If **imaging** in isolation, disconnect the induction chamber from the anesthesia machine, saturate spray with Oxivir both the interior and exterior of the induction chamber, and enter it into the biosafety cabinet and let it sit for 5 minutes.
- ___ After ensuring that all supplies and equipment are available and decontaminated, remove a cage with its water bottle in place from the rack and **saturate spray the cage and water bottle** with Oxivir.

- ___ Place the sprayed cage with bottle in the biosafety cabinet and allow it to sit for 5 minutes. Multiple cages may be sprayed and loaded into the biosafety cabinet, but only one cage may be opened at a time within the biosafety cabinet.
- ___ **Saturate spray gloves and sleeves** with Oxivir. A pan of Oxivir solution may be used to decontaminate gloved hands by immersion before handling mice in each microisolator.
- ___ Ensure that all activities are conducted at least 4 inches within the air curtain of the biosafety cabinet (i.e., greater than the height of a cage card holder).
- ___ Nothing should touch the interior surfaces of the microisolator, including any surface of the biosafety cabinet.
- ___ Remove the water bottle and place it on the work surface. Carefully unclamp and remove the microisolator lid and invert it onto the biosafety cabinet work surface and place the wire lid on top of the inner surface of the lid. The wire lid can be used to assist in handling rodents.
- ___ Saturate spray with Oxivir sleeves and gloved hands before handling mice, and between each cage.
- ___ Work surfaces/hoods are decontaminated after use by saturate spraying with Oxivir and allowed 5 minutes contact.
- ___ **Depart in your PPE.**

Use Procedures Outside of Bidirectional Isolation:

- ___ If reentry of mice is required to isolation, don a **Tyvek suit** and follow the entry procedures described above. Plan your day so that returning to isolation is not required. If other housing and use areas are accessed after departing from isolation and you must **re-enter** isolation, you must first don a **Tyvek suit in addition to all new PPE** in accordance with entry procedures above.
- ___ Isolation housing and use areas have limited personnel access, additional PPE required, monthly monitoring for opportunistic infectious agents, and dedicated equipment and supplies.
- ___ Some isolation housing and use is **unidirectional** (e.g., with IVIS 100 in isolation, or limited to immune deficient colony production), consequently, mice in unidirectional isolation **may not exit except** for post mortem tissue derivations in **necropsy**.
- ___ Some isolation housing and use is **bidirectional**, permits mice to exit to common procedural areas (e.g., IVIS 200, MRI, gamma or x-irradiator, CT/PET/SPECT) and return to isolation housing.
- ___ Signage on the isolation housing and use door must indicate whether mice can exit to common procedural areas and return to isolation housing.
- ___ Whenever mice in isolation must exit bidirectional isolation for a **common procedural area**, PPE must be donned in advance in accordance with **entry procedures** described above, the microisolator to be transported is identified and saturate sprayed with Oxivir, and the sprayed microisolator transferred to the common procedural area by **exiting in your PPE**.
- ___ If **several** microisolators are to be transferred to a common procedural area, a **two person transfer procedure** should be utilized to exit with (a) the interior individual donning PPE in advance in accordance with entry procedures described above, (b) keeping the door **closed** while the interior individual **identifies and saturate sprays the microisolators** with Oxivir on an interior cart, (c) the door is **opened**, and the sprayed microisolators transferred to the exterior individual and an **exterior cart**, (d) the door is closed, and the exterior cart is used to deliver the microisolators to the common procedure area.
- ___ Immune deficient mice that must exit bidirectional isolation and **return** to isolation housing must be delivered back to bidirectional isolation using a **two person transfer procedure** with (a) the interior individual donning PPE in advance in accordance with entry procedures described above, (b) keeping the door **closed** while the exterior individual saturate sprays the microisolators with Oxivir, (c) the door is **opened**, and the sprayed microisolators are transferred to the **interior cart**, (d) the door is closed, and the interior cart and microisolators are **sprayed again** by the interior individual and allowed to sit for **5 minutes** inside isolation.
- ___ All equipment and work surfaces in the common procedural areas should be decontaminated prior to and after each use.
- ___ Staff intending to work in common procedural area with mice from bidirectional isolation must in addition to the disposable gown and shoe covers, don in sequence (a) bouffant, (b) surgical mask that covers the nose, (c) push your thumb through the seam of the sleeve of the gown, (d) put on a pair of gloves ensuring cuffs overlap the gown, (e) Tyvek sleeves, and (f) put on a second pair of gloves ensuring gloves overlap sleeves and no skin is exposed.

___ Work in common procedural areas involving mice from bidirectional isolation should precede work involving other mice from other housing areas. If multiple cohorts from separate housing rooms are present in the common procedural area, work with cohorts must occur in sequence, with work involving mice from isolation, including those administered uncharacterized human specimens, preceding the other cohort, rather than concurrently. **Only one cage should be opened at a time.**

Demonstrate:

- ___ Isolation signage, unidirectional, bidirectional isolation housing and use areas
- ___ Emphasize that on **animal orders** or relocation requests the appropriate isolation housing and use room should be indicated for the intended use
- ___ Moffitt Resource Scheduler be used to reserve biosafety cabinets
<https://moffitt.resourcescheduler.net/mapview> in Isolation.
- ___ Appropriate isolation **entry**, donning and decontamination of PPE
- ___ Dedicated equipment and supplies
- ___ Appropriate decontamination and **two person transfer** entry of secondarily enclosed supplies
- ___ Disinfection of biosafety cabinet and other work surfaces (e.g., induction chamber, equipment)
- ___ Appropriate movement within the **biosafety cabinet**, >4" from air curtain, use of Oxivir soaked towels
- ___ Prior to departure, remove outer gloves and Tyvek sleeves and place in red biohazard bin. Depart in your remaining PPE. If Tyvek suit had been donned, remove outer gloves prior to exit.
- ___ Rentry procedures requiring **additional PPE**
- ___ Return to bidirectional isolation **requires** two person transfer

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