STANDARD OPERATING PROCEDURES

DIVISION OF COMPARATIVE MEDICINE
UNIVERSITY OF SOUTH FLORIDA

TITLE:	**Germ-free and Gnotobiotic Mice Care and Use**
SCOPE:	All Authorized Personnel
RESPONSIBILITY:	Facility Manager and Technical Staff
PURPOSE:	To Outline the Proper Procedures for Safely Caring for and Using Germ-free & Gnotobiotic Mice

I. PURPOSE

1. To outline the proper procedures for safely and effectively conducting husbandry, handling and use of **germfree and gnotobiotic** (GF) mice housed in bioexclusion isolation individually ventilated caging that is positively pressurized and hermetically sealed (IsoIVC-P), using a glovebox isolator with integral hydrogen peroxide vaporizer (i.e., Bioquell Qube) to decontaminate the exteriors of all caging, supplies and equipment prior to opening any IsoIVC-P.

2. To ensure, longitudinally, the continual **absence** of microbes in germfree mice, and the consistent presence of a **defined** microflora in gnotobiotic mice.

3. To ensure that occupied and autoclave sterilized unoccupied IsoIVC-P used to house GF mice, and autoclave sterilized reverse osmosis (RO) drinking water reservoir bottles are all **only opened inside the Qube** after completion of a **HPV loaded sterilization cycle**.

4. To ensure that only IsoIVC-P housing "**like microflora**" mice (e.g., germfree mice only, or similar gnotobiotic mice only) are entered into the Qube **together** in preparation of a Qube hydrogen peroxide vapor (HPV) loaded cycle.

II. RESPONSIBILITY

1. The Veterinarian, Assistant Directors, and Facility Managers are responsible for ensuring:
    a. All CM personnel are trained to perform the practices described.
    b. Implementation of the GF isolation procedures described.
    c. Appropriate equipment, personal protective equipment (PPE), and supplies are available and used.

2. It is the responsibility of all CM personnel to read, understand, and follow the procedures outlined below, and to document their training in these procedures.

3. Access to GF isolation housing and use is limited to essential Comparative Medicine (CM) personnel only with documented in-person training presented to the IACUC and uploaded to ARC.

III. PROCEDURES

III.A. Autoclave Sterilization of IsoIVC-P

1. **Fully assembled IsoIVC-P** (i.e., bottom, top, wire, bottle, sipper, cage-level HEPA, extruded feed, cellulose bedding, nestlet, igloo), are **sterilized as a unit,** held firmly **closed** on a GF sterilization transport rack, but unlatched, with white safety buttons up, and each supply air valve actuated open.

2. Only **autoclaved, irradiated, extruded** (not pelleted) chow (e.g., **5VOF-IRR 25#, Irradiated Purina Select Rodent Lab Diet 50 IF/6F Auto**) is used in GF isolation.

3. Only **autoclaved, irradiated, paper cellulose** (not recycled) bedding (e.g., **Shepard's Alpha Dry-IRR**), is used in GF isolation.

4. Only **autoclaved reverse osmosis (RO**)-purified drinking water in red-capped glass reservoir bottles is used in GF isolation.

5. After washing equipment in accordance with SOP 1005 *Cagewash Operation* and SOP 1004 *Monitoring Cagewash Efficacy*, each clean, assembled IsoIVC-P used in GF isolation must have a **cage-level HEPA filter** appropriately seated in the cage top.

6. Before installing in the cage top, each cage level HEPA filter must first be **numbered** with a sharpie, prior to assembling each IsoIVC-P in preparation of a GF autoclave sterilization cycle.

7. Cage level HEPA filters are discarded after **5 autoclave sterilization cycles**.

8. To track autoclave sterilization cycles experienced by a cage level HEPA filter, number the HEPA filter's white frame with a sequence of numbers (1-5) each time it is to be inserted into the cage top, indicating that an additional sterilization cycle will be experienced.

9. To appropriately seat, align the cage level HEPA **tab to its slot**, pressing on the **perimeter** (not center) of the HEPA filter housing, and ensure that the blue filter **cover snaps securely** closed.

10. Check to ensure the **red flexible gasket** that surrounds the top at its periphery is approximately seated into the groove in the IsoIVC-P top. This will ensure the IsoIVC-P is hermetically sealed when fully assembled and clamped shut.

11. Assembled IsoIVC-P are sterilized using a **GF autoclave cycle** of 2.45 psia pre-vacuum, 121°C, 20 minutes, 2.45 psia drying vacuum while IsoIVC-P are **unlatched**, with **white safety buttons up**, with each IsoIVC-P held tightly in place by the spring-loaded runners of the GF transport rack, with each IsoIVC-P **supply air valve actuated open** to permit steam to enter each interior.

12. GF autoclave sterilization cycles of IsoIVC-P are **validated as sterile** in accordance with SOP 1002 *Monitoring Autoclave Sterilization* using a Steris Verify Steam Test Pack with Self Contained **Biological Indicator** and **Integrator** strip placed in the **center "indicator" IsoIVC-P** of a GF transport rack in the autoclave sterilized load.

13. In addition, a **dated Integrator strip** must be **taped to the front** of each GF sterilization transport rack prior to sterilization and left in place when delivered to GF housing.

14. After IsoIVC-P autoclave sterilization, using gloves and sleeves that are saturate-sprayed with Oxivir-Tb, the center "indicator" IsoIVC-P is carefully removed and discarded, and the **Integrator strip dated**, recorded, and **taped to the front** of a remaining IsoIVC-P on the GF sterilization transport rack to indicate sterilization date of the delivered load.

15. The Biological **Indicator** from the "indicator" IsoIVC-P is set in the incubator, read and recorded per SOP 1002.

16. If more than one autoclave IsoIVC-P load is processed in a single day, loads must be distinguished by labeling Biological Indicators and Integrators (e.g., date-A, date-B, date-C).

17. In addition, for each autoclave sterilized load, the dated **autoclave printer strip is taped to the front** of the GF sterilization transport rack to document its completion of a GF sterilization cycle.

18. After removing the indicator IsoIVC-P, using gloves and sleeves that are saturate-sprayed with Oxivir-Tb, the GF sterilization transport racks of sterile IsoIVC-P are taken directly to GF isolation for **two-person entry**, see below.

19. **Each delivered** GF transport rack of IsoIVC-P must have both a **dated autoclave printer strip** and a **dated Integrator strip** that has changed in accordance with SOP 1002**, taped to the front** of one IsoIVC-P when delivered to GF housing, and left in place until IsoIVC-P use.

20. If Integrator strips or Biological Indicators after incubation have not changed according to SOP 1002 and SOP 1013, **notify** your supervisor and GF isolation staff immediately. Such supplies are **rejected** and returned to dirty side.

21. Inside GF isolation, each IsoIVC-P is left held tightly in place by spring-loaded runners, allowed to cool.

22. Each IsoIVC-P is then carefully **latched shut** by raising the closing clamps on each side, with the clamp tabs secured under the top rim of the box. The clamps are then pushed inwards, and the white safety buttons on each side are pushed downwards, **locked**. Ensure the **white tab** presses smoothly and becomes fully **down**.

23. Locked sterile IsoIVC-P are then placed on the GF housing rack for use, fully docked if the air handling unit AHU of the rack is actively pressurized and ventilating, but **not fully docked if the AHU is off**.

III.B. Autoclave Sterilization of Reservoir Bottles

1. Autoclaved RO drinking water is prepared in **reservoir bottles** in accordance with SOP 1006 *Autoclave Sterilization* and SOP 1013 *Monitoring Steam Sterilization of Liquids* except that the **GF liquid sterilization cycle** must be 122°C, 90 minutes, 2.45 psia vacuum (i.e., no pre- or post-pulse stages).

2. Baskets of reservoir bottles (i.e., Corning 1L glass bottles) are filled with RO drinking water, **capped loosely** with red high temperature-resistant caps, and are **Tyvek covered** over the top.

3. Filled reservoir RO water bottles are validated as sterile per SOP 1013 using a **MagnaAmp** indicator suspended inside one filled uncapped "indicator" bottle, and a **dated Integrator strip taped onto the top Tyvek cover of each basket** in each autoclaved load.

4. After sterilization, using gloves and sleeves saturate-sprayed with Oxivir-Tb, the "indicator" bottle with the MagnaAmp is opened and discarded, the MagnaAmp is set in the incubator (later read and recorded), and the covered basket(s) of bottles with dated Integrator strip delivered to GF isolation housing for two-person entry**.**

5. **Each delivered** load of reservoir bottles must have a **dated Integrator strip** that has changed in accordance with SOP 1013.

6. All supplies destined for GF isolation housing must be sterilized & dated, or be capable of being HPV-sterilized later in the Qube, prior to two-person delivery.

7. If Integrator strips or Biological Indicators after incubation have not changed according to SOP 1002 and SOP 1013, **notify** your supervisor and GF isolation staff immediately. Such supplies are **rejected** and returned to dirty side.

III.C. Two-Person Delivery of Sterilized Equipment & Supplies to GF Isolation

1. Whenever **autoclaved** Iso-IVC, reservoir bottles, or other equipment and supplies are delivered to GF isolation, a **two-person transfer procedure** is utilized with:

    a. Both the interior, receiving staff (i.e., shoe covers, gown, bouffant, mask, sleeves, gloves) and exterior, delivery staff (i.e., sleeves, gloves) don PPE in advance and assist with entry procedures described below.

    b. Keep the door **closed** while the exterior individual **saturate sprays** the exterior of the sterile equipment & supplies with **Oxivir-Tb**,

    c. The door is opened, and the sprayed, sterilized equipment & supplies are either transferred to an interior cart, or the GF transport cart is rolled over the **antimicrobial adhesive mat** just inside the door,

    d. The door is closed, and the cart, rack, equipment, and supplies are **sprayed again** by the interior individual, and allowed to **sit for 5 minutes** inside GF isolation housing.

e. Inside GF isolation housing, using Oxivir-Tb saturate-sprayed gloves & and sleeves, baskets of GF water reservoir bottles are left Tyvek **covered** and placed on a shelf with the **dated** Integrator strip **clearly visible**.

f. When baskets are cool, each reservoir bottle red cap is tightly closed, and the Tyvek covering replaced.

g. The locked IsoIVC-P are removed from the GF sterilization transport rack, and placed on the IsoIVC-P housing rack with actively pressurized and ventilating AHU ensuring each inadequate docking indicator **yellow flag closes**.

h. If the **AHU is off**, the IsoIVC-P should **not be fully docked** on the rack.

i. The dated Integrator strip taped to the exterior front of one IsoIVC-P is **left in place** and used to indicate the date of the delivered load.

j. The dated Integrator strip is removed and **discarded** when the “indicator” IsoIVC-P is used, just prior to its entry into the Qube “glove-box” isolator.

k. All autoclave sterilized IsoIVC-P and Tyvek-covered, red capped, RO drinking water reservoir bottles must have **autoclave print out paper** strips and appropriately turned, **dated Integrator strips** both taped to the exterior for validation of sterility prior to acceptance and entry into GF Isolation.

III.D. Preparation of GF Isolation

1. GF isolation is prepared in advance of occupancy with all equipment and supplies **dedicated** to GF isolation, and validated as decontaminated using HPV in accordance with SOP 1016 *Hydrogen Peroxide Vapor Decontamination* and SOP 1162 *Bioquell Z-2 Hydrogen Peroxide Vapor Generator System*.

2. Occupied and unoccupied IsoIVC-P and RO drinking water reservoir bottles are **only opened inside** the Qube after a bio-decontaminating HPV loaded cycle.

3. Occupied IsoIVC-P are **monitored** for microbes whenever opened.

4. Specimens from **germfree** mice are frozen (IDEXX), while specimens from **gnotobiotic** mice are placed in a vendor provided kit solution (Transnetyx).

5. All specimens are shipped overnight for analysis.

6. Portable **equipment** (e.g., scale, caliper, surgical instruments, anesthesia machine) and consumable **supplies** (e.g., syringes, needles, gavage needles, specimen collection tubes) must be delivered to GF isolation in sterile packs, or after autoclave sterilization, and all are also HPV sterilized in the Qube prior to use.

7. The antimicrobial adhesive mat placed inside the door has its contact sheet removed at least daily.

8. If additional consumable supplies, equipment, reagents or biologics are needed during study, they must be in **nonporous containers** (e.g., Nunc tubes, autoclave-able bags) and either sterilized by autoclaving, and/or by HPV inside the Qube prior to use.

9. Containers of supplies are delivered using a **two-person** transfer procedure as described above.

III.E. Personnel Entry into GF Isolation

1. Access to GF housing and use is limited to essential personnel, and only those with **documented** in-person GF isolation training presented to the IACUC and uploaded to ARC.

2. Work in GF isolation **must precede all other work** in any other animal facility area.

3. Prior to entry, ensure that all portable **equipment and supplies** required for work have been **dedicated to GF isolation** housing and use.

4. The GF isolation room door must remain **locked**.

5. Signage on GF isolation must indicate that germ-free or gnotobiotic mice are housed in GF isolation and describe the PPE and procedures required for entrance.

6. After donning disposable shoe covers and gown, complete GF isolation personnel entry procedures below.

7. At the GF isolation room door, all CM staff must don or already be wearing a disposable **gown** and **shoe covers**, and then **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**, and (e) don **Tyvek sleeves** ensuring sleeves overlap gloves and no skin is exposed.

8. Then **enter** GF isolation ensuring shoe covers make **contact** on the **antimicrobial adhesive mat** just inside the door.

9. Before handling anything, **saturate spray your sleeves and gloves** with Oxivir and wait 5 minutes before handling anything.

10. Note that Oxivir-Tb and other decontaminating sprays or solutions **containing alcohol**, are **never used inside the Qube system**.

11. Only **sterile low particulate wipes** that do not contain alcohol (e.g., Klerwipe-CR) impregnated with either Biocide A, B, C, or D may be used inside the Qube system.

12. Sprays should **not** be used inside the Qube system.

13. Do **not** use solvent containing solutions to clean the Qube control panel screen or front access door glass. The control panel screen will be damaged if cleaned with a solvent containing solution.

14. **Occupied IsoIVC-P** must **never** be left **undocked** from the bioexclusion housing rack, since each primary enclosure is a sealed unventilated microenvironment.

15. Plans for exterior bio-decontamination of occupied Iso-IVC inside the Qube must be finalized, and all supplies and equipment assembled in advance, prior to undocking an occupied Iso-IVC.

III.F. Qube System "Glove-box" Isolator

1. Whenever possible, **work in at least pairs**.

2. The Bioquell Qube is an aseptic workstation, a rigid "glove-box" isolator, with an integral HPV bio-decontamination system, which is loaded via the front access window.

3. The front access window is opened and shut by carefully pulling or pushing on the **rim of the glove ports**. Warning, **do not push** on the sides of the window as this could cause damage.

4. The Qube system has 2 large chambers, a hydrogen peroxide vapor (**QHPV**) module, and an extension (**QEXT**) module. Each of these 2 larger chambers is flanked by a smaller, material transfer device (**QMTD**) used for exiting processed material.

5. The complete Qube system is controlled via a control panel on the QHPV module.

6. The QEXT enables the operator to work on or store one load while simultaneously HPV decontaminating another load in the QHPV.

7. The QMTD is used to transfer the completed load out of the Qube system.

8. Qube system interior lighting is "white", but changes to "blue" during a HPV cycle.

9. When not in use, to ensure diurnal lighting of GF isolation housing, the interior lights of both the QHPV and the QEXT should be **switched off**.

10. During **bio-decontamination mode**, the QHPV provides assurance of a 6-log microbial reduction of the load placed inside the QHPV module.

11. Bio-decontamination can be of a load placed inside the QHPV (i.e., **QHPV loaded cycle**), or of the empty Qube system with all interior doors open (i.e., **System empty cycle**).

12. During "use" or **processing mode** (after HPV decontamination), the Qube system provides a unidirectional airflow working zone that meets the classification

requirements of **ISO 14644-1 Class 5** (equivalent to EU cGMP Grade A and US FED STD 209E Class 100).

13. During “use” or processing mode, the Qube system maintains an **airflow rate** of **573 m3/h** and chamber **pressure set points** of **75 Pa** for the QHPV, **50 Pa** for the QEXT, and **36 Pa** for each of the QMTDs (i.e., a positive pressure gradient from the QHPV to the room).

14. During a bio-decontamination cycle a pressure setpoint of **50 Pa** is maintained.

15. The bio-decontamination **QHPV loaded cycle** (i.e., either a QHPV loaded cycle or a system empty cycle) is a four-stage process that includes (1) **conditioning**, when the vaporizer increases to operating temperature, (2) **gassing**, when the hydrogen peroxide liquid is drawn from its bottle, flash vaporized and distributed around the chamber(s), dew point is reached and micro-condensation forms on Qube interior surfaces, (3) **dwell**, when the HPV is maintained at a constant level and distributed inside the Qube, and (4) **aeration**, when the HPV is catalytically converted to water vapor and oxygen.

16. If a QHPV loaded cycle or System Empty cycle ends in an **alarm**, all doors will **automatically lock**, a safety coverall in case HPV ppm remains inside the Qube chamber(s).

17. In the case of an alarm, consider the HPV ppm level present inside the chamber. An **aeration only cycle** will clear remaining HPV from the chamber, but **will not unlock** the front access window after aeration cycle completion.

18. In the case of an alarm, the front access window can only be opened if either another HPV cycle is started and successfully completed, or the door interlock is **disabled** administratively.

19. If disabled, after opening and closing the front access window, the door interlock must be manually **reset**.

20. A key to all **alarm codes** is found in the Qube user manual.

21. Both the QHPV and QEXT modules provide **accessory electrical outlets** inside each chamber that are used by installing adapter connections.

22. Both the QHPV and QEXT have **side doors** fitted with **pneumatic inflatable seals**, which automatically inflate when the door is manually closed. All doors are **interlocked**, ensuring that doors cannot be opened during bio-decontamination mode.

23. Each side door has an **indicator light** which is either lit **continuous green** when it is **enabled** and the door can be opened, **not illuminated** when it is **disabled** and cannot be opened, **flashing red** when there is an **alarm** involving the door seal, or **flashing green** when an **action** is required of the door (e.g., “open the door” prior to the start of a system empty cycle, or “close the door” at the end of a system empty cycle).

24. To **open a side door** between a chamber, depress the footswitch located below it on the handle side, the seal will deflate, the indicator light will flash green, then manually lift the door open.

25. During “use” or **processing mode**, internal side doors of the QHPV module can only be opened if the entire Qube system has been bio-decontaminated, and **only one** internal door can be opened at a time.

26. Items can be **exited** from the QHPV to the left, or from the QEXT to the right via the adjoining QMTD.

27. Only one door of the **QMTD module** can be opened at a time. After opening the QMTD external door, there is a **2-minute clean-up time** before the internal door of the same QMTD can be opened again.

28. The **front access window** of a QHPV module can be **opened only** if the interior side doors are closed and sealed first with each green indicator light illuminated.

29. The Qube system uses 35% hydrogen peroxide in 150ml bottles. Each bottle contains a Radio Frequency Identification (**RFID**) tag which enables the bottle’s batch number, volume remaining, and expiration date to be read by the Qube.

30. If there is **sufficient** hydrogen peroxide for the requested cycle, the bottle icon surround on the QHPV control panel is colored **blue**. The volume left in the bottle is displayed in the center of the bottle icon on the control screen.

31. The bottle module door can be opened manually only if there is no bottle loaded.

32. If there is **insufficient** hydrogen peroxide for the cycle (i.e., indicated by an **amber** surround of the bottle icon on the control screen), unlock the bottle module door by selecting the bottle changing function on the control screen, and the **bottle module** door will open.

33. Unscrew and remove the bottle’s cap leaving the membrane in place. Set the bottle into the holder and rotate, ensuring the bottle “locates” onto the register and the RFID can be read. Close the bottle module by pushing gently.

34. The control panel outline color of each module represents its condition; **GREEN** meaning aseptic; **AMBER** not aseptic; **BLUE** when in cycle; **RED** during alarm conditions.

35. An **Aeration Only Cycle** only runs the aeration phase. It may be run if the Qube alarms mid-HPV cycle, the cycle is aborted, and remnant HPV requires removing.

36. A **Pressure Test Cycle** monitors for chamber leaks on either the QHPV or the complete Qube system. If unsuccessful, an alarm icon will appear in the navigation bar and the cycle will end prematurely.

III.G. Germ-free and Gnotobiotic Mouse Husbandry Using the Qube System

1. Whenever possible, **work in pairs**.

2. IsoIVC-P used to house mice of a **presumed similar microbiota status** (e.g., germfree or comparable gnotobiotic) may be entered **together** into the Qube system for change-outs.

3. Ideally, whenever possible, germfree mouse husbandry and gnotobiotic mouse husbandry should occur on different work days.

4. **Whenever IsoIVC-P** housing **germfree** mice are to be changed out **using the M35** Model Qube (i.e., largest Qube with 4 chambers, including a QHPV, QEXT, and two QMTDs) a **System Empty Cycle** must **precede each** and every **QHPV Loaded Cycle**.

5. After completion of a system empty cycle, the QHPV, QEXT, and QMTDs are considered in **aseptic hold**.

6. Confirm on the control screen that a **green halo** surrounds the images of the QHPV, QEXT, and QMTDs, indicating that all remain in aseptic hold, in a **sealed, HPV-decontaminated** condition.

7. The **aseptic hold period** is the validated time that aseptic conditions are maintained in the QEXT displayed at the top of the control screen in **days and hours**. The **time elapsed** since completion of the system empty cycle (e.g., maximum 120 hours) ends whenever there is a system breach, a door opened or a pressure or airflow alarm. If aseptic hold is **lost** (e.g., power surge or failure), an **amber halo** will surround the affected chamber(s). If aseptic hold is lost, begin again with item 4, above.

8. The smaller M1 Model Qube with a single QHPV chamber can be used sequentially for either germfree or gnotobiotic mouse husbandry.

9. The QHPV has an integrated HPV decontamination system, and is loaded via the front access window. On the control panel, activate the “open front access window” button, gently push in on the **rim of the sleeve port** until a “click” is heard, the window seal will deflate, the window will release, then gently pull on the rim of the sleeve port to open.

10. Load the QHPV with items to be decontaminated (e.g., unoccupied autoclave sterilized IsoIVC-P with feed, bedding, empty bottle and sipper, and environmental enrichments, autoclave sterilized RO drinking water reservoir bottles, and occupied IsoIVC-P) , ensuring good gassing presentation, and that **occluded surface areas** of the load are **minimized**.

11. Prior to closing the front access window, ensure sleeves are folded in the correct position, and that **gloves** are suspended by the magnet in the front window with glove fingers **fully extended**.

12. Complete a QHPV Loaded Cycle.

13. Work carefully, systematically, comparably to guidance provided in ***SOP 400 Rodent Husbandry***, using reservoir bottles to fill drinking water bottles, transferring mice to a clean environment, and reporting any concerns.

14. Daily observations are recorded on the ***Room Status Sheet*** within the Room Log Book, with health surveillance occurring twice per day on weekdays and daily on weekends.

15. Observations and communications should be noted on the ***Room Status Sheet*** **CMDC #41** and the ***Animal Health and Environmental Concern Form*** **CMDC #77**.

16. Refer to ***SOP #006***, entitled ***Animal Health and Environmental Surveillance***, for specific procedures of reporting and recording health concerns.

17. IsoIVC-P is changed as a unit. IsoIVC-P that is excessively soiled may be changed at the discretion of the animal care technician. IsoIVC-P housing gnotobiotic mice are routinely changed every two weeks. IsoIVC-P housing germfree mice are routinely changed every two weeks, but can be extended to three or four weeks at the discretion of the veterinarian, due to the absence of microbiota-effects on primary enclosure environmental quality.

18. After transfer of mice to clean IsoIVC-P, **soiled IsoIVC-P are not** to be **disassembled** within the Qube, but left assembled and clamped, removed from the room fully intact, and taken to cage wash for breakdown and sanitation.

19. After completion of husbandry tasks, all IsoIVC-P must be **clamped shut** prior to opening the Qube's front access window and returning occupied IsoIVC-P to the bioexclusion housing rack.

20. Bioexclusion rack air handling unit (AHU) **prefilters** must be changed out every 2 weeks or as needed, carefully bagged and removed from the room, and this recorded on the ***Room Status Sheet***.

21. Bioexclusion racks must be changed out every 6 months, and this recorded on the ***Room Status Sheet***.

22. Complete and record other room duties, which are comparable to those specified in ***SOP 400 Rodent Husbandry***.

NOTE: If at any time there is a medical emergency (e.g., moribund, dystocia, bleeding, irretractable seizures, lethargy, dehydration, etc.), the clinical veterinarian is to be notified immediately.

III.H. Taconic Shipper Receipt Using the Qube System

1. Whenever possible, **work in at least pairs**. Three staff are helpful for receipt of GF mice delivered inside a Taconic shipper.

2. The QHPV has an integrated HPV decontamination system, and is loaded via the front access window. On the control panel, activate the “open front access window” button, gently push in on the **rim of the sleeve port** until a “click” is heard, the window seal will deflate, the window will release, then gently pull on the rim of the sleeve port to open.

3. Load the QHPV with items to be decontaminated, ensuring good gassing presentation, and that **occluded surface areas** of the load are **minimized**.

4. Prior to closing the front access window, ensure sleeves are folded in the correct position, and that **gloves** are suspended by the magnet in the front window with glove fingers **fully extended**.

5. **Receiving** and unpacking **germ-free mice** delivered in a **single Taconic shipper requires 3 HPV cycles** to safely unpack, test, and (presumably) confirm the germ-free status of each shipper.

6. **Work flow** while unpacking the Taconic shipper proceeds from **left to right** from the **QHPV** into the **QEXT** into the right **QMTD**.

7. Confirm that **2-3 individuals** are scheduled for receipt, one working in the QHPV, one working in the QEXT, and preferably one working in the GF isolation room, supporting the others, including **recordkeeping**.

8. Confirm that **appropriate glove sizes** are in place in each of the chambers for the individuals scheduled to work in the QHPV and QEXT.

9. Staff assigned to **support work** in the GF isolation room wears **sleeves and gloves** saturate-sprayed with Oxivir-Tb, dried, then wiped dry with autoclave sterilized towels, and then with Steris 6% hydrogen peroxide wipes.

10. Staff working inside either of the **chambers** rely on the **sleeve and glove integral** to the QHPV or QEXT front access window.

11. **Before** the scheduled receipt of a Taconic shipper of germ-free mice, run (**1st cycle**) a **System Empty Cycle**.

12. Also, **before receipt**, confirm that all autoclaved equipment and supplies (e.g., Iso-IVC, reservoir bottles, Nunc specimen **tubes in an Iso-IVC**) required for receipt are on hand in GF isolation.

13. After completion of a system empty cycle, the QHPV, QEXT, and QMTDs are considered in **aseptic hold**.

14. Confirm on the control screen that a **green halo** surrounds the images of the QHPV, QEXT, and QMTDs, indicating that all remain in aseptic hold, in a **sealed, HPV-decontaminated** condition.

15. The **aseptic hold period** is the validated time that aseptic conditions are maintained in the QEXT displayed at the top of the control screen in **days and**

hours. The **time elapsed** since completion of the system empty cycle (e.g., maximum 120 hours) ends whenever there is a system breach, a door opened or a pressure or airflow alarm. If aseptic hold is **lost** (e.g., power surge or failure), an **amber halo** will surround the affected chamber(s). If aseptic hold is lost, begin again with item 11, above.

16. **Before** the scheduled receipt of a Taconic shipper of germ-free mice, **load the QHPV** with all autoclaved supplies and other supplies not autoclaved but needed for unpacking and Iso-IVC housing GF mice. Ensure minimal occluded surface areas for effective HPV sterilization.

17. Run (**2nd cycle**) a **QHPV Loaded Cycle**.

18. The **QHPV Loaded Cycle** HPV-sterilizes all supplies needed for unpacking, including dated, autoclaved, labeled and uncapped Nunc specimen tubes (1 for each shipper to confirm germ-free status), water reservoir bottles (1 for each Iso-IVC), fully assembled Iso-IVC, a Nunc tube rack, and a pair of forceps.

19. After completion of the 2nd cycle, while working in the QHPV, reservoir bottles are opened one at a time and used to fill Iso-IVC water bottles.

20. The HPV decontaminated Iso-IVC, filled water bottles, Nunc specimen tubes, rack, and forceps are then moved from the QHPV, through the middle inner door, and **staged inside the QEXT**.

21. The **empty** reservoir bottles are **not** transferred from the QHPV into the QEXT, and will exit after the QHPV is otherwise emptied and the middle inner door is closed preserving the **QEXT aseptic hold**.

22. Filled water bottles and Iso-IVC tops are stacked in the QEXT **on the wire shelf**, out of the way, and the **QEXT right side** wire grid floor is used for staging the Iso-IVC with wires. Iso-IVC can also be stacked. Loosely cap and then position the Nunc tubes in the rack, and hang the forceps from the wire shelf.

23. The **QEXT left side** is unoccupied and **uncluttered** permitting the eventual entry of the shipped static cages containing GF mice, as these are unpacked and removed from the Taconic shipper after completion of the 3rd cycle, below, which is another QHPV loaded cycle.

24. On **receipt of the Taconic shipper**, using two-person GF isolation entry procedures above, while in circulation outside of GF isolation, remove the shipper from its vented Rubbermaid crate, decontaminate the shipper exterior with Oxivir-Tb saturate spraying, **avoiding the HEPA filtered vents**, then place the shipper inside GF isolation on an internal cart.

25. **Inside GF isolation**, dry the shipper with sterile towels, then decontaminate the shipper exterior using Steris 6% hydrogen peroxide wipes, being careful to avoid the HEPA filtered vents, and to not disrupt any seals, and then allow it to dry.

26. Using **flexible port caps**, securely **seal** the HEPA filtered vents of the Taconic shipper, then place it, a pair of **scissors,** and **forceps** into the QHPV.

27. The **shipper** should be **positioned** to the **QHPV left side**, so that after cycle completion static boxes can be passed through the middle inner door without unnecessarily contacting the shipper.

28. Run (**3rd cycle**) a **QHPV loaded cycle**, to HPV-sterilize the sealed shipper, a pair of scissors and forceps.

29. After completion of the 3rd cycle (i.e., shipper-loaded QHPV cycle), work as a **group of 3**, with 1 individual in the **QHPV**, 1 individual in the **QEXT**, and 1 individual in the **GF isolation room**, also recordkeeping.

30. The **individual** in the **QEXT**, **unclamps** all 3 Iso-IVC, **fills** all 3 Iso-IVC water bottles using water from the reservoir bottles, **positions** unclamped Iso-IVC with filled bottles, ready to receive GF mice, and positions the Nunc tube(s) in the rack for collecting specimens of at least two fresh fecal pellets, presumably to confirm GF status of the shipper.

31. Iso-IVC tops can be **stacked** out of the way in the QEXT on the wire shelf or base grid, and **wire tops** can be used to **temporarily "hold"** mice in Iso-IVC, as gradually trios are created during unpacking.

32. The **individual in the QHPV** opens the Taconic shipper with the scissors, making a broad **"U"-shaped incision** through the clear plastic top, avoiding the sealed HEPA filters, then **folds open** the **created plastic flap**, making accessible the static cages containing germ-free mice.

33. **Even after completion of the 3rd HPV cycle**, the loaded QHPV cycle containing the shipper, the **shipper exterior** is considered **contaminated** at all contact points, deep in folds, under the flexible port caps and its flaps.

34. The individual in the QHPV then opens the "middle" side door, and passes a static cage of mice into the QEXT, attempting to **avoid shipper contact** by static boxes or gloved hands, since portions of the shipper may remain contaminated, including contact points of the shipper's bottom, folds, and overlapping flaps under the flexible port caps.

35. Static cages and mice are considered germ-free. If the static cage holds male mice, one will be entered into each Iso-IVC, if female mice, two will be entered into each Iso-IVC.

36. A single Nunc tube containing **specimens** of two fresh fecal pellets, preferably from different mice shipped in different static cages, is collected to confirm germ-free status of the shipper.

37. In sequence, the QHPV individual exits the 3 static boxes of mice, one by one, into the QEXT for the creation of breeding trios in each of the Iso-IVC. To make space in the QEXT, **emptied** static **boxes**, emptied water **reservoir bottles**, and the

Nunc rack and **sealed tube** containing specimens can be **exited** via the **right QMTD** to the third individual working in GF isolation. Note, that there will be a **2-minute clean-up time** before the internal door of the QMTD can be opened again.

37. Once each Iso-IVC contains a breeding trio and a filled water bottle, the Iso-IVC are sealed, clamped shut, with the **white safety button** pushed downwards, **locked,** and exited through the right QMTD.

38. After **all Iso-IVC** are completed and locked, the QEXT front window can be opened and all materials exited. If a second Taconic shipper is received the same day, begin again with item 8, above.

39. After, remove all items, clean the interior of the Qube chambers, wiping clean with sterile Steris 6% hydrogen peroxide wipes, which do not contain alcohol (do not use Oxivir-Tb inside the Qube chambers).

III.I. Inhalational Anesthesia Procedures in the Qube System

23. Whenever possible, **work in pairs**.

24. IsoIVC-P used to house mice of a **presumed similar microflora status** (e.g., germ-free) may be entered **together** into the Qube system for assessments, procedures, or change-outs.

25. Procedures requiring **isoflurane** anesthesia is conducted in the **QHPV** chamber with the inhalational anesthesia tubing installed through the tri-clover port, and in accordance with SOP 033 *General Anesthetic Techniques* and SOP 1102 *SurgiVet® Anesthesia Machine.*

26. Autoclave the anesthesia **supply tubing** beyond the two breathing system filters, the nosecone, and the induction box using a GF Iso-IVC sterilization cycle.

27. Open the QHPV front access window. Disconnect the main supply tubing just beyond the stopcock. Thread the main supply tubing through the tri-clover port, then reconnect the tubing to the stopcock.

28. Ensure the **silicone gasket** is appropriately seated before locking the tri-clover clamp. Then lock the tri-clover so that its **hinge** is at **eight o'clock** and its **clamp** is at **two o'clock** ensuring the wire mesh floor grid will fit around it.

29. Install **two** (2) new Pall MGF50 breathing system **filters** in the anesthesia line, one just prior to the induction box, and the other just prior to the face mask. At the completion of procedures, discard these two filters. The Pall MGF50 is an efficient inhalational anesthesia filter that prevents the introduction of microorganisms or airborne contaminates (e.g., excludes from the medical gas line Human Influenza A H1N5, HIV, Hepatitis C, Mycobacterium tuberculosis, and pathogenic prion proteins).

30. The QHPV has an integrated HPV decontamination system, and is loaded via the front access window. On the control panel, activate the “open front access window” button, gently push in on the **rim of the sleeve port**, the window seal will deflate, the window will release, then gently pull of the rim of the sleeve port.

31. Load the QHPV with the items to be gassed, ensuring good gassing presentation, and that occluded surface areas of the load are **minimized**.

32. Prior to closing the front access window, ensure sleeves are folded and in the correct position, and **gloves** are suspended by the magnet in the front window and **fully extended**.

33. **Receiving** and unpacking germ-free mice delivered in a single **Taconic shipper** requires **3 HPV cycles** to safely unpack, test, and presumably confirm the germ-free status of each shipper.

34. **Work flow** while unpacking the Taconic shipper proceeds from **right to left**. Confirm that **3 individuals** are scheduled for receipt, one working in the QHPV, one working in the QEXT, and one working the GF isolation room.

35. Confirm that **appropriate glove sizes** are in place in each of the chambers for the scheduled individuals working the QHPV and QEXT.

36. The **day before** the scheduled Taconic shipper receipt, run (1) a **system empty cycle**, followed (also the day before) by (2) a **loaded QHPV cycle** (i.e., that HPV-sterilizes all needed autoclaved supplies and equipment, including Nunc specimen tubes, a tube rack, forceps, 3 water reservoir bottles, and 3 assembled Iso-IVC).

37. The autoclaved and HPV decontaminated supplies, equipment, reservoir bottles, and Iso-IVC are then moved into and **staged inside the QEXT** for use the next day. Two Iso-IVC and 2 reservoir bottles should be placed **on the wire shelf**, up, out of the way, so that a single Iso-IVC and single reservoir bottle are ready for use on the right side of the woven wire base rack, and the left side of the QEXT can permit entry of the static cages of mice from the Taconic shipper the next day.

38. The QHPV and QEXT are left **overnight in aseptic hold**.

39. The next morning, confirm that on the QHPV control screen a **green halo** surrounds both images of the QHPV and the QEXT, indicating that both remain in aseptic hold, in a **sealed, HPV-decontaminated** condition.

40. If aseptic hold was **lost overnight** (e.g., power surge or failure with delayed generator start-up), an **amber halo** will surround the affected chamber(s).

41. The **day of receipt** of the Taconic shipper, after two-person entry into GF isolation and saturate spraying the shipper with Oxivir-Tb, **dry** the shipper, then **seal** both of the shipper HEPA filters using the flexible port caps.

42. Load the QHPV and run the 3rd HPV cycle, a **loaded QHPV cycle**, to HPV-sterilize the sealed shipper, and a pair of scissors.

43. Simultaneously, while running the loaded QHPV cycle, **working in pairs**, one in the QHPV and one in the QEXT, the individual in the QEXT, **unclamps** all 3 Iso-IVC, **fills** all 3 Iso-IVC water bottles using the 3 red-capped reservoir bottles, **positions** all 3 unclamped Iso-IVC now ready to receive mice, and positions the Nunc tube in its rack for collecting a single specimen of two fresh fecal pellets to confirm germ-free status of the shipper. Tops can be **stacked** out of the way in the QEXT on the shelf or base wire grid, and Iso-IVC wire tops used to contain mice as germ-free trios are created.

44. After completion of this "3rd cycle", the individual in the QHPV opens the Taconic shipper with the scissors, making a broad **"U"-shaped incision** through the clear plastic avoiding the covered HEPA filter on the top of the shipper, then folds open, the created plastic flap, making accessible the static cages containing germ-free mice.

45. Working in pairs, the individual in the QHPV opens the side door, and passes a cage of mice through to the individual in the QEXT. If male mice, one will need to be entered into each of the 3 Iso-IVC. If female mice, two mice will need to be entered into each Iso-IVC.

46. A single Nunc tube containing **specimens** of two fresh fecal pellets, preferably from different mice shipped in different static cages, is collected to confirm germ-free status of the shipper.

38. In sequence, the QHPV individual exits the 3 static boxes of mice, one by one, into the QEXT for the creation of breeding trios in each of the Iso-IVC. To make space in the QEXT, **emptied** static **boxes**, emptied water **reservoir bottles**, and the Nunc rack and **sealed tube** containing specimens can be **exited** via the **right QMTD** to the third individual working in GF isolation. Note, that there will be a **2-minute clean-up time** before the internal door of the QMTD can be opened again.

47. Once each Iso-IVC contains a breeding trio and a filled water bottle, the Iso-IVC are sealed, clamped shut, with the **white safety button** pushed downwards, **locked,** and exited through the right QMTD.

48. After **all Iso-IVC** are completed and locked, the QEXT front window can be opened and all materials exited. If a second Taconic shipper is received the same day, begin again with item 8, above.

49. After remove all items, clean the interior of the Qube chambers, wiping clean with sterile Steris 6% hydrogen peroxide wipes, which do not contain alcohol (do not use Oxivir-Tb inside the Qube chambers).

IV. Preparation of Fecal Slurries

1. Only mice that are SPF/VAF for infectious agents on the exclusion list are used as donors for this procedure.

2. Fecal pellets are collected fresh in sterile Eppendorf tubes, prior to entering bedding, and stored immediately at -20°C. Approximately 10 pellets should be collected for each slurry preparation.
3. All work for fecal slurry preparations should be done in a biosafety hood, with all sterile tools and sanitized surfaces.
4. Work surfaces are decontaminated with Oxivir-Tb before and after use and in between different flora samples.
5. Each unique fecal slurry requires a 100 um cell strainer, two 50 ml conical tubes, sterile pipette tips, pipette, 5-10 ml syringe or handheld electric pipette with 5 or 10 ml serological pipettes, sterile cryotubes, label maker, sterile PBS, and Mr. Frosty container for long term storage in -80°C.
6. Pellets are hand homogenized with a sterile syringe plunger, inside the collection tube, with 200-300 ul of sterile PBS as diluent.
7. After pellets have been mixed into a paste, the paste is then transferred into a 50ml conical tube and further mixed with 5-7 ml of sterile PBS.
8. After a uniform consistency, with no clumps evident, the mixture is then transferred through a 100 um cell strainer, attached to the top of a new 50ml conical tube.
9. The mixture is slowly strained, with the help of a syringe plunger. The final volume should be at least 10 pellets into 10 ml of sterile PBS.
10. Aliquots of the resultant supernatant are then pipetted off into sterile cryotubes (1 ml each) and then labeled with PI, date, volume, and “fecal slurry”.
11. The cryotubes are placed in -20°C for short term use, or into a Mr. Frosty container which is then placed into a -80°C for long tern storage.
12. Samples of each slurry made should be submitted to both IDEXX and Transnetyx for microbiome analyses. Each unique slurry sample should be placed into an orange-top tube provided by Transnetyx, or an Eppendorf tube for IDEXX submission. For submitting fecal slurry to Transnetyx, place 200 ul of the fecal slurry into the buffer. If submitting pellets, provide 2 pellets for each tube. Note: Sterile pipette tips should be used when pipetting samples into Transnetyx or IDEXX collection tubes.
13. When a tube of fecal slurry is thawed for use, it should not be re-frozen.
14. Fecal slurries should be thawed just prior to use and mixed well before and between gavage administered doses.

Approved: **Date:**