

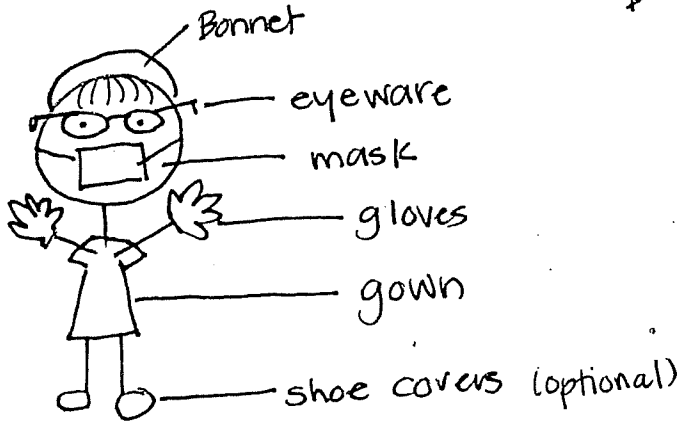
GNOTOBIOTIC TRAINING ... a Guide ☺

SEMI RIGID ISOLATORS

Created by Jessica K Lang
& Developed

• ENTERING THE ROOM

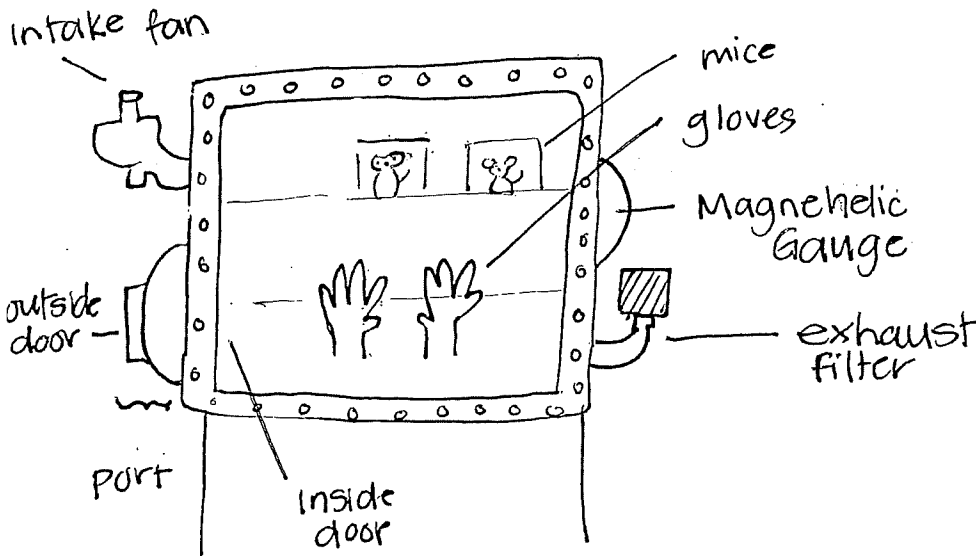
- ↗ Entering not near isolators = no PPE
- ↘ WORKING WITH ISOLATORS = PPE
- ↳ PORT OPENING = FULL PPE



* ALWAYS GLOVES LAST!

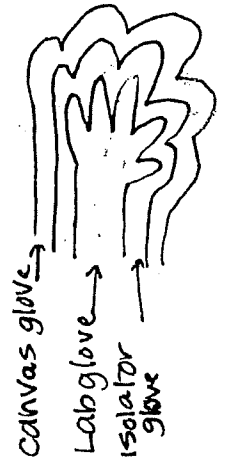
- touch as little as possible!

ISOLATOR ANATOMY



INSIDE THE ISOLATOR

ALWAYS WEAR 3 LAYERS OF GLOVES.



Dr. Garrett's Semi-Rigid Isolator Protocols

Created & Developed by Jessica K. Lang latest revision 12.13.18

Contributions by Kathryn G. Rosinski

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Intake Fan

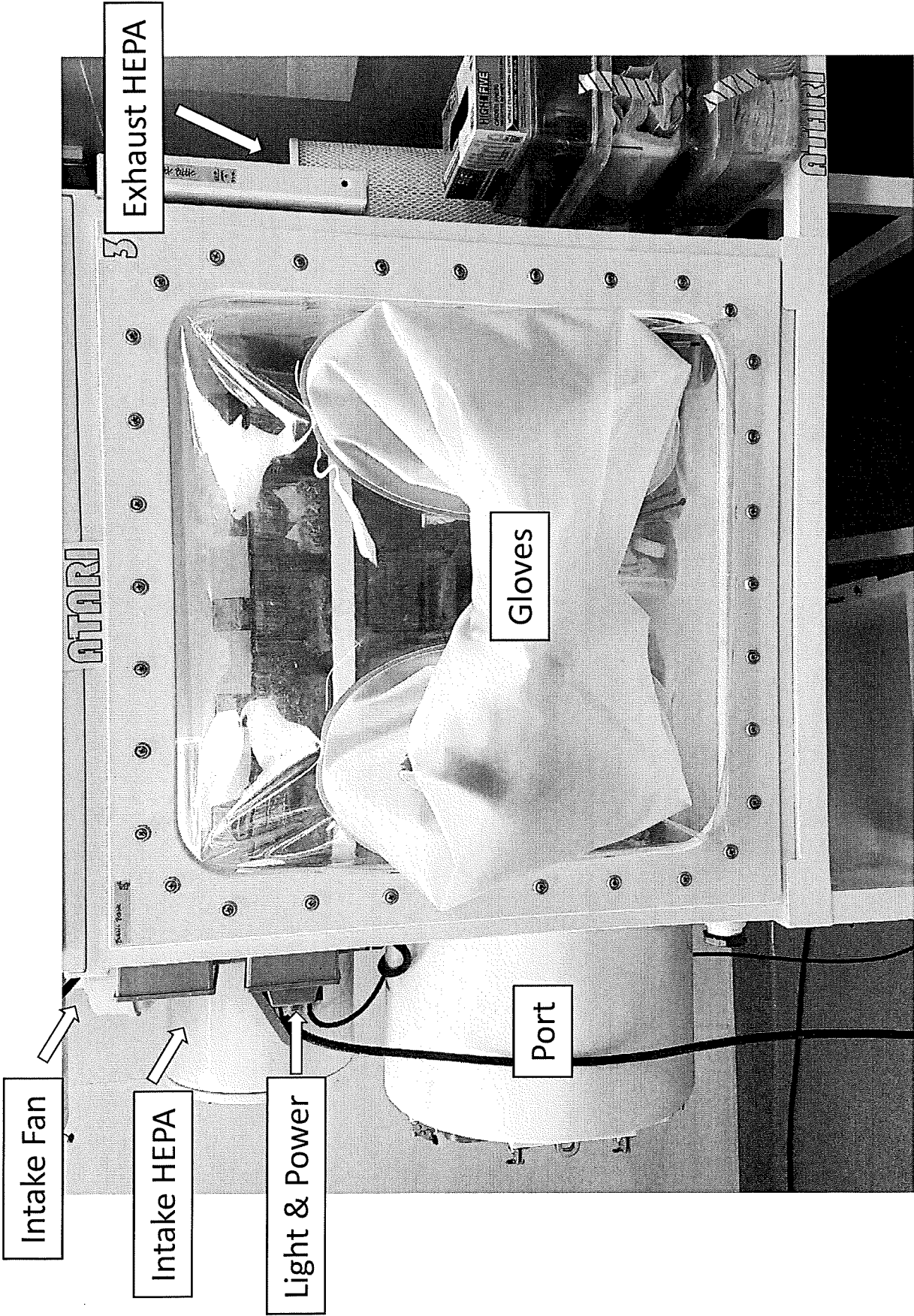
Intake HEPA

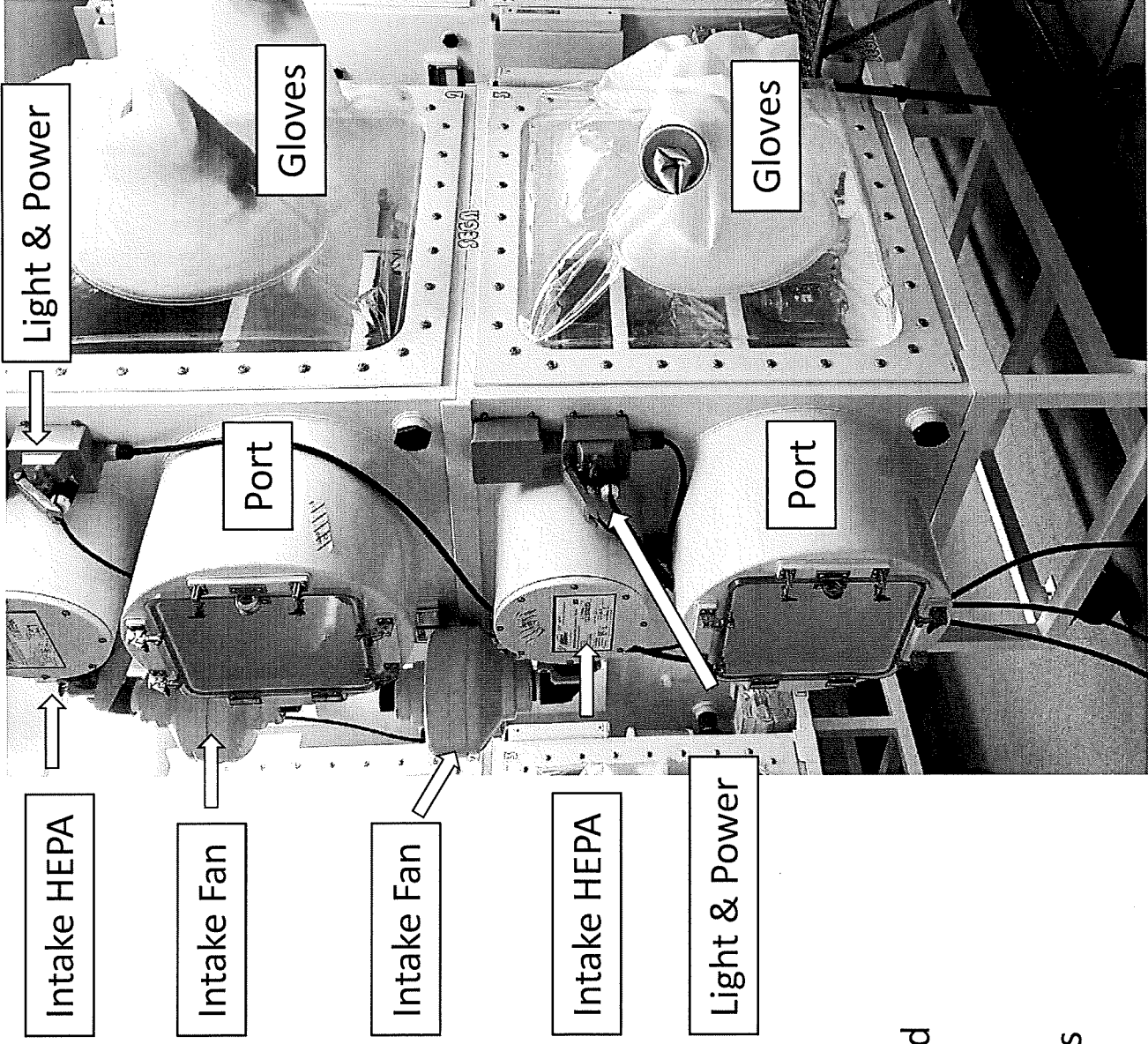
Light & Power

Port

Gloves

Exhaust HEPA





Isolators are stacked in pairs and move as a quad.

However, each Isolator operates independently as its own unit!

Preparing Sterilant:

Mixing Alcide Exspor

Refer to MSDS for more info

1:4:1 Dilution – PLEASE MIX IN THIS ORDER ONLY!

- 1 part base
- 4 parts water
- 1 part activator

Examples:

Base	Water	Activator	Purpose
25 mL	100 mL	25 mL	closing up port only (atomizer gun)
50 mL	200 mL	50 mL	spraying in/dunking small items (tubes, forceps)
100 mL	400 mL	100 mL	closing up several ports (atomizer gun)
200 mL	800 mL	200 mL	dunking in packs, entering water

****Note:** Allow 15 minutes to settle before using. Has a 12 hour time for use.

Preparing/Bagging Feed & Bedding:

- Tape longer sides & bottom of a medium autoclave pouch (7.5 x 3 inch) using autoclave tape.
- Fill bags with feed or bedding*
- For food, fill a bit more than $\frac{1}{2}$ full (as feed expands during the autoclave cycle).
- Remove excess air by pushing down.
- Close pouch using the sticky seal.
- Using autoclave tape, tape over seal again.
- Tape longer sides of a large (12 x 18 inch) autoclave pouch.
- Stuff the medium bag into the large bag face down (so there will be no visible window).
- Repeat. (3 medium bags can fit in a large one).
- Remove excess air by pushing down and close pouch using the sticky seal.
- Using autoclave tape, tape over seal again.
- Date, label and initial bag.
- Place in an autoclave tray. 4 large bags will fit in a tray.
- Wrap in blue drape. Secure with tape.
- Date label tray.
- Once autoclaved use gloves and handle minimally.



Preparing Water:

- Rinse old water jugs with warm water.
- Fill with tap water $\frac{3}{4}$ of the way full.
- Cover with green cap ensuring it is covered fully.
- Place piece of autoclave tape with date across cap.
- Autoclave on appropriate cycle.
- Allow jugs to cool before removing from autoclave.
- Check for indented green cap- if cap is not indented, reautoclave.



Verifying the Autoclave:

Indicator Strips: are placed in feed and bedding bags and can be brought into the isolator.

Spore Tubes: cannot be entered into the isolator as they need to incubate 24 hours prior to entry.

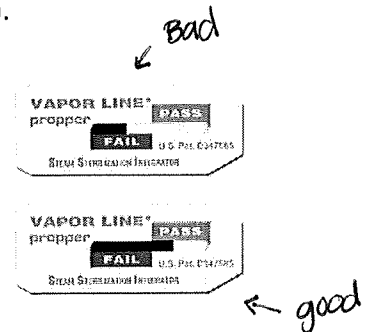
- Each cycle should have at least one indicator strip inside a food/bedding bag.
 - Located in the middle of the autoclave load (ie inside a prepared bag).
 - This bag will be packed with the window visible.

- Each autoclave cycle should have a biological indicator spore tube.

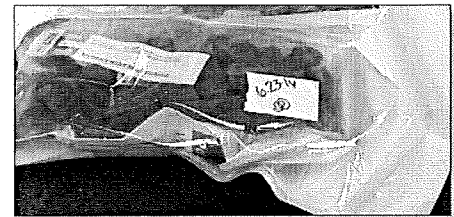
- Wear clean gloves when handing any post autoclaved supplies.

- The test bag will be opened after autoclaving.
(prior to using any supplies from the sterilized load)

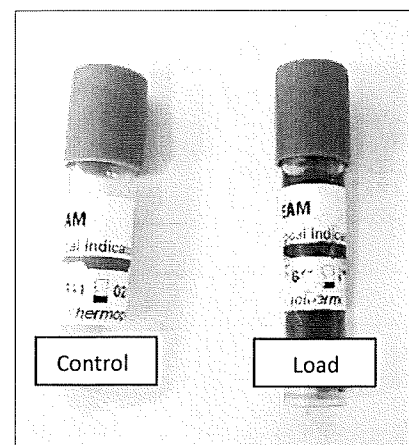
- Tear open test bag and remove the indicator.
- Activate the vial by crushing the inner tube using the vial crusher (on the incubator).
- Incubate tube for 24 hours.



- If any spores survive, the vial medium will turn yellow.
- IF the load fails the test (yellow) discard all supplies from the **whole load** and re-autoclave.
 - if the load contains any feed, discard and re package fresh food.
- IF the load passes the test (purple), supplies are able to enter into isolator.



- Always include a control test.
 - a test that has not been into the autoclave.
- Make sure to crush the control tube as well.



New Isolator Supply List:

Fill new isolator as full as possible to minimize entry of supplies in the future.

- 15 cages
- 15 wire tops



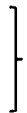
These supplies are autoclaved in blue drape material.

- 15 water bottles
- 15 sipper tops
- Canvas gloves
- Feed
- Forceps
- Scraper
- Bedding
- Ear Punch
- Eppendorf tubes
- Scissors
- Nalgenes (2)
- Paper Towels & Nestlets
- Pencil & note cards
- Garbage bags



These supplies are autoclaved in double autoclavable pouches then inside a mouse/rat cage.

- Food
- Bedding



In large trays wrapped in blue drape.

- Water jugs



Autoclaved: Dunked/sprayed in.

Recycling an Isolator:

Time needed: 3 hours plus at least 24 hours wait time *does not including prep of supplies*

Supplies needed: New gloves/rings, new filters, new isolator supplies.

- Wear PPE (gloves, gown, mask and face shield) at all times during recycling.
- All supplies should be pre autoclaved (if possible) and the load verified via biological indicator prior to entry.

- Turn on isolator blower (this will remain on during the whole recycling process).
- Prepare your sterilant (make extra large batch).

During sterilant wait time- 15 minutes:

- Remove front panel of box (where the gloves are attached) using a screw driver/electric drill.
- Clean inside panel with warm water and soap. Rinse with water and wipe dry.
- Replace gloves and re-tape.
- Set aside.

- Clean dust and debris from inside (wipe down with 70% ethanol) and dry.
- Saturate all isolator surfaces with sterilant (spray or with a cloth). It should be soaking wet.
- Spray inside holes and exhaust filter piping.

- Attach a new filter (screw in).
- Re-thread gasket bulkheads and dunk and screw into holes.
 - The exhaust filter gasket bulkhead will remain inside isolator (do not screw on).

- Saturate autoclaved supplies with sterilant (dunk/spray thoroughly) try to handle quickly- open cage/package and coat supplies with sterilant asap, and place supplies inside isolator.
- Saturate front panel of isolator.
 - Including the gloves (turn inside out) and the seal around the screws.
- Place a dunked spray bottle filled with sterilant inside isolator.
- Screw in screws.

- Spray inside of the isolator with the interior spray bottle.
 - Thoroughly covering all surfaces with sterilant.
- Close inside port & outside port and allow to sit overnight.

Entering & Exiting supplies (Opening/Closing Port):

Supplies Needed: Cart and spray bottle/air compressor, sterilant.

Entering Supplies:

- Prepare sterilant- wait 15 mins.
- Open outside port door.
 - Make sure that inside door is CLOSED before opening outside!
 - Open the hinges all the way (if not, they can scratch the door).
- Wipe out any leftover sterilant.
- Coat inside of isolator with wet cloth with a swirling motion. Ensure to cover all surfaces including the port door and gasket.
- Check supplies any possible perforations (rips/holes etc.).
 - For water jugs: make sure green cap is sunken in to verify integrity of cap seal.
- Thoroughly cover ALL surfaces of the supplies to be entered.
 - dunk, spray, use cloth etc.
 - ** Caution: supplies will be slippery!
- Place supplies inside port.
- Spray using spray bottle/air compressor ensuring total sterilant coverage on all surfaces of port, including the seal.
- Wait a minimum of 40 minutes before opening inside door.

Exiting Supplies:

- Ensure outside port is closed!
- Open hinges and place items to exit inside the port and close inside door.
- Items can be left inside the port until it needs to be opened to enter supplies.

OR

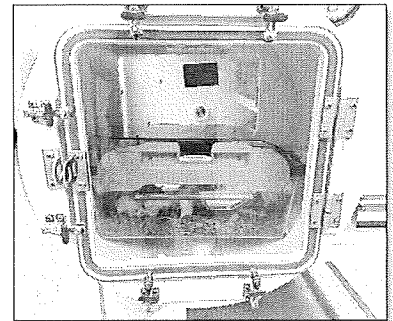
- Remove items asap!
- Then, close up port by spraying closed using sterilant, adding in any appropriate supplies needed for entry (food, bedding, water etc.).
 - this MUST be done! If it is not sterilized closed, the next user could open the inside port and accidently and contaminate the isolator.

IF IT CANNOT BE CLOSED ASAP- use the **NOT STERILE** sign!

Transfer of Animals Between Isolators:

Supplies Needed: A pre autoclaved container (Nalgene/IVC cage/empty water bottle) in the donor isolator, sterilant.

- Pre spray receiving isolator with sterilant using spray bottle/atomizer.
- Allow a minimum of 40 minutes for sterilant to set.
- Place donor mice in a pre-autoclaved container (already inside the donor isolator).
- Remove from isolator.
- Place non sterile sign on isolator.
- Dip and spray outside of the container.
 - Ensure that you have covered all surfaces with sterilant.
- Place container in recipient port.
- Spray port closed.
- Wait no more than 10 minutes before you enter mice.
 - MONITOR THE MICE!
- Open inside door, remove container.
- Close door & house mice appropriately.



Transfer of Animals from Isolator to IVC:

Supplies Needed: IVC cage in the donor isolator, sterilant.

- Pre spray the recipient port.
- Place mice to be transferred in an IVC cage (already inside the donor isolator).
- Remove from isolator.
- If mice are being used for a later time, dock IVC cage appropriately on rack.
- Close both ports asap.

OR

- Pass mice inside the IBS in a sterile fashion via dunk tank.
- Perform experiment.

Transfer of Animals From a Taconic Shipper:

Time: 40 minutes

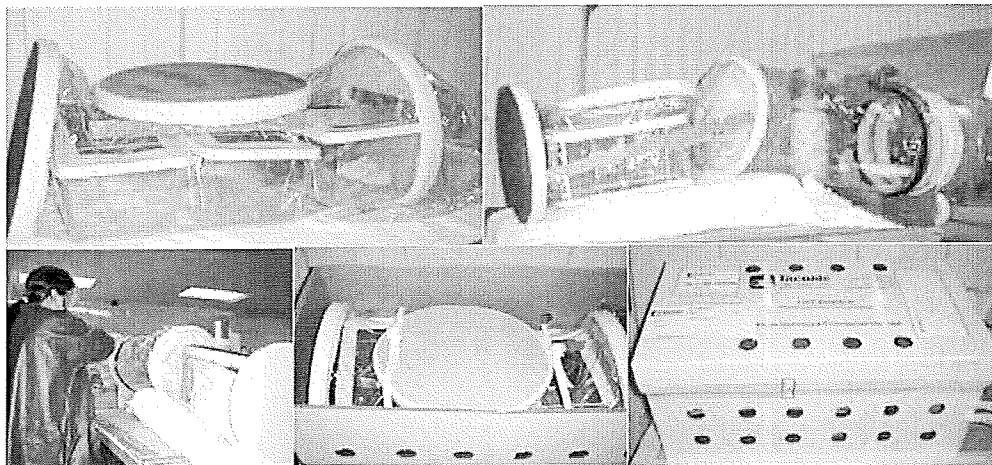
Supplies Needed: Adaptor port connector, sterilant.

- Remove door from the isolator port (it slides upwards off hinges).
- Cover all surfaces of port connector with sterilant.
- Attach the cylinder adaptor by using sterilant as a lubricant.
- Secure by taping with vinyl tape.

- Take vinyl shipper from the cardboard box (use care to keep cages and oval filter upright).
- Open end of connecting sleeve.
- Unfold sleeve and remove yellow vinyl tape that was folded and sealed prior to shipment.

- Swab inside of sleeve and the face of the Mylar diaphragm with a sterilant.
 - Using caution not to apply too much pressure to Mylar diaphragm.

- Pull sleeve partway onto the port adaptor of the destination Isolator.
- Spray sleeve portion of shipper with sterilant by spraying inside the nipple.
- Spray until the sleeve is inflated and close with nipple.
- Secure with tape, to make a complete tape seal of the shipper to the isolator port.
- Wait a minimum of 40 minutes.



Taconic Germfree Shipper

- Remove the yellow tape from the circumference of the Mylar diaphragm.
- Carefully, work the Mylar diaphragm to a horizontal position (using external manipulation).
- Pull the diaphragm through the port and into the Isolator.
- Rotate the cages and slide toward the Isolator port (using external pressure).
- Pull in cages and remove the bands that are securing the cages lids.
- Remove the animals and place in isolator caging.
- Collect stool from mice & place tubes in port.
- Discard all refuse (including the cages they came in) and place inside the port.
- Close inside door.
- Remove items from port.
- Replace door.
- Close port as per standard protocol.

Standard Isolator Husbandry SOP:

- Wear PPE (gloves/gown) & spray gloves with Quatricide® prior to entry.
- Change gloves between isolators.

Cleaning cages:

- Clean a minimum of once/week & spot change as needed.
 - condensation, or flooded cages then immediately change the cage.
- Always put cages back in the same place in the isolator!
- Only open one cage of animals at a time.
- Lift wire bar with forceps or gloves at corner and grasp with hand and set aside.
- Pick mice up with forceps or gloves at base of tail or move to the side to clean cage
 - check sex, and health status of each mouse & cross verify census.
- Scrape cage and dirty content in a bag (leave a bit of old bedding behind in cage).
- Rinse cage with water and wipe clean with a paper towel.
- Add new bedding & enrichment materials.
- Repeat the cleaning procedure of all cages.
- Sweep up all loose bedding and feed using scraper and paper towels.
- Always make sure isolator is clean and organized before you exit. Do not leave a mess!



Changing/refilling water:

To open water jugs: make sure the water just is sitting level on floor. Never tilt new water jug when opening – it can EXPLODE! Gently break the seal of the green closure.

- Add new water to existing water bottle.
- Only change to a new bottle if there is a lot of debris.
- Make sure there is an air pocket (space) between the stopper and the top of the water.
- Replace stopper and tighten.
- Check each bottle to make sure water comes out of the stopper before placing it in the cage.

Changing/refilling feed:

- Break up feed using scraper or forceps.
- Always add new food to the hopper (do not discard old food).
- If needed, you can transfer feed between cages ***as long as it is the standard diet***

Isolator Maintenance:

Weekly

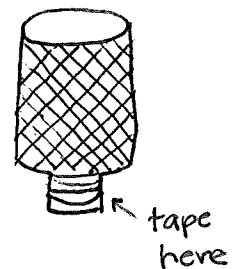
- Wash the walls, shelving and floor inside the isolator with paper towels and water.
- Inspect isolator panel for rips and tears.
 - Especially the gloves!
- Wash the outside of the isolator and dust the top.
- Make sure isolators are well stocked with food, bedding, towels etc.
 - Record what is needed in weekly work flow.
 - Food must be used within 3 months of entry into the isolator (or 6 months from mill date).
- Record weekly census of mice.
 - Replace breeder pairs at around 5-6 months of age.
 - Set up new breeders at 6-7 weeks of age (or earlier to save space).

Exhaust Filters:

- Can be replaced if you feel resistance from bubble.
- Check that nothing is blocking the inside pipe,

To Replace Exhaust Filters:

- Turn off blower.
- Close inside gasket bulkhead.
- Spray sterilant inside PVC pipe.
- Add new plumber tape treading on new exhaust filter.
- Coat treading on filter with sterilant.
- Screw in new exhaust filter.
- Turn on blower and set the magnehelic gauge at 2.0.



Quality Assurance:

- Collect fresh stool in an eppendorf tube from a random mouse every time the port is opened.
- Label with isolator name and date.
- Store at 4°C.
- Perform ~monthly qPCR on newest sample.

Collect water/environmental samples from each mouse;

- Add a small amount (1-2 drops) of sentinel water to a culturette.
- Dip the swab into the vial and dampen it with water.
- Swab several of the mice and the interior of their cages; place the swab into the vial.
- Add a fresh stool sample to culturette for gram stain (done monthly).

Perform 16S qPCR Protocol:

- Follow QIAGEN QIAamp® DNA Stool Mini Kit (Cat# 51604) for DNA extraction.
- Check DNA concentration on nanodrop (ng/uL on nanodrop).
- Perform 16S qPCR.
 - make sure to include a negative control (from SPF mice) and a positive control (water) for each test.

Gram Staining:

4-step Staining Test Procedure for Gram Negative Staining

1. Flood the fixed smear with primary stain for 1 minute – **Gram crystal violet.**
2. Remove the primary stain by gently washing with cold tap water.
3. Flood slide with mordant and retain on the slide for 1 minute – **Gram iodine or stabilized gram iodine.**
4. Remove the mordant by gently washing with cold tap water.
5. Decolorize until solvent running from the slide is colorless – **Gram delcolorize.**
6. Wash slide gently with cold tap water.
7. Flood slide with counter stain and stain for 30 to 60 seconds – **Gram safranin or gram basic fuchsin.**
8. Wash slide gently with cold tap water.
9. Blot with blotting paper or paper towel or allow to air dry.
10. Examine smear under immersion lens.



Description	Temperature (°C)	Pressure (psi)	Sterilize Time (mins)	Dry Time (mins)
Supplies	128.0 °C	26 PSI	30 mins	10 mins
Liquid	121.1° C	20 PSI	60 mins	-

Supplies include: IVC cages, isolator cages, food and bedding bins