

GNOTOBIOTIC TRAINING ... a Guide ☺

created & developed by
Jessica K. Lang,

Tecniplast ISOcage IVC System

PERSON #1 works inside IBS
STERILE!



PERSON #2 works outside IBS
not sterile



"SPRAYING IN"

After donning appropriate PPE, person #1 touches nothing, while person #2 sprays person #1

PERSON #1



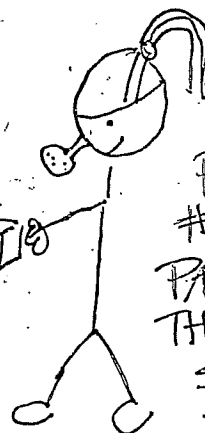
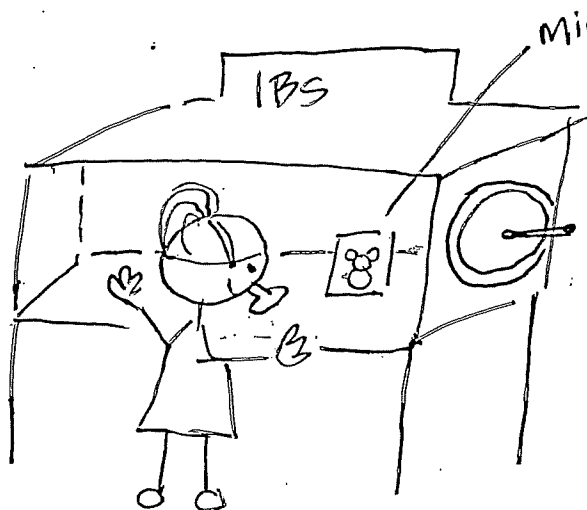
sterilant

PERSON #2

helps person #1



WORKING IN HOOD



PERSON #2
PASSES THROUGH supplies & mice

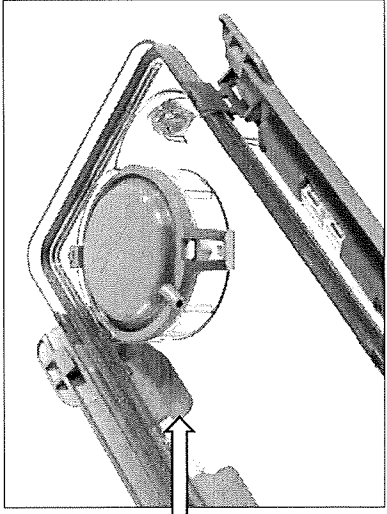
PERSON #1 stays inside IBS

Dr. Garrett's Tecniplast Protocols

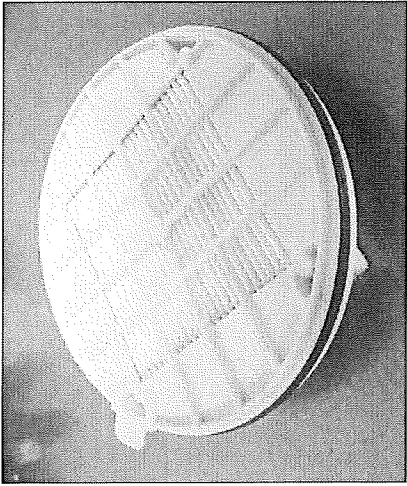
Created & Developed by Jessica K. Lang latest revision 12.13.18

Contributions by Kathryn G. Rosinski

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HEPA cover



HEPA filter

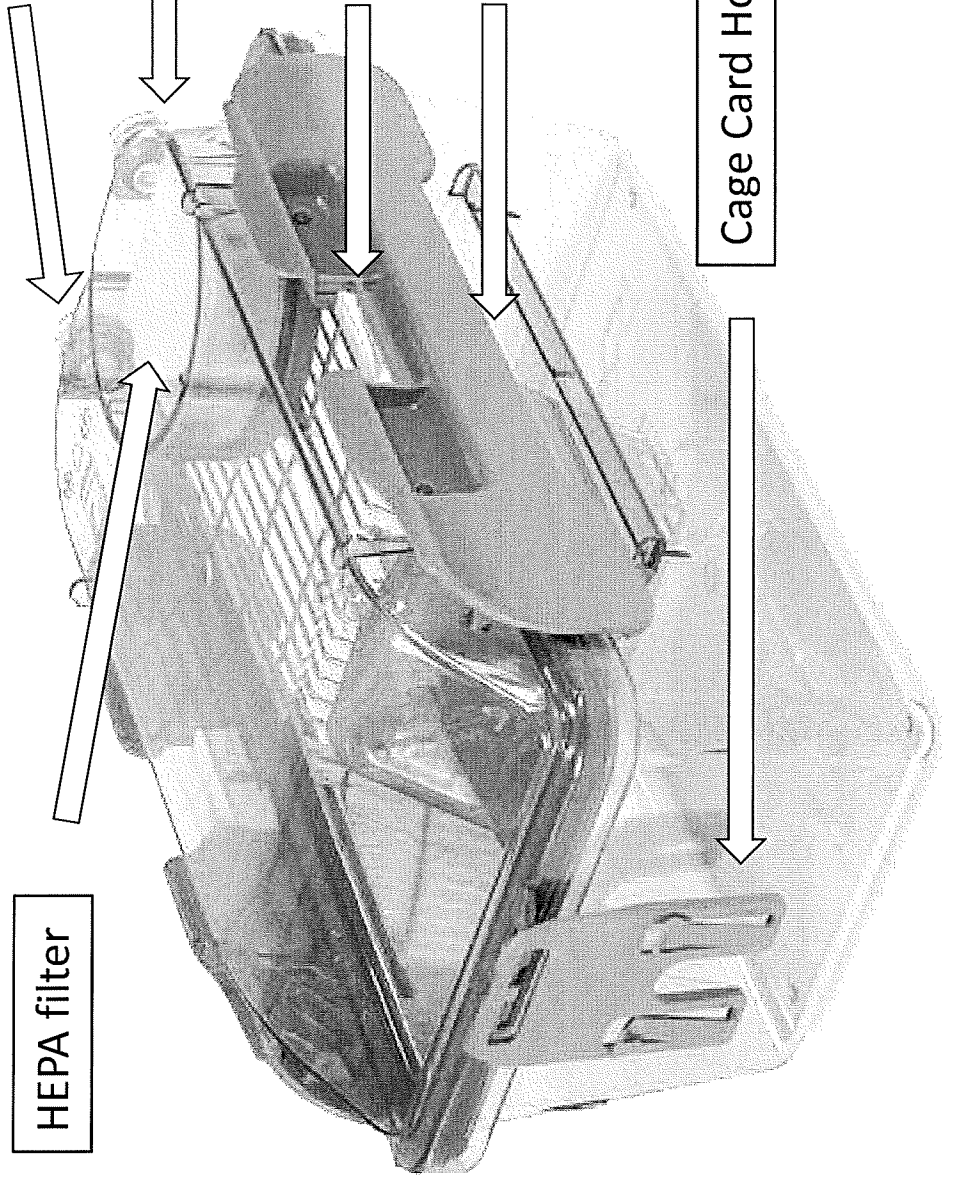
Exhaust Air Outlet

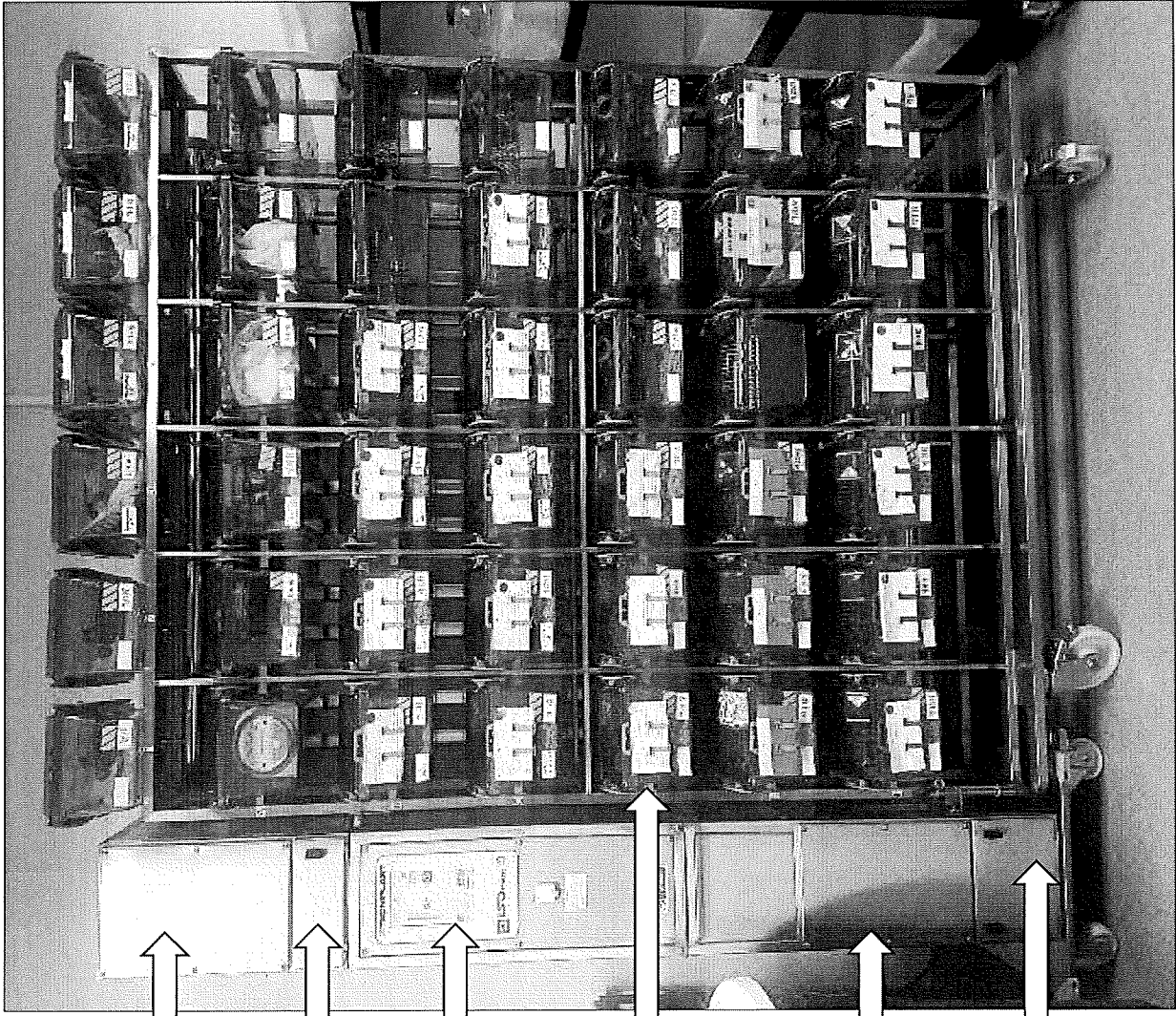
Inlet Air Valve

Cage Lock

Clamps

Cage Card Holder





Supply HEPA housing

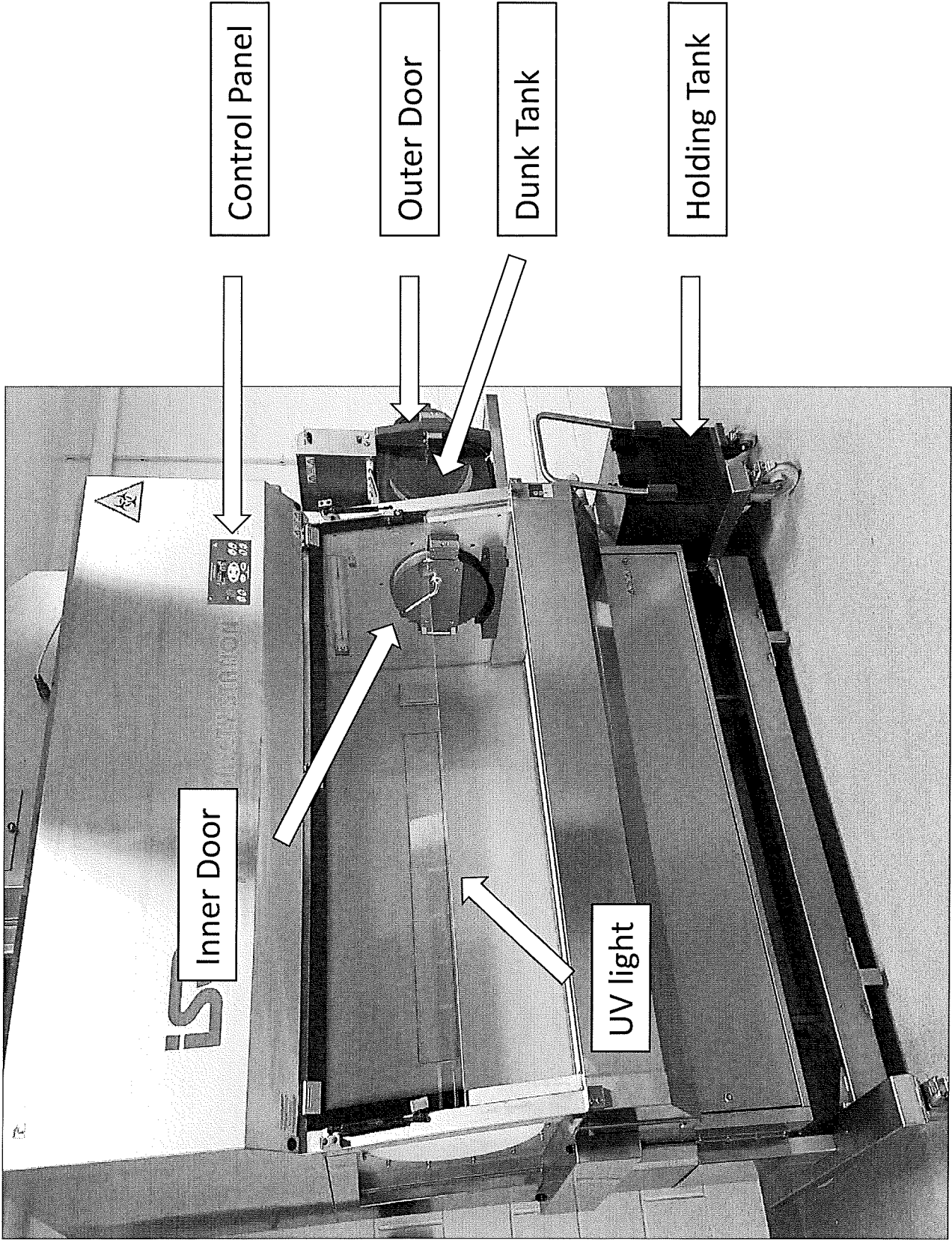
Supply prefilter housing

Control Panel

IsoCage

Exhaust HEPA housing

Exhaust prefilter housing



Control Panel

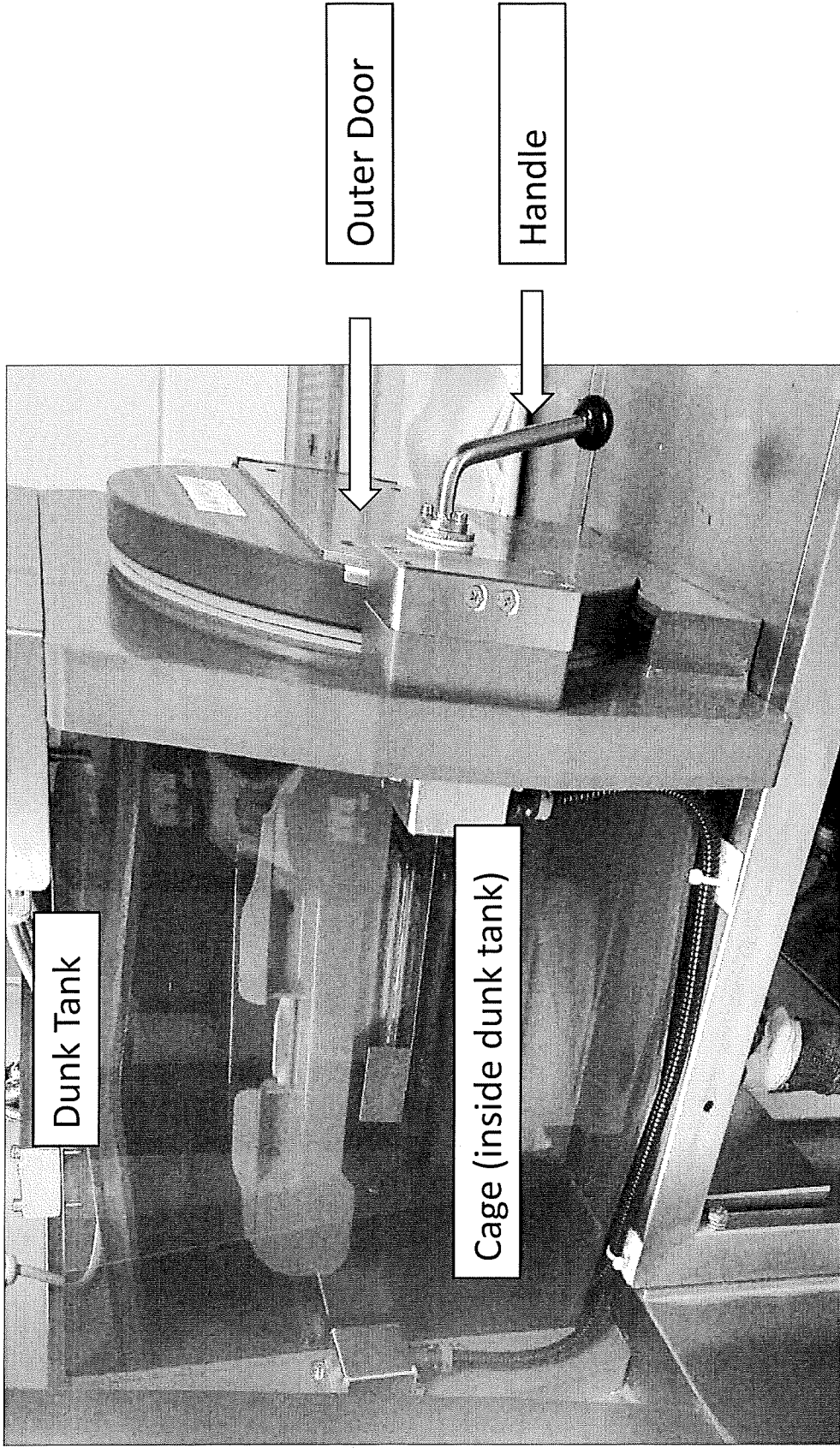
Outer Door

Dunk Tank

Holding Tank

Inner Door

UV light



Outer Door

Handle

Dunk Tank

Cage (inside dunk tank)

Preparing Sterilant:

MB-10®(a chlorine dioxide product) comes in 6 gram tablets (used for tank solution) and 1.5 gram packages (for spray bottle preparation).

When preparing solutions always wear a gown, gloves, mask and eye protection.

A 200 ppm concentration of MB-10® is used for all work processes. Check the tank solution concentration before each use.

MB-10® can be used for 7 days. Sensitive to light, store in a cool dark location.

Preparing Tank Solution:

The tank solution is changed approximately once per week.

- Add 32 L of water to the tank using water.
- Add 16 of the 6 gram tablets to the water to make up a 200 ppm solution.
- Allow the tablets to dissolve for 10 to 15 minutes before use.

Preparing a Spray bottle Solution:

- Add 500 mL of tap water in a graduated cylinder.
- Add one 1.5 gram tablet of MB-10® to the water.

OR

- Add 2000 mL of tap water in a graduated cylinder.
- Add one 6 gram tablet of MB-10® to the water.
- Allow the tablet to dissolve for 10 to 15 minutes before use.
- Use as needed.

Check Concentration of MB-10®:

- Dip an MB-10® test strip in the tank.
- Check via colour comparison (indicated on the strip bottle).
- If the strip confirms 200 ppm it is safe to use the tank.
- If the strip indicates a concentration below 200 ppm dispose of the solution and make up a fresh tank of solution.

Connecting the Disinfectant Tank to the IBS:

- Attach the tank to the unit with the fixing bar.
- Connect the inlet and outlet pipes to the quick connections on the disinfectant tank.
- Connect the wire and screw into place.

Tank should only be disconnected from the tank for purposes of cleaning the tank or for refilling the tank with disinfect solution.

Cleaning the Disinfectant Tank:

The disinfectant tank should be physically cleaned once every two weeks.

- Disconnect the tank from the IBS.
- Turn on the tap in the floor sink and fill the tank to the top with water.
- Drain tank using the pump.
- Once empty, rinse the tank with copious amounts of water for several minutes.
- Use a wet clean cloth and wipe down the interior of the tank. Remove any debris or residue.
- Rinse the tank and make sure you remove all the water from the bottom.
- Wipe the tank dry with a clean cloth.
- Refill with tap water and reattach to IBS.
- Run through twice to rinse with water.
- Wipe down inside port.
- Add 16 x 6 gram tablets to the reservoir tank.
- Allow the tablet to dissolve for 10 to 15 minutes before use.
- Replace tape on front of dunk tank (with red shield) with new date & initial.

Preparation of ISOCage Biosafety Station (IBS) Hood:

The IBS is designed to be used by a two person team.

- Person 1 wears “sterile” PPE and works in the IBS and never removes hands from the IBS area.
- Person 2 works outside the IBS and never places their hands inside the IBS area.

IBS Start Up:

- The IBS must always be plugged in.
- When not in use the IBS should be in ½ mode OR have UV cover on if being sterilized between uses.
- Turn on the IBS. Press the green ventilation button. Enter the password (1, 2, 3). Press the green arrow button to confirm.
- If the IBS is in ½ mode, press the ½ mode key. Confirm yes to the prompt “reduced air speed off” by pressing the green arrow key. Enter the password (1, 2, 3). Press the green arrow key to confirm. The unit will ramp up to full ventilation. Alarm may sound. It will go off on its own.

Wait approximately 15 minutes before using the IBS. Ensure the unit is running in full ventilation mode.

Before you begin:

- Check the disinfectant solution concentration in the solution tank with an MB-10[®] test strip.
- Check that the disinfectant tank has an adequate volume of MB-10[®].
(it will not start if it is below the level),
- Check that timer on the dunk tank is set for 90 seconds of contact time.
- Spray down hood and allow sterilant to sit for at least 15 minutes.

IBS Clean Up:

Once all changes are complete and cages are back on the rack, both Person 1 and 2 can work in the IBS for clean-up. One of the priorities in clean-up is to remove the MB-10[®] residue from any stainless steel contact. Remove all supplies from the IBS.

- When work is complete remove everything from IBS.
- Mop up excess sterilant using a cloth.
- Remove white floor panels and the pre filters and set aside.
 - if pre filters have debris, use vacuum to clean.
- Remove front grate and set aside.
- Clean inside of the hood using appropriate cleaner.
- Wipe clean.
- Spray again with water and wipe clean.
- Replace filters, grate and floor panels.
- Shut down IBS into ½ mode OR UV sterilize.

IBS Shut Down ½ Mode:

- When IBS is clean, turn the IBS ventilation to ½ mode.
- Press the grey ½ mode button. Confirm yes to the prompt “reduced air speed on” by pressing the green arrow key.
- Enter the password (1, 2, 3).
- Press the green arrow to confirm.
- The unit will go into ½ ventilation mode.

OR

UV Sterilize:

- Turn off unit (press green power button & enter the password (1,2,3)).
- Place metal cover over the opening and lock into place.
- Press UV button (it will flash yellow).
- Set start delay and run time.
- Leave room when UV decontamination begins.

Sterile Operation of IBS:

The IBS operates as a 2 person system.

Person 1	Person 2
<i>Works sterilely <u>INSIDE</u> IBS</i>	<i>Works <u>OUTSIDE</u> the IBS</i>
◦ New gloves	◦ New gloves
◦ Respirator	◦ Respirator
◦ New gown	
◦ Sleeve covers	

****** MB-10® spray bottle should be in port before person 1 dons their PPE ******

- Person 1 sprays their left hand and sleeve with MB-10® and enters into IBS.
- Wait 3 minutes for sterilant to become active.
- Pick up spray bottle inside IBS with left hand and spray right hand and sleeve.
- Enter into IBS.
- Spray hands and surfaces ensuring to cover all with sterilant.
- Wait 3 minutes before touching any additional supplies or opening port.



****** Person 2 can aid person one by “spraying them in” by spraying both hands/sleeves at the same time while on the outside of the isolator. Once person 1 is inside, they need to wait the 3 minute contact time and will spray their hands again.***

- Person 1 does not leave IBS for any reason during a procedure!!!
- Should they have to exit- they must change ALL their PPE for a new set and wait the contact times as listed above.

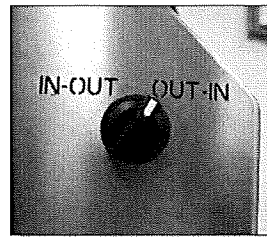
Using the Dunk Tank & Chamber:

All materials entering the IBS must go through the dunk tank (if possible OR sprayed in) for a timed disinfectant process before entering the IBS.

The doors of the dunk tank are interlocked. When one side is open, the opposite door cannot open.

- Turn the switch to OUT-IN when processing items into the IBS.
- A green light is present when in OUT-IN mode. When the light is green you can open the door. (A red light will show inside the IBS.)

An emergency push button (reset cycle) is available on the controller of the dunk tank in the event a cycle needs to be stopped. The cycle will stop and the chamber will empty when the button is pushed.



Notes

- IBS will not work if the hood is not turned on.
- Tank may also not fill if there is not enough:
 - Sterilant in the holding reservoir.
 - Adequate volume displacement from small items (tank will not drain).
 - should this occur: push red reset button & add an additional item (eg. sterilized bottle of water) to the tank and try again.

To process items into the IBS use the following steps:

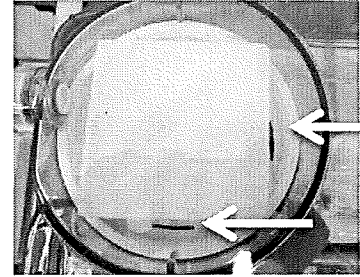
- Person 1 sprays themselves sterily inside the IBS as per standard protocol.
- A green light will show on the outside controller side and a red light will show inside the IBS. Open the dunk tank door and place an item in the dunk chamber and close the door. The cycle will begin automatically. A red light will flash on the controller during the cycle.
- The cycle is set for 90 seconds of contact time.
- When the cycle is complete, the green indicator light goes on in the IBS. This indicates the door to the dunk chamber can open from the IBS side.
- Person 1 can open the door inside the IBS and remove items from the chamber.
- Close the door once all items are removed from the dunk chamber.
- Person 2 can start another cycle as soon as Person 1 has closed and locked the door.
- Person 2 continues rotating each item through the dunk chamber until all required items are in the IBS.

Preparation of Supplies:

Items may include: clean IsoCage, autoclaved water, spray bottle filled with MB-10®, additional container.

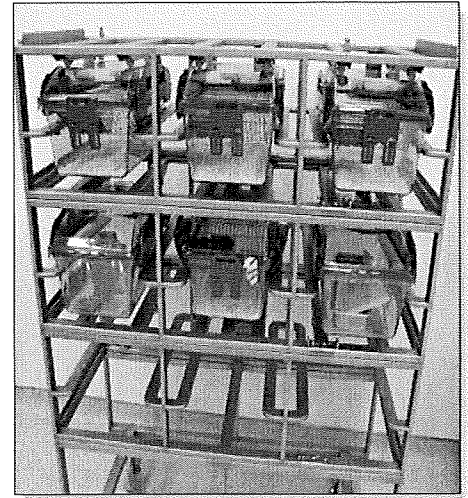
Clean IsoCage :

- Prepare IsoCage bottom with ¼” of bedding and 1 square of nestlets/shack (breeder).
- Check the HEPA filter on the IsoCage lid.
 - Each HEPA filter can be autoclaved a maximum of 5 times. A mark is placed on the white plastic of the HEPA filter before each autoclaving.
 - If the filter has 5 marks, discard.
 - Use a new filter & place a mark on the new filter.
- Place the wire bar lid on the cage. Fill the food hopper with rodent diet.
- Add an indicator strip to food hopper.
- Place an empty water bottle (with sipper top removed) in wire top.
- Check that no debris is on the cage gasket or lid gasket then place the lid on the cage.
- Insert the cage on a decontamination rack sitting on a decontamination trolley or rack.
- Date cage with autoclave tape prior to autoclaving.



Use of the Decontamination Rack:

The IsoCage when closed properly, is a hermetically sealed container (no oxygen inside). If the unit is autoclaved without air entry into the cage, the cage could implode. The decontamination rack allows air flow into the cage during autoclaving. When an IsoCage is seated properly on a decontamination rack, the inlet valve on the cage is opened providing an exchange of air and equalization of pressure in the cage during an autoclave cycle.



An autoclave trolley is used to stack the decontamination racks.

- Stack decontamination racks on one autoclave trolley. Each rack holds 3 cages.
- Place the first rack on top of the trolley. Insert the stacking pins into the rack locking system. Repeat the step for two additional decontamination trolleys.
- To insert a cage on the decontamination rack, slide the cage across the runners until it attaches to the cage valve opening device and the cage locking device engages.

******Open the cage latches by pulling up on both white tabs and pulling the blue tabs out to the side******

- Include a spore test taped to the outside middle of one of the cages.
- Autoclave the decontamination rack on appropriate cycle.
- Remove the racks from the sterilizer. Disengage the rack locks and lift the first rack onto the trolley and lock in place. Lift the 2nd rack onto the trolley and lock in place.
- **Approximately 30 minutes later, pull the cages away from the cage valve opening device and secure the clamps on the cages.**

****The cages must cool down before release from the cage valve opening device. Failure to do this step will result in the inability to open the cage lid. The internal pressure will be too high!**

- The cages can remain on the decontamination trolley or rack until needed. Always confirm the results from the spore test before using any cages on a trolley.

Verifying the Autoclave:

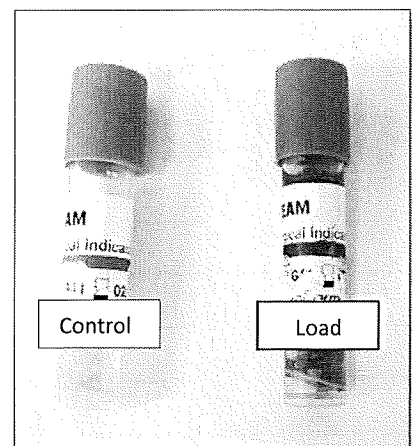
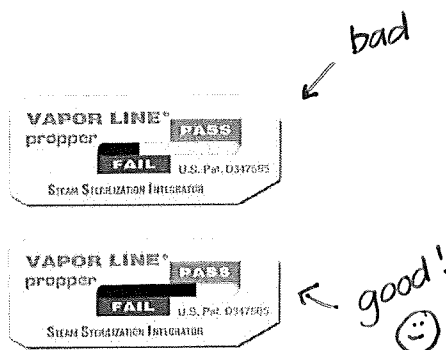
Indicator strips: are placed in feed and bedding bags and can be brought into the isolator.

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

- Each IsoCage should have an indicator strip placed in food hopper.
 - this will be visible from outside of the cage.
- Each autoclave cycle should have a spore tube taped to the outside of the cage.

Prior to using ISO cages :

- Check that indicator strip has passed.
 - Spore tube must be incubated for 24 hours to verify sterility.
 - Activate the vial by crushing the inner tube using the vial crusher (on the incubator).
 - 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.



IVC Cage Change Procedures:

Cages will be checked daily for water and food levels, sick animals and new litters.

Cages will be changed bi-weekly (every 2 weeks).

Any manipulation of the cage or the mice must be considered high risk and extreme care and attention to detail is critical. Germ free mice can be inoculated with bacteria or microbiota and will be referred to as gnotobiotic but ALL will be treated as “germ free” for manipulations.

SPRAY YOUR HANDS WITH MB-10® BEFORE EACH TOUCH OF ANY PART OF ANY CAGE OR SURFACE IN THE IBS!

IBS Set Up for Cage Change:

A large number of items must be processed through the dunk tank for entry into the IBS.

Wherever possible an item is autoclaved in an IsoCage for entry into the IBS.

Person 1 will work in the IBS.

Person 2 will work outside the IBS.

Once person 1 has donned appropriate PPE and have entered sterilely as previously described they are not able to remove their hands from the hood.

- Items to into the IBS:
 - Spray bottle full of fresh MB-10® (always enter first!) & tray with cloth.
 - Autoclaved water jug.
 - Sterile IsoCages for changing.
 - IsoCage with animals*.

**IsoCages with animals must not be off the rack for more than 20 minutes!*

- Place smaller items (spray bottle, water jug, tripour/Nalgene) into the dunk chamber.
- Close the door and begin dunk chamber cycle.
- Once cycle is complete, person 1 pulls supplies into IBS.
- Person 2 may pass through the next item to be entered (IsoCage with animals).
- Using the MB-10® exited from the dunk chamber, thoroughly spray the gloves, the work surface, back wall, left side and right hand port area including the chamber.
- Open the door to retrieve the next load (passed through by person 2).
- Spray your hands before opening port door and pull through additional supplies.

Cage Change: (Spray hands with MB-10® before each touch of any surface).

**Note* Remember that cages with animals must not be off the rack without opening them in the IBS for more than 20 minutes.*

- Spray hands with MB-10®.
- Open the lid of the clean cage and place the lid vertically.
- Move the wire bar to sit on lid OR slide backward on cage.
- Transfer the mice one at a time into the clean cage using forceps or hand.
- Place the wire bar back on the clean cage as soon as all mice are transferred.
- Replace wire top on cage.
- Make sure that the top rim of the cage bottom is absolutely clear of any bedding or debris before placing the lid on any IsoCage. Any debris may create a problem with the gasket.
- Fill up water bottle taking care not to touch as little as possible.
- Spray hands with MB-10®.
- Replace the lid on the clean cage and engage the clamps.
- Cage can be passed back through chamber to exit or via mayo stand on side of IBS.

START: Pre spray hood

- Sterilant bottle
- Sterilant tray (napkin)
- Water bottle
- Clean cages



Person 2: Unsterile (outside hood)

- Passes through cages of mice/supplies as needed

Person 1: Sterile (inside hood)

- Don appropriate PPE
- Spray into hood (person 2 can help!)
- Wait contact time
- Spray hands/use napkin
- Open new cage
 - Fill water
- Open cage with mice
 - Flip top vertically
- Move mice to new cage
 - Shut cage
- Spray hands & Repeat

