



Chapter 9 Quarantine

Wash your hands upon entering the lab, put on gloves, and wash your hands before you leave. When working in quarantine assume that everything is contaminated.

9.0 Introduction

Fish health is of foremost importance to the on going operation of the lab and the continued welfare of the H221 and J083b colonies. Many laboratories colonies of *Danio rerio* including ours are of chronic, sub-acute infectious agents, which at anytime, if not contained, can become a problem. Two such agents have been diagnosed in our fish are a *Mycobacterium* spp. the causal agent of piscine tuberculosis, and *Microsporidian* spp. Pathogenicity unknown. Most fish pathogens are not harmful to humans, however there is evidence (published) that suggests that fish tuberculosis (*Mycobacterium* spp.) can be transmitted to immune-compromised humans. Based upon these findings wear protective gloves, and wash your hands when you enter the quarantine laboratory and just before you leave.

9.1 Fish Importation Overview

Adult zebrafish and embryos housed in another fish lab including our quarantine room may not be introduced into the H221 and J083b facilities with out consent of the lab manager. New/foreign fish will be housed in quarantine for a 4-week period in the Q-room (G617) during which time the laboratory manager will observe the fish for diseases. The fish need to be found free from harmful pathogens, specifically those associated with Zebrafish (*Danio rerio*). Fish in quarantine are housed in an isolated system and must remain so until clearance has been achieved from the laboratory manager only at that time may surface disinfected embryos be moved to the main facility. **Plan ahead:** space is limited in the Q-room and it will take time to clear the fish, so plan your studies with these considerations in mind.

Fish importation Process

- 1) If you want to bring fish in to the quarantine room contact the laboratory manager via email dgw5079@u.washington.edu. In your email include the following information: the **source** of the fish such as a NOAA, or a university in the latter case include the name of the laboratory, **import type** either adult or embryos (including whether or not the embryos were bleach disinfected), **number of fish**, **genetic information** (gene if applicable mutation etc), **date of birth** if known at the time, and **comments/plans** such as need to ID carriers, spawn as soon as possible, etc. The manager will send you a conformation email including the tank number(s) to be used in quarantine, and a stock number. Imported fish will be tracked in a database.
- 2) When the fish arrive take them directly to the quarantine laboratory or incubate if applicable. The 4-week observation will begin as soon as you notify the laboratory manager except in the case of very small fish. During the observation period you may see notes and labels on your tank and fish will be readily culled.
- 3) If the fish pass the 4-week observation period the manager will give you a time window (~2 weeks) to spawn the fish and disinfect embryos see below. More time can be arranged. The time period will be written on the tank and emailed to the fish owner. If there is a problem with the fish the laboratory manager will discuss options with you.
- 4) Once a population is established in the main colonies quarantine fish need to be promptly eradicated.



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Source	Import Type	No# Fish	Genetic ID	D.O.B	Comments/Plans
Example U.W. Alder Lab	Adult	4	gol ^{bl}	9/22/05	Need to id hets and bring progeny to main lab

Table 9.0 Example of the information needed prior to importing fish in to the quarantine laboratory

9.2 Surface Disinfecting Embryos

All of the equipment needed to bleach disinfect embryos is found in the quarantine laboratory.

- 1) Obtain the bleach solution from the shelf to the left above the sink. Note on bleach solution its 0.1 ml of 6.8% bleach in 170 ml of clean system water. Use chemically pure sodium hypochlorite PO₄ <0.0005%; Ca <0.001%.
- 2) Place the embryos in a clean dish, remove the water they are in and add the bleach solution to cover the embryos, or use a pipette to add the embryos to the bleach solution.
- 3) Gently swirl the embryos in the bleach solution and allow them to stand for 5 minutes.
- 4) Rinse the embryos two to three times in sterile system water and transfer the eggs to a clean disposable petridish.
- 5) Repeat the bleaching procedure steps 1-4.
- 6) Bleach the embryos within the first day. Bleaching may toughen the chorion to the point that fish may not be able to hatch. Because of this, the chorion may need to be removed either mechanically or with pronase treatment prior to normal hatching.
- 7) Embryos that have been properly cleared and bleached can be removed safely from the quarantine room. Be careful not to recontaminate the embryos by contact with nonsterile water or glassware.

Caution There will be no free exchange of equipment from the Q-room to any other facility. Should there be a need for equipment, nets, etc or supplies please ask the facility manager. There can be no transfer of fish or any objects (nets etc) from one tank to another in the quarantine room.

9.3 Water Tower & Flow-through System

The fish system in the quarantine laboratory is different than any other system in that water is not recirculated instead water periodically enters the tanks and then leaves the system. This timed-flow-through system is needed to isolate the individual tanks on the system from each other, so that water is not shared between tanks. When using this system be sure not to transfer any items nets, fish etc from one tank to the next.

The system starts primary filtration of tap water in 9 canister filters. The first two are 10 µm fabric filters, next are two short filters that are reserved for special filtration needs depending on source waters quality, next are 4 carbon filters and finally 1 deionizing resin filter. After primary filtration the water enters the tower that has a float valve that shuts off the water when it reaches a 180-gallons. From here conductivity and pH monitor/controllers operate dosing pumps adjust the water quality parameters to 1000 µS and a pH 7.0. Water in the tower is recirculated bottom to top (mixed) by a distribution pump that also filters tower water through an additional carbon filter and a 25-watt ultraviolet water sterilizer see Figure 9.0 and 9.1.

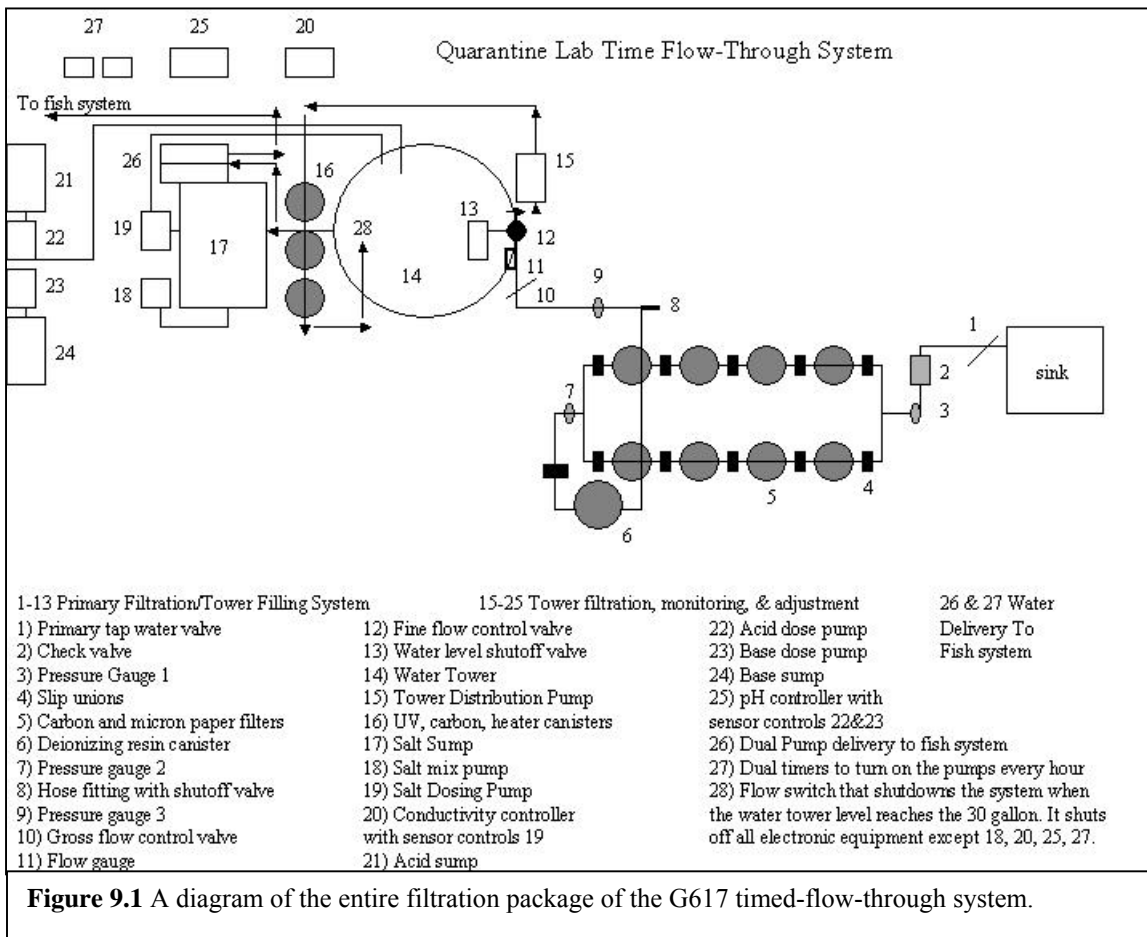


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Figure 9.0 Left: primary filtration package Center: Salt sump Right: flow-through fish system

One of two fish system pumps controlled by timers draw water from the tower in 1-hour intervals and delivers it to the tanks on the fish system. Each hour 77 liters of tower water is delivered to the 33 9.0 liter fish tanks (2.3 liters/tank). Water leaves the tanks, enters a gutter and is delivered to a floor drain underneath the fish rack.





Monthly Filtration Maintenance

Maintenance should be performed anytime there is a PSID (pounds per square inch drop) of 25 psi between the pressure gauge #1 and gauge #2. or #1 and #3 while water is passing through the filter canister (i.e. while the tower is filling). Through out these instructions please refer to **Figure 9.1**.

1. First record the reading from all three pressure gauges (3, 7, 9) and note whether the pressures were recorded while the system is running (water passing through the filtration) or not. Record this information along with any relevant maintenance notes on the maintenance log located above the canister filters.
2. Shut off the primary tap water valve (1) then the gross flow control valve (10).
3. Next relieve the pressure in the canisters (5 and 6). To do this first remove the nozzle from the system hose and place it in the floor drain below the fish rack. Then open the hose valve (8) and all valves on the system hose. Water should drain out the hose from the canister in to the floor drain. Additional pressure can be relieved by pressing and holding down the red bottoms on top of the canister lids. CAUTION do not proceed to the next step until the pressure as read on gauges #1 and #2 has dropped to 5 psi or less.
4. Loosen the canisters (5) by turning the canisters counterclockwise with a large wrench just below the canister lids. Loosen only. Then unfasten the grey couplers between each canister being careful not to disturb or lose the o-ring in each coupler.
5. Remove the canisters. Unscrew the canister body the rest of the way from the canister lid.
6. The first two canisters are 10 μm felt filters. 2 clear empty filters reserved for special filtration needs. The next 4 are carbon filters.
7. Remove the filter from the canister housing and throw them in to the garbage if they are felt filters. For carbon filters unscrew the cap from the pipe filter and pour out the carbon in to the garbage then refill it with fresh carbon. Rinse off carbon dust and screw the pipe filter lid back onto the pipe. Rinse out the inside of the canister housing, remove the o-ring near the canister housing threads, wipe it off with a towel, relubricate it with Teflon, and reinstall it in to the groove just below the threads.
8. Install the new filter in to the canister making sure the hole in the bottom of the filter sets over the centering ring in the bottom of the canister housing. Then screw the housing back on to the canister lids making sure the hole in the top of the filter lines up with the outlet in the middle of the canister lid. Tighten until the housing is snug against the canister lid.
9. After all of the #5 canisters have been serviced reinstall the canister in the same order as they were when you started: 2 felt ► 2 empty ► 4 carbon by locking together the slip unions, make sure the slip union o-rings are still in place.
10. Make sure all lines are connected. Close the hose valve (8) and remove the hose from the drain. Then open the primary valve (1) and then gross flow control valve (10). Hold down the pressure relief valve one-by-one on top of the canister lids until water is breached from them.



11. Check the system for water leaks. Record maintenance dates on the tape on the sump housings. Fill out the maintenance log including pressure gauge readings after maintenance.
12. The Deionizing canister (6) needs to be replaced only when it is suspected of causing a 25 psid i.e. after maintenance on the other canisters (5) or when the acid sump of the pH dosing system requires frequent refills. DI canister typically last for several months. For maintenance on the DI canister follow steps 2-11. It is easiest to perform this maintenance concurrently with the other canisters.

Water Tower Filtration Maintenance

Water tower filtration maintenance solely refers to the carbon canister located adjacent to the water tower (on the same dunnage racks). Maintenance is performed once a month. Through out these instructions please refer to **Figure 9.1**

1. First unplug the distribution pump and UV from power bank #2.
2. The close the valve in front of the distribution pump (15) and open the globe drain valve, which is in front of the carbon filter (16) hanging just below the dunnage rack. Drain the water from this valve in to a container and pour it down the sink.
3. Using the handle of a large wrench for leverage turn the carbon canister (16) lid counterclockwise until loosened then unscrew the rest of the way by hand. Remove the lid.
4. Inside the canister pull out the filtration chamber and pull off the cap at the top of it. Pour the old carbon into the trash and rinse off the chamber. Pour fresh carbon in to the chamber and rinse off the carbon dust in to the sink. Place the cap back on top of the chamber.
5. Place the chamber back in to the canister making sure the hole in the bottom of the chamber rest over the hole in the bottom of the canister (centered).
6. Remove the o-ring from the inside of the canister lid. Wipe it off with a paper towel, relubricate it with Teflon and place it snugly back in to the canister lid. Wipe off the canister lid threads on the canister housing. Then screw clockwise the canister lid back onto the canister housing threads until it is tight (hand-tightened is sufficient).
7. Close the drain valve and open the pump distribution valve (15). The water lines and canister will be gravity feed filled with water.
8. Plug in the distribution pump and UV. Check the system for leaks and record the service on the maintenance log.

Every Year: Replace the UV bulb.

Tank Use

To clean a tank shutoff the water valve to the tank it's located on the black water line. Remove the water and green airline from the tank lid. With the tank still in place lift the front of tank to drain water out the back of the tank. Additional water can be removed from the tank if need be by siphoning it with a rubber hose with a screen attached to the intake to prevent fish from being sucked up. Water from the siphon can be drained in to a



bucket, and emptied in to the floor drain. Pull out the dirty tank. Fill a clean tank a $\frac{1}{4}$ full with system water from the white sink faucet. Using a clean fishnet transfer the fish to the clean tank. Rinse off the dirty fish net with water and place it in the net soak do not reuse. Place a clean lid on the tank and install a clean airline tube. Take the dirty tank to the sink remove the lid and empty out any remaining water. Thoroughly rinse the dirty tank and accessories with water, and place them in the bleach bath or on the dirty dish shelf. Spray the shelf where the dirty tank resided, and the water line with 70% isopropyl alcohol. Install the new tank on the shelf and insert the water and airlines through the lid. Open the water line valve. Adjust the via air valve on the manifold if necessary. Note: water only turns on every hour for a few minutes there is not a continuous flow of water to the fish tanks.

Safety Features & Emergency Shutdown

The tower has a float switch located at the bottom of it that will shut down all of the equipment if the water level in the tower drops down below 30 gallons to prevent gas bubble disease and to protect equipment. The equipment will turn back on after the water levels rises in the tower. To shutdown the power manually turn off the switch on plug bank #2 next to the tower. Meters and dose pumps control water quality parameters in the tower, so it is important to keep the meters and probes properly calibrated. In case of failure in the water level shutoff valve there is an overflow drain in the top of the tower that will remove excess water from the tower to the floor drain.

9.4 Tasks

For all daily and weekly duties please refer to the laboratory animal and room care log (ARCL). Fill out the sheet as you finish each task.

Daily

- *Check all tanks for any fish mortality.* If any are found remove the fish, place it in a plastic bag in the freezer, and fill out the mortality log in the notebook above the sink.
- *Check that all 3 red LED on the three power banks* on the wall on either side of the water tower. If any of the lights are off let the laboratory manager know.
- *Check and record the conductivity controller reading* and enter it in to the ARCL.
- *Keep the laboratory clean and organized.* Put dishes away disinfect countertops and shelves etc with isopropyl.
- *Check the salt dosing sump.* Keep it filled above the 30-liter mark on the sump. To do this determine the volume you want to refill then add 1700 ml of instant ocean per 15 liters of water (3400 ml/30 liters etc). First use the DI hose to fill the sump to the desired level. Then take down the salt beaker from the top shelf of the fish system and add the salt to it from the instant ocean salt bucket. Then slowly dispense the measured amount of salt in to the salt sump. Add salt slowly there is a mixing pump in the sump that can get jammed or ruined if salt is added too quickly.
- *Check acid and base sumps refill if necessary* details below.
- *Record the temperature* from the thermometer on the refrigerator in to temperature log. Both are located on the refrigerator door.



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- *Clean dishes.* To clean disinfected equipment put on a pair on rubber gloves, and a lab coat. Remove the items one at a time from the bleach bath and place them in the sink. Using the DI water hose thoroughly rinse inside and out all of the dishes. Generally, water should pass across every surface of every item at least 3 times. Minimize the backsplash from the hose to the immediate sink area. Then place the wet dishes on the drying shelf next to the bleach bath. Let the items dry before using again. To add items to the bleach bath first make sure it can be bleached, generally hard plastics are bleachable, but not metal.

Weekly

- *Switch out bleach bath.* Be careful anytime you use sodium hypochlorite (bleach). It is highly toxic to fish in very small quantities. Open the valve at the back of the bath this will drain it. Do the dishes. After its drained close the valve and use the DI hose to fill the bath to the gallon mark. Then add 1.5 liters bleach. Write the date the bath was switched out on the lid.
- *Change out net soak.* To do this remove any nets from the net soak bucket, rinse them off with water and place them in the clean net beaker. Then empty the net soak down the floor drain. Rinse the bucket out with water and dump it down the drain. Then fill the bucket halfway full with DI water. Then get a bottle of net soak off the shelf above the sink. Take off the cap and then use it to measure out 15 ml (3 cap-fulls) add this cap by cap to the water in the bucket. Finally, place the lid on the bucket and date it.
- *Change out dechlorinator.* Empty the bucket down the floor drain then refill it ~3/4 full with water, add 50 ml (30 mg) of sodium thiosulfate and date the lid. Sodium thiosulfate is located on top of the refrigerator.
- *Ammonia.* Test the water tower and fish system using the ammonia test kit on top off the refrigerator. Follow the directions provided with the test kit. Collect the tower water sample from the white faucet labeled system water above the sink. For the fish system collect the sample from one of the heavier stocked tanks (~20 fish). Never place testing equipment directly in the water to be sampled, instead collect the water in a clean “neutral” container and then add it to the sample container.

Acid and Base Dosing System

As always never mix any part of an acid with a base. The dosing system controls pH in the water tower to a range of 6.8-7.2 with a set point of 7.0 (ideal reading). The two dosing pumps acid (hydrochloride-red labeled) and base (sodium bicarbonate-green labeled) are controlled by a Pinpoint pH monitor with a cell sensor in the water tower. One pump draws acid from an acid sump and the other pump draws base from the base sump. It is these sumps that need to be checked daily and refilled as necessary both should be refilled before they drop below the 10-liter mark. Both the acid buffer and sodium bicarbonate can be found on top of the fish rack.

Acid: 250 ml hydrochloride/10 liters water



To fill the acid sump, measure out 250 ml acid buffer (hydrochloride) into a clean 1.0-liter beaker be careful not to stir up the fine powder (scoop and pour slowly). Obtain a clean 5.0-liter beaker and fill it with 4.0L of water then slowly pour in half the acid buffer (~125 ml). Stir the mixture with a short piece of PVC pipe. Pull back the lid on the acid sump and slowly pour the acid from the beaker in to the sump. Repeat this for the next 4.0 liters of water and 125 ml acid then add 2.0 L of pure water to the sump from the same 5.0-liter beaker. Place the lid back on the sump, date the lid including the volume of acid you added to the sump.

Base: 225 ml sodium bicarbonate/10 liters

To fill the base sump, measure out 225 ml of sodium bicarbonate into a clean 1.0-liter beaker be careful not to stir up the fine powder (scoop and pour slowly). Obtain a clean 5.0-liter beaker and fill it with 4.0L of water then slowly pour in half the sodium bicarbonate (~112.5 ml). Stir the mixture with a short piece of PVC pipe. Pull back the lid on the base sump and slowly pour the base from the beaker in to the sump. Repeat this for the next 4.0 liters of water then add 2.0 L of pure water to the sump. Place the lid back on the sump, date the lid including the volume of base you added to the sump.

9.5 Q-Room Feeding

Q-Room Feeding

The quarantine room needs to be kept completely separate from the main colonies. Follow procedures below carefully and always feed this laboratory last.

- 1) Take ~50 ml of harvested Artemia, paramecia (if any nursery fish) in paper cups covered with aluminum foil, a prox card that will gain you access to the comparative medicine wing G617, and the door key. Keep the prox card and key in your pocket when you're not using it do not place it on any surface in the quarantine room.
- 2) Wash your hands upon entering the lab, put on a pair of latex-free gloves and a lab coat.
- 3) Feed all adult fish dry salmon food first, and then feed Artemia. Feed any larval fish.
- 4) Take water quality readings on the flow-through fish system, nursery if there is any fish present and on the water tower. Record the temperature, pH, and conductivity information on the door. Note, clean all probes off with isopropyl before moving water meters to different fish systems. On the door there is a feeding checklist fill this out. The water tower has separate water meters they are located on the wall next to the tower.
- 5) Dispose of any paper cups in the trash can Remove the lab coat, gloves and wash your hands before leaving the lab. Do not take any items (dishes, tanks, etc) from the quarantine back to the main colonies in H and J laboratories.