

Sentinel Fish Program

A. Summary

The sentinel program is designed to detect and monitor the presence of fish pathogens in the main room fish facility and to assess the efficacy of the filtration system and UV lights at preventing the spread of disease. Sentinel tanks are set up at pre- and post-filtration locations on the two water systems, which feed separate halves of the fish room. Two tanks are placed at each location representing three and six month exposure periods. Sentinel fish are sampled quarterly for diagnostic testing.

B. Definitions

1. Side A sentinels are located in the main room side A (north side) at A06C27 and A06C28. They receive post-filtration and post-UV water from a commingling of Systems 3 and 4.
2. Side B sentinels are located in the main room Side B (south side) at B15C08 and B15C09. They receive post-filtration and post-UV water from System 2.
3. System 3,4 sentinels are located in the aquaculture room and receive water from the Side A sump. This water is pre-filtration and pre-UV pooled effluent from all tanks on Side A.
4. System 2 sentinels are located in the aquaculture room and receive water from the Side B sump. This water is pre-filtration and pre-UV pooled effluent from all tanks on Side B.

C. Materials

1. Eight clean, dry 3.75 L aquaria with lids, drains, and air straws
2. Barcode label for each tank listing fish strain, stock number, and fertilization date. The four barcode labels for the new sentinel fish will be identical. The four labels for the fish that have been sentinels for the previous three months will be identical. Write the original tank of origin on each barcode label. See section K, Supplementary Documents, for an example.
3. "Sentinel Tank" label for each tank listing location in sentinel system, date fish were placed in the sentinel tank, date to be sampled, and "tank may be cleaned but DO NOT MOVE TANK". See section K, Supplementary Documents, for an example.
4. 60 AB wildtype strain zebrafish, 3 to 5 months of age, large enough not to flow out an adult drain, of the same stock number, and raised together in the same tank up to this point. The tank of origin should have previously been shown to be negative for microsporidia by PCR assay.
5. Eight 50 mL screw cap tubes each containing 42 mL of Dietrich's fixative
6. Fixing Zebrafish for Histopathology SOP and associated materials
7. Euthanasia of Fish for PCR or Fixation SOP and associated materials

D. Procedures - Setup

1. Assemble eight 3.75 L aquaria with drains, lids, and air straws.
2. Label each tank with the appropriate barcode and sentinel tank labels. Place the barcode label at the top of the front of the tank. Place the sentinel tank label at the bottom of the front of the tank.
3. Remove the ball and spring from the drains of the System 3,4 and System 2 sentinel tanks only.
4. Fill the tanks half full with fish water.
5. Using a new net for each tank, transfer fish from the tanks that have been on the water systems for 3 months (have been sampled as sentinels once) to the appropriately labeled new tanks. Place these tanks back on the system, one in each of the four locations (see Section B. Definitions and Figure 1). These fish will become the six month exposure sentinels at the next sampling date.
6. Using a new net, transfer 15 fish of the new sentinel stock (AB, 3-5 months old, same stock, raised together up to this point) to each of the remaining 4 new tanks. Place these tanks on the system, one in each of the four locations. These fish will be the three month exposure sentinels at the next sampling date.

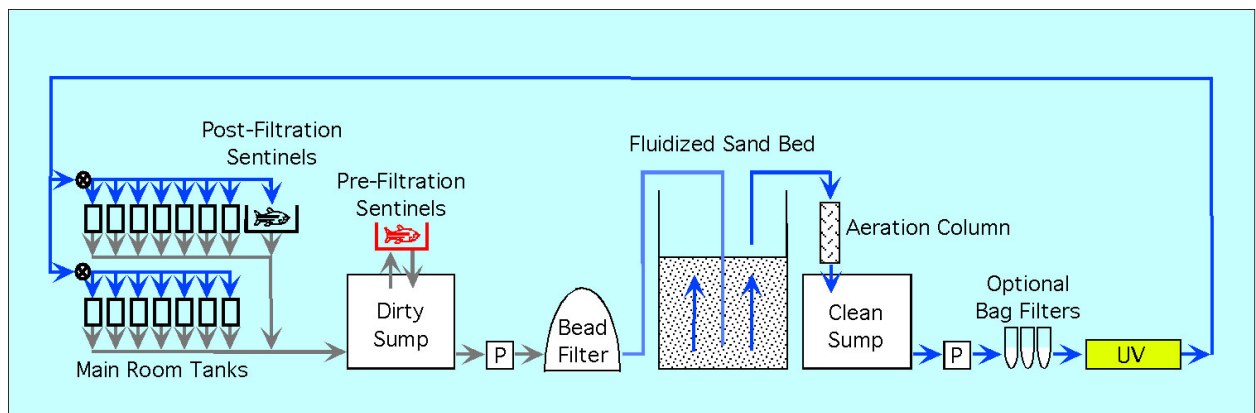


Figure 1

E. Procedures – Monitoring and Maintenance

1. Husbandry and daily care of sentinel tanks is equivalent to all adult 3.75 L aquaria in the main fish room.
2. Visually inspect the aquaria twice daily during feedings. Report any morbidities or mortalities to an animal technician or the veterinarian immediately.
3. If a tank is dirty, use a new net to transfer the fish to a clean, appropriately labeled tank, and return the fish to the original location. Note that the ball and spring should be removed from the drain of new System 2 and System 3,4 sentinel tanks. Place the removed ball and spring into the drain of the dirty tank that is being replaced. All dirty drains must contain a ball and spring before being placed in the basket in the dirty side of the tunnel washer room.
4. Bleach and clean dirty tanks as all other tanks.

F. Procedures - Sampling

1. Sample sentinel fish for diagnostic histopathology every three months.
2. Euthanize and fix five fish from each of the eight sentinel tanks: Side A three and six month exposure tanks, Side B three and six month exposure tanks, System 2 three and six month exposure tanks, and System 3,4 three and six month exposure tanks. The sample of five fish should include any morbid fish identified in the tank. Handle each sentinel tank separately to avoid mixing the populations. Euthanize the fish according to Euthanasia of Fish for PCR or Fixation SOP. Fix the fish according to Fixing Zebrafish for Histopathology SOP.
3. Fix all five fish from one sentinel tank as a group in one 50 mL screw cap tube containing Dietrich's fixative.
4. Label each tube of fixed fish with the sentinel tank location (i.e. Side A, Side B, System 3,4, or System 2), and exposure time (i.e. 3 or 6 months).
5. Additional sentinel fish may be sampled for necropsy exam, bacteriology, or other diagnostics at the veterinarian's discretion.
6. After sampling, identify the four sentinel tanks that have been on the water system for six months (sampled twice for histopathology) and euthanize any remaining fish in the tank by hypothermal shock (see Euthanasia by Hypothermal Shock SOP). The fish that have been on the system for three months return to their locations in the water system and will become the six month exposure tank at the next sampling time.
7. Follow section D. Procedures – Setup to prepare the tanks that will be the three month exposure tanks at the next sampling time point.

G. Monitoring Requirements

Sentinel fish are sampled quarterly. The fish are assigned a case number in the Pathology Database. Fish identification information, system location, exposure time, and histopathology results are entered into the Pathology Database under this case number

H. Record Management

1. All sentinel results are archived in the Pathology Database according to case number.
2. Electronic copies of the Pathology Reports for all sentinel cases are available on the ZIRC Public Server.
3. Sentinel results are made available to the public on the ZIRC website Animal Health Report (http://zebrafish.org/zirc/documents/health_report.php)
4. Hard copies of the Pathology Reports for all sentinel cases are archived in the ZIRC Fish Health Reports binder.

I. References

N/A

J. Other SOPs Cross-Referenced Here

1. Fixing Zebrafish for Histopathology SOP and associated materials
2. Euthanasia of Fish for PCR or Fixation SOP and associated materials
3. Euthanasia by Hypothermal Shock SOP

K. Supplementary Documents

1. Example barcode and sentinel labels

<p>Sentinels - Main Room Side A In tanks since 17 April 2009 To be sampled 7/17/09 & 10/16/09 Tanks may be cleaned but DO NOT MOVE TANKS</p>	<p>AB- ZS 6398 DOB: 25-Feb-09 AB-3 ix AB-3</p>  <p>6398</p>
<p>Sentinels - Main Room Side B In tanks since 17 April 2009 To be sampled 7/17/09 & 10/16/09 Tanks may be cleaned but DO NOT MOVE TANKS</p>	<p>AB- ZS 6398 DOB: 25-Feb-09 AB-3 ix AB-3</p>  <p>6398</p>
<p>Sentinels - System 2 In tanks since 17 April 2009 To be sampled 7/17/09 & 10/16/09 Tanks may be cleaned but DO NOT MOVE TANKS</p>	<p>AB- ZS 6398 DOB: 25-Feb-09 AB-3 ix AB-3</p>  <p>6398</p>
<p>Sentinels - System 3,4 In tanks since 17 April 2009 To be sampled 7/17/09 & 10/16/09 Tanks may be cleaned but DO NOT MOVE TANKS</p>	<p>AB- ZS 6398 DOB: 25-Feb-09 AB-3 ix AB-3</p>  <p>6398</p>
<p>Sentinels - Main Room Side A In tanks since 17 July 2009 To be sampled 10/16/09 & 1/15/10 Tanks may be cleaned but DO NOT MOVE TANKS</p>	<p>AB-5 ZS 6375 DOB: 12-Feb-09 AB-4 ix AB-4</p>  <p>6375</p>
<p>Sentinels - Main Room Side B In tanks since 17 July 2009 To be sampled 10/16/09 & 1/15/10 Tanks may be cleaned but DO NOT MOVE TANKS</p>	<p>AB-5 ZS 6375 DOB: 12-Feb-09 AB-4 ix AB-4</p>  <p>6375</p>
<p>Sentinels - System 2 In tanks since 17 July 2009 To be sampled 10/16/09 & 1/15/10 Tanks may be cleaned but DO NOT MOVE TANKS</p>	<p>AB-5 ZS 6375 DOB: 12-Feb-09 AB-4 ix AB-4</p>  <p>6375</p>
<p>Sentinels - System 3,4 In tanks since 17 July 2009 To be sampled 10/16/09 & 1/15/10 Tanks may be cleaned but DO NOT MOVE TANKS</p>	<p>AB-5 ZS 6375 DOB: 12-Feb-09 AB-4 ix AB-4</p>  <p>6375</p>