

Example Sentinel SOP with quarterly sampling

I. Sentinel Tanks

A. Location in Aquaculture System

Separate sets of sentinel fish receive water that has returned from the main room tanks to the "dirty" water or pre-filtration sump (see Figure 1). Sentinel control tanks (post-filtration and UV) are located in the main room racks on each side of the room. The Side A sentinel control tank receives water from System 1. The Side B sentinel control tank receives water from System 2.

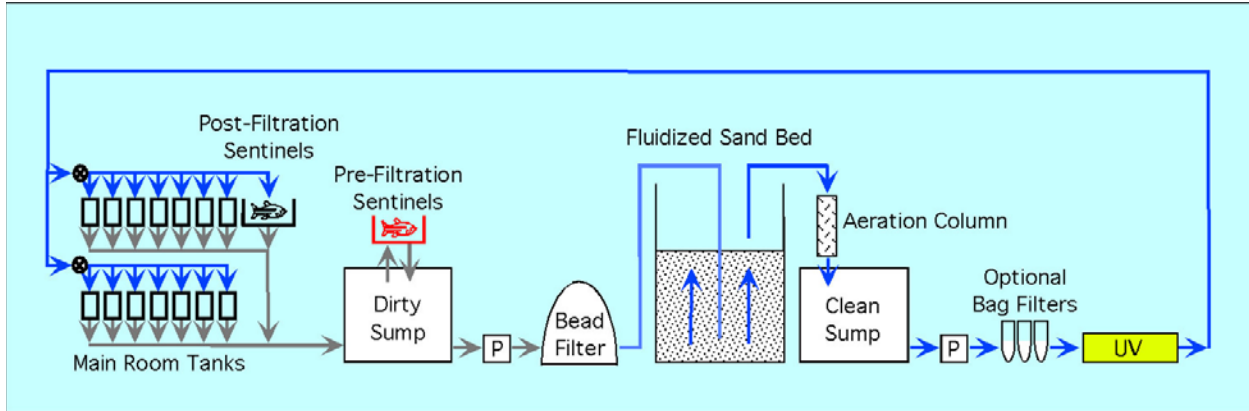


Figure 1

B. Tank Size - Sentinel fish are housed in 3.75 L aquaria.

C. Tank Cleaning - Tanks are bleached and cleaned between sampling and exposure periods. Tanks may be cleaned but not moved during the three-month exposure period.

II. Sentinel Fish

A. Strain: AB-1 or AB-2 wildtype strain. The same stock of fish should be used in all sentinel and control tanks for a particular sampling period.

B. Age: Fish are 3-6 months of age when placed in sentinel tanks. Fish should be large enough not to flow out the drain. Use baby mesh over drains if fish are small.

C. Number: Ten fish per sentinel tank.

D. Monitoring: Fish are visually inspected twice daily during feeding. Any morbidity or mortality is immediately reported to the staff.

E. Feeding: Fish are fed twice daily same as main room adult fish (1x *Artemia* naupli and 1x dry master mix).

III. Sampling/Exposure Period - Fish are sampled following a three-month exposure period. Five fish are typically sampled from each sentinel tank. Sentinel fish are euthanized and fixed in Dietrich's fixative. Additional fish are sampled for necropsy exam, bacteriology or other diagnostics if warranted.

IV. Examination - All fish are processed for histopathology with routine H&E staining. Special stains are performed as warranted.