



OFFICE OF LABORATORY ANIMAL CARE

Working Instructions

WIN Number:	210	Zebrafish Health Surveillance Testing	Revision #:	0
Date Effective:	10/16/23		Supersedes:	0

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PROCEDURE

All work instructions must be demonstrated by the trainer and performed by the trainee.

1. Schedule Testing:

a. If sending LIVE fish:

- Notify VRL prior to shipping by emailing john.firreno@vrl.net and larissa.walker@vrl.net, so they can follow up with the lab.
- Add a comment to the Portal order form under special instructions indicating that live fish are included.

b. If sending euthanized fish, no need to notify the lab prior to shipping

c. Schedule the collection time with the zebrafish facility manager.

d. Ship animals early in the week to be received by Friday of the same week.

e. Do not ship animals during weekends or holidays.

2. Specimen Collection:

- **General note:** select samples randomly across all rooms and systems, and prioritize including sick fish.
- **Fish for "Zebrafish Core Panel PCR":**
 - Coordinate with the zebrafish facility manager to select roughly 60x fish for submission.
 - Euthanize fish via rapid chilling in a 4°C ice bath for at least 10 minutes following opercular cessation.
 - Pool samples by placing up to 5 freshly euthanized fish per 15mL conical tube.

- Label tubes with species, collection date, room number and/or system ID number.
- Store conical tubes in the OLAC freezer until ready to pack for shipping.
- Fish for Histopathology:
 - Coordinate with zebrafish facility manager to select 5x fish for submission, prioritizing sick fish for histopathology (histopath > PCR).
 - Euthanize fish via rapid chilling in a 4C ice bath for at least 10 minutes following opercular cessation, then incise the abdominal cavity with a scalpel or scissors to expose viscera without damaging them.
 - Place euthanized fish into a rigid leak proof container with 10% buffered formalin, with at least a 10:1 formalin: tissue ratio
 - Label bottle with species, collection date, room number and/or system ID number.
 - Store containers at room temperature until ready to pack for shipping.
- Environment for "Zebrafish Core Panel PCR": *(Annually, or as requested by DVM)*
 - All environmental samples should be pooled by type (sludge, biofilm, etc).
 - Sludge:
 - Identify a visible accumulation of sludge (dark waste/biomatter) inside the sump tank or on the filter sock/sponge of each system sump.
 - Collect 1mL of the detritus with tank water into a rigid sealable conical tube.
 - Pool samples from all system sumps into a single conical tube.
 - Store the tube at ambient temperature until ready for shipment.
 - Biofilm:
 - Randomly select 5-10 system tanks and temporarily disconnect the overflow tubing.
 - Insert a collection swab into the tubing to sample biofilm.
 - Place swab into conical tube.
 - Reconnect overflow tubing
 - Repeat previous (3) steps for the remaining tanks and pool samples into a single tube.
 - Label tube with species, collection date, room number, and system ID number(s).
 - Store the tube at ambient temperature until ready for shipping.
- a. Pack samples for shipment:
 - Seal all containers with Parafilm to prevent leaking.
 - Seal the bottom end of a heat seal bag.
 - Place sample containers in the heat seal bag with enough absorptive material to prevent leaking, i.e., paper towels.
 - Seal the top of the heat seal bag.
 - Seal the bottom of another slightly larger heat seal bag.
 - Place the first bag inside the second bag.
 - Seal the top of the second heat seal bag.

3. Shipment:

- a. Print the submission form from VRL's website, <https://vrlsat.com/>. Tests- Z.Fish PCR Panel 2- 10Z13 and Histopath- 805ZF.
- b. Follow the instructions on the VRL website to print a FedEx shipping label.
- c. Ship priority overnight
- d. Place the heat-sealed bag(s) into an appropriately sized insulated box with a frozen ice pack, if needed.
- e. Place the completed submission form on the outside of the insulated box. Tape box closed.
- f. Attach the FedEx label to the outside of the box.

4. Sentinel Results:

- a. Save all results in the Shared Drive/Vet Folder/Zebrafish.
- b. Email results to the vetstaff@lists.berkeley.edu and to PI (principal investigator)/lab.

REFERENCE DOCUMENTS

REVISION HISTORY			
REVISION NUMBER	AUTHOR(S)	EFFECTIVE DATE	REVISION(S)