



University Animal Care Committee Standard Operating Procedure		
Document No: 15.4	Subject: Fixing of Zebrafish	
Date Issued: May 5, 2016	Revision: 1	Page No: 1

Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the correct procedure for the fixation of zebrafish following euthanasia or after being found dead, for the purposes of a diagnostic necropsy and/or histopathology.

1) **Introduction and Definitions:** Post-mortem autolysis and putrefaction (post-mortem decomposition caused by non-pathogenic bacteria) occurs rapidly following death. This makes fish that are found dead poor specimens for histopathology. For optimum preservation and diagnostic value fish must be alive and euthanized just prior to fixation. Fish designated for histopathology should be euthanized according to the SOP 15.3 "Anesthesia and Euthanasia of Zebrafish".

2) **Materials:**

- Supplies for euthanasia (see SOP 15.3 "Anesthesia and Euthanasia of Zebrafish")
- Fine forceps
- Dissecting scissors
- Plastic spoon
- Fish net
- Paper towel or absorbent surface
- Sealable glass jar
- 10% buffered formalin (~10ml for a single adult zebrafish)
- Tape
- Permanent marker

3) **Procedures:**

Preparation of Bleach Solution:

- Euthanize zebrafish according to the SOP. If found dead, skip to next step.
 - Use the plastic spoon to remove the fish from the euthanasia tank or a fish net to remove a dead fish from a tank. Use the side of the tank to drain off excess water. Dead fish should be discarded in the appropriate manner (placed in a plastic bag and stored in freezer for disposal).
 - Place the fish on its side on the paper towel or other absorbent surface.
 - Use the scissors to cut off the tail fin, by cutting through the tail muscle, but behind the anal fin. The tail should be left intact if there is a lesion on the tail or if the fish is less than 1.5 cm in length.
 - Using the fine forceps, pull the skin over the coelom upwards, to avoid disrupting the internal organs, and make a small cut in the raised skin with the scissors. Ensure the skin is opened to allow formalin to permeate the internal organs.
 - Use the forceps to pick the fish up by a fin and place it in the sealable glass container containing 10% buffered formalin. The volume of the fixative should be at least 10x the volume of the specimen. Tighten the lid.
 - Use the tape and permanent marker to label the container with the date, ACS number, PI, protocol number and facility. Fill out a pathology submission form with indicated information including history, clinical signs associated with the case and any treatments provided.
 - Bring samples and paperwork to Animal Care Services for submission for histopathology.
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References:

- 1) Dr. Nicole Compo, DVM
- 2) Zebrafish Husbandry Course, 5th International, Leading Advances in Zebrafish Husbandry, September 27-30, 2016, Bugaggiato (VA), Italy
- 3) CALAS Symposium June 11-14, 2016, Toronto, Ontario

Revised: October 22/2020
