RUTGERS UNIVERSITY
Office for Research

RUAC STANDARD OPERATING PROCEDURE

TITLE: Iodine and Bleach Dip for Zebrafish Embryos		
SOP Category: Aquatic	**RUAC SOP #: 5.12**	**Page: 1 of 3**
Effective Date: 6/17/2025	**Approval:**	
Revisions:		

SCOPE:

This document describes the procedures to be followed when zebrafish eggs are disinfected/sanitized by dipping them in a dilute iodine and bleach solution. This SOP applies to all Animal Care Staff (ACS), ACS Supervisors (ACSS), Veterinary Staff (VS), and Research Staff members (RS) at the Rutgers University facilities.

OBJECTIVE:

The objective is to describe how to surface-sanitize zebrafish embryos at 24 hours post-fertilization (hpf). This process is performed for eggs exiting the quarantine room to be entered into the main room.

PROCEDURES:

Materials




- Petri dishes
- Instant Ocean Egg Water (60ug/mL) squirt bottle
- Stock 10% Ovadine (1% available iodine, *Pentair Aquatic)*
- Stock reagent grade 5% Sodium Hypochlorite (*Sigma*)
- 6-well Plate
- P20 pipette and tips to make solutions
- 50mL Conical tubes (2)
- Straining Cylinder:
    - 100-micron nylon cell strainer (eg. *Corning* cell strainer part#CLS431752)
    - Or made using marine-safe glue to adhere nylon mesh (100-400 microns) to the bottom of cut-off conical tube or small PVC pipe.

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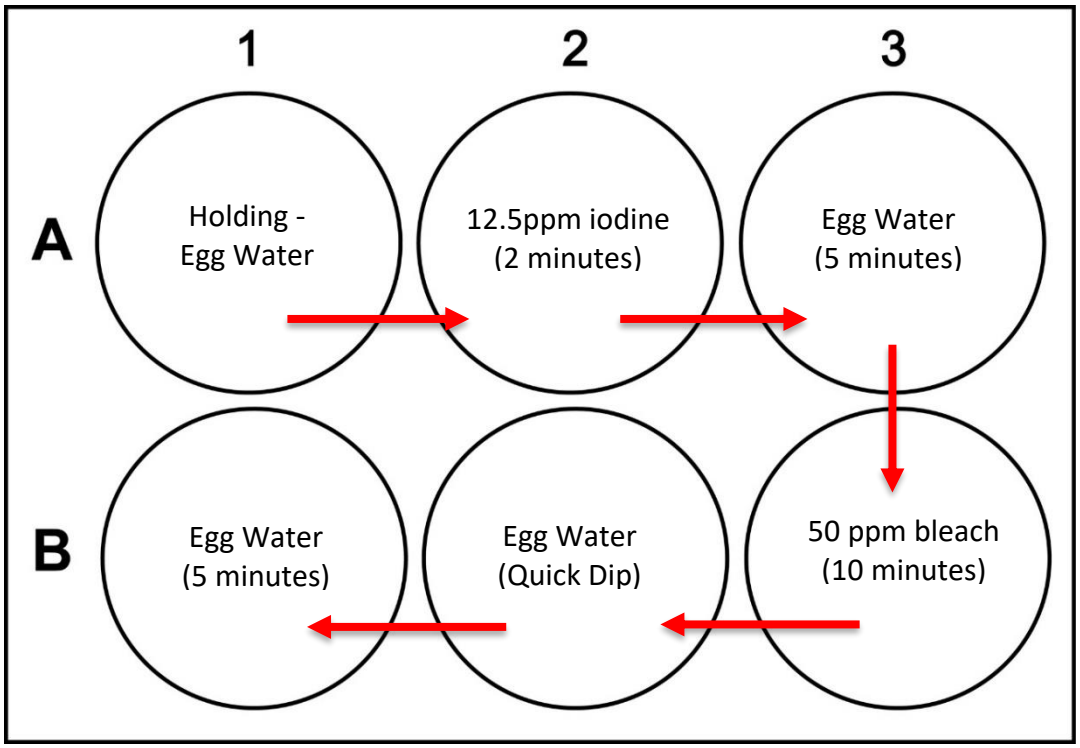
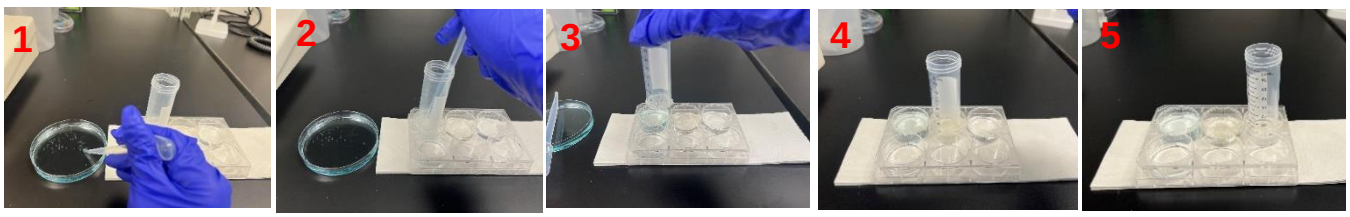
Treatment Outline:

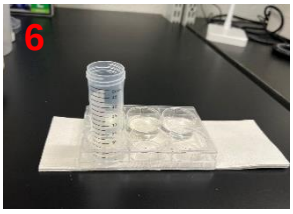


Procedure:

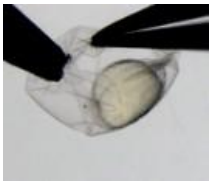
1. Prepare the following solutions in a 50mL conical tube (use within 30 minutes):
   • 50 ppm bleach solution:10µL of 5% reagent grade bleach in 10mL of egg water.
   • 12.5 ppm Iodine solution: 12.5µL of 10% Ovadine (1% available iodine) in 10 mL of egg water.
2. In the 6-well plate, add the solutions to each well (see above plate outline):
   - A1, A3, B1, B2: egg water
   - A2: 12.5ppm iodine solution
   - B3: bleach solution
3. Place the straining cylinder in the first well (A1) and move/pipette the eggs into it from the petri dish (pictures 1&2).
4. Move the straining cylinder to the iodine solution well (A2) and leave it for 2 minutes (picture 3&4).
5. Move the straining cylinder to the next well (A3) with egg water for 5 minutes (picture 5).



6. Move the straining cylinder to the bleach solution in well (B3) below and leave it for 10 minutes.



7. Move the straining cylinder into the next well (B2) of egg water for a quick dip and then into the final well (B1) of egg water for a 5-minute wash (picture 6).
8. Move the eggs from the straining cylinder into a new petri dish by using the egg water squirt bottle.
9. Place the petri dish with the eggs into the Main Facility incubator (RB-20).
10. Check dishes and remove any non-viable eggs at least once at 1- 2 dpf.
11. At 3 days post-fertilization, manually remove the chorion with forceps from any embryos that did not hatch. Use two forceps to grasp the chorion and gently tear it away under a bright field microscope.



    a. Remove chorion debris with a pipette.
    b. Replace 25-50% of the egg water for a water change.
12. Record the number of fish on the fish usage log for the appropriate protocol.
13. At 5dpf, move larvae into the tank with the 300-micron screen baffle and place them on the nursery row on the main system.

REFERENCES:

Zebrafish Embryo Disinfection with Povidone–Iodine: Evaluating an Alternative to Chlorine Bleach. *Zebrafish* 2016 Jul Vol 13.

Zebrafish International Resource Center, Embryo Surface Sanitation: https://zebrafish.org/wiki/_media/protocols/ess/customer_egg_bleaching.pdf