CZAR - Embryo Ovadine Protocol

TITLE: Embryo **Ovadine** Protocol

PURPOSE: To describe the procedures for Ovadine treating embryos prior to transporting between various fish facilities on campus in order to reduce the risk of spreading pathogens between systems. All embryos going into the CZAR nursery incubator should either be bleached using the bleaching protocol, or Ovadine treated using this protocol. The bleaching protocol followed by the Ovadine protocol must be used for embryos coming out of the CZAR QX room.

All users must read the "Sanitizing Zebrafish Embryos" policy prior to putting embryos into the nursery incubator.

Ovadine Sanitation Protocol – can be used on embryos 6-48 hpf. This protocol must be used after the bleaching protocol on all embryos prior to exiting the QX.

Materials needed:

- Embryos should be ~6-48 hpf.
- 1% Ovadine (in 1 ml cryovials in each injection room, replaced WEEKLY, please check dates).
- 3 mating tanks with RO/DI water (right hand tap) filled to about 2/3 full as rinse baths.
- One tea strainer
- One 50 ml tube for every 100 embryos to be sanitized.
- E3 (without methylene blue)
- E3 with methylene blue
- A timer set to 2 minutes
- Transfer pipets

Protocol:

1. Dilute 50 µL Ovadine into 40 ml of E3 (w/o methylene blue) in each 50 ml tube.
2. Transfer ~100 embryos into tea strainer, rinse with running RO, blot off excess water from bottom of tea strainer on a paper towel, then use 35-40 ml of 12.5 ppm Ovadine solution to rinse the embryos into a petri dish.
    a. If embryos are stuck in the strainer when all the solution has been used, use a transfer pipet to suck up solution from the petri dish to wash them into the dish.
3. Swirl the embryos in the dish to make sure they are all covered in Ovadine solution.
4. Start the 2-minute timer.
5. When time is up, pour the embryos into the tea strainer, rinse into the strainer with E3 (no methylene blue).
6. Dip the embryos into each of the 3 – RO rinse baths in succession, dipping up and down 2-3 times per bath.
7. Rinse embryos with E3+methylene blue into a clean petri dish. Label dish.
8. Repeat for each batch of 100 embryos, using a new tube of Ovadine solution for each batch.
9. Pour out RO rinse baths and refill with fresh RO after 200-300 embryos have been processed.
10. Check embryos 24 hours later and pick out any dead or mutant embryos. Count embryos into plates of 25 for submission to the nursery or to be raised in the QX.