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Exposure of snails to miracidia

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Introduction
Schistosome eggs hatch readily when placed in water, depending upon the tissues from which they are obtained. Concentrated egg pellets, when diluted in copious amounts of water, can yield large numbers of miracidia for experimental infection of snails.

Video: Schistosomiasis Resource Center Video SOP | Collecting *S. mansoni* (NMRI) miracidia - YouTube

Equipment
Waring blender (Eberbach E8580.00)
Centrifuge
Dissecting microscope
Darkened side-arm flask or Erlenmeyer flask

Materials and reagents
0.85% NaCl solution, pre-chilled (saline)
50 mL conical centrifuge tubes
Pasteur pipettes
Fine-tipped (drawn) Pasteur pipettes
Artificial Pond H_2O (water) https://www.afbr-bri.org/schistosomiasis/standard-operating-procedures/artificial-pond-water/
Petri dish or Syracuse watch glass

Eggs of *S. mansoni*, *S. haematobium*, and *S. japonicum* hatch readily if placed in water. Allowing them to hatch in a petri dish works well, but it may be difficult to clean up the preparation well enough to obtain miracidia easily. A side-arm flask is beneficial for obtaining a miracidial suspension relatively free of tissue debris. **Keep tissues and eggs cold during the procedure.** Cold temperatures will reduce egg-hatching activity.

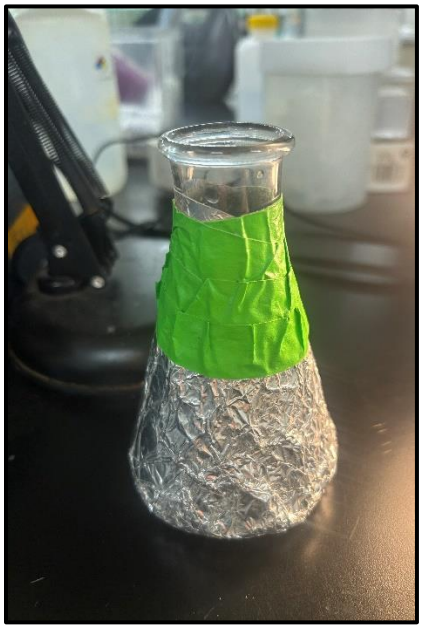
Procedure
1. Mince tissue containing eggs for 30 seconds in saline, pre-chilled, using a low-speed setting on a Waring blender.
2. Centrifuge homogenate for 5 minutes at 100 x g set at 4°C
3. Gently pour off the supernatant. Gently rinse the layer of blood from the top of the pellet using saline in a squirt bottle without loosening the pellet.
4. Pour a small amount of water into the centrifuge tube and shake vigorously to suspend the egg pellet.
5. Place the egg suspension in a 1-liter darkened side-arm flask in which the side-arm is not darkened (pictured) Alternatively, suspension can be added to an Erlenmeyer flask covered with

aluminum foil. Fill the flask to the top with water. Place the stopper on top if using the side arm flask.

6. Place the flask into a container with enough tap water (pre-warmed to 26ºC) to submerge the bottom of the flask
7. Shine a light on the exposed side-arm, taking care not to overheat the side arm. Since miracidia are phototropic, they will begin to collect in the water of the side-arm in 20-30 minutes, at which point they can be removed with a Pasteur pipette.
8. In the meantime, place snails to be exposed into a beaker with enough water to cover the snails. For exposing a large number of snails, we place 50 snails in ~50 mL of pond water in a 250 mL beaker. Individual snail exposures can be done using a 24 well plate.
9. Withdraw a pipette full of miracidial suspension and place it in a petri dish with additional water. The volume of miracidial suspension and water dilution will depend on the density of the miracidia and comfort with counting.
10. Add water back into the side-arm flask to keep the volume constant.
11. With a drawn Pasteur pipette and using a dissecting microscope, withdraw the appropriate number of miracidia* and place into the beaker of snails.
12. Incubate snails with miracidia for at least 2 hours at room temperature to ensure miracidial penetration. Ensure all snails remain in the water.

Comments

Large numbers of miracidia can be obtained from the livers of mice infected for 6-7 weeks with 150-200 *S. mansoni* cercariae per mouse, or 45-50 *S. japonicum* per mouse. Miracidia can be obtained from eggs from feces of infected mammals. Investigators have observed tissue-dependent hatch rates for feces-, liver-, and gut-derived eggs. Hamsters infected for 3 ½ – 4 months with ~350 *S. haematobium* cercariae will have eggs in the liver and the gut.



Using a darkened side arm flask assures a cleaner miracidial preparation than one from which miracidia are not selectively attracted to light.

*The target number of miracidia for exposure is 5-7 per snail.

References

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