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Exposure of snails to miracidia

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

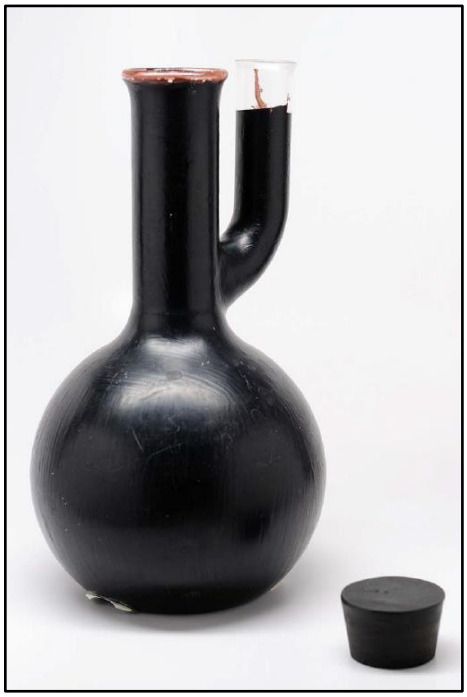
Equipment
Waring blender
Centrifuge
Dissecting microscope
Darkened side-arm flask (with side arm not darkened)
Hand-held counter

Materials and reagents
50 ml conical centrifuge tubes
Pasteur pipettes
Fine-tipped (drawn) Pasteur pipettes
Artificial Pond H_2O

Eggs of *S. mansoni*, *S. haematobium*, and *S. japonicum* hatch readily if placed in artificial pond 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 the miracidia easily. A side-arm flask is beneficial for obtaining a miracidial suspension relatively free from tissue debris.

Procedure
1. Mince tissue containing eggs for 30 seconds in 0.85% NaCl, using a low-speed setting on a Waring blender.
2. Centrifuge homogenate for 5 minutes at 100 x g.
3. Pour off the supernatant.
4. Place the egg suspension in a 1-liter darkened side arm flask in which the side arm is not darkened (pictured) and fill the flask with pond water.
5. Place the side arm flask into a container filled with water pre-warmed to 26ºC.
6. Shine a light on the exposed side arm, taking care not to overheat the side arm. Since the 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 by a Pasteur pipette.
7. In the meantime, place snails to be infected into a beaker with enough water to cover all snails.

8. Withdraw a pipette full of miracidial suspension and place it in a petri dish with additional pond water.
9. Add water back into the side-arm flask to keep the volume constant.
10. With a drawn Pasteur pipette and using a dissecting microscope, withdraw the appropriate number of miracidia* and place into the beaker of snails.
11. Incubate snails with miracidia for at least 2 hours to ensure miracidial penetration. Ensure all snails remain in the water for the duration.



Comments

Large numbers of miracidia can be obtained from the livers of mice infected for 7 weeks with 150-200 *S. mansoni* cercariae per mouse, or 20-30 *S. japonicum* per mouse. Miracidia can be obtained from eggs from feces of infected mammals, but they usually do not hatch as quickly in water as do those from tissues (liver and intestines). Hamsters infected for 3 ½ – 4 months with *S. haematobium* 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 infection is 5 per snail. At this stage we have no control over the miracidia movement. Some snails may get infected with 5 miracidia which is ideal, and some may get more than 5 which could be fatal for the snail. Some may get less than 5, which may take longer to become positive for cercariae.

References

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3. Tucker, M. S., Karunaratne, L. B., Lewis, F. A., Frietas, T. C., and Liang, Y-S. 2013. Schistosomiasis, in Current Protocols in Immunology 19.1.1-19.1.57, John Wiley and Sons, Inc., (R. Coico, Ed). Published online November 2013 in Wiley Online Library (wileyonlinelibrary.com). doi: 10.1002/0471142735.im1901s103.

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