

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 H2O (water)

Eggs of *S. mansoni*, *S. haematobium*, and *S. japonicum* hatch readily if placed in aged tap 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. If one has a side-arm flask, the following procedure works well in obtaining a miracidial suspension relatively free from tissue debris.

Procedure

- Mince tissue containing eggs for 30 seconds in 0.85% NaCl, using a low speed setting on a Waring blender.
- Centrifuge homogenate for 5 minutes at 100 x g.
- Pour off the supernate and resuspend the pellet in water that has been pre-warmed to 26°C.
- 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 water.
- 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.
- Withdraw a pipette full of miracidial suspension, and place it in a petri dish with additional water.
- Add water back into the side-arm flask to keep the volume constant.
- With a drawn Pasteur pipette and using a dissecting microscope, withdraw the appropriate number of miracidia and place with the snails in a small volume of water.
- Incubate snails with miracidia for at least 2 hours to ensure miracidial penetration.

**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.

References

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