



BIOMEDICAL RESEARCH INSTITUTE

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Percutaneous exposure of mice to *Schistosoma mansoni*

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Introduction

Percutaneous exposure of mice to *S. mansoni* via the tail offers significant advantages over other means of infection. Skin penetration is the natural route of cercarial infection, and there is no need to anesthetize the mice, which are secured by restrainers. An investigator can obtain a relative estimate of the percentage of cercarial penetration by counting remaining cercariae after the mouse tail has been removed, and the mouse returned to its proper cage.

Materials

Mouse restrainers (Fischer Scientific, Broome-Style Rodent Restrainer)
12 x 75 mm plastic test tubes (5cc)
Test tube racks to hold 5cc tubes of cercarial suspensions
Mouse exposure apparatus- two "egg crate" ceiling light diffuser panels and spacers
Micropipette (Eppendorf 200 μ l+) and tips
Adhesive tape (Johnson and Johnson zonas porous tape)
4" x 4" gauze pads
Kim wipes
Artificial pond water

Procedure

1. Prepare the animal procedure room by cutting two pieces of 3" adhesive tape for each mouse. Place small amounts of Kim wipes inside of the restrainers to prevent urine and fecal contamination of the cercarial suspensions.
2. Pipette a pre-calculated* number of cercariae into the 12 x 75 mm tubes, then fill the tube with pond water to within approximately 10 mm of the rim.
3. Place tubes spaced apart in the bottom rack, then place the spacers in the corners and add top rack (see image below).
4. Pick up a mouse by its tail and gently, but quickly, pull it backward into the restrainer (Fig. 1 left) until it is completely inside the restrainer and the tail is entirely through the opening in the bottom.
5. Hold the tail securely with pinched fingers from one hand while tightening the lid of the restrainer with the other hand. *Be careful not to cramp the mouse in the restrainer but secure it sufficiently that it cannot escape.*
6. With the mouse in the restrainer, attach two small pieces of adhesive tape to anchor the tail in place.
7. Wipe the tail clean with a gauze pad moistened with pond water.

8. Insert the tail into the cercarial suspension, resting the restrainer on the top of the rack (Fig. 1 right).
9. After 1 hour remove the mouse, release it from the restrainer and return it to its cage.
10. Collect 5 random tubes from the mouse exposure apparatus and place them on a tube rack in the blue transport box.
11. Combine all 5 samples into a gridded watch glass and add a small volume of iodine to fix (kill) and stain the cercariae for visualization. Using a microscope and counter, count the number of cercariae quantify the penetration percentage.

$$\text{Penetration percentage} = \frac{(\text{Mean Cercarial Infection} \times 5) - \text{Counted Cercariae}}{(\text{Mean Cercarial Infection} \times 5)}$$

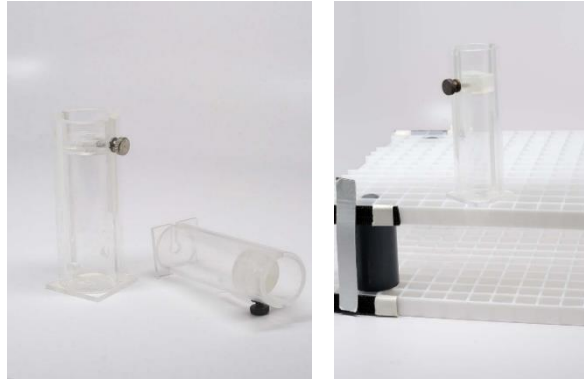


Figure 1: Left: Broome-type mouse. Variations of these can be purchased commercially or can be engineered from plastics companies. Restrainers with diameter openings of about 25mm can restrain mice up to 20 gr, whereas larger ones (up to 30mm diameter openings) are needed for larger mice. Right: materials for mouse tail exposure to *Schistosoma* cercariae. Shown are a base and upper level of fluorescent light panels (available in standard hardware stores), separated by spacers and anchored with Velcro strips. The height is adjusted to 75mm, to accommodate 75 x 20mm test tubes (not shown). A single mouse will be restrained in the Broome-type restrainer, with its tail extending into the test tube.

Comments

*Refer to the Counting Cercariae protocol for pre-calculated number of cercariae. We use infection rates of 180-200 cercariae per mouse. This infection rate is ideal for life cycle propagation. Chronic infections (≥ 8 wk) require fewer cercariae (Tucker et al. 2013).

One can count the number of non-penetrating cercariae by emptying the contents of the test tube into a counting dish after the mouse tail has been removed. One can usually obtain a reasonable estimate by counting the contents from 4-5 randomly chosen tubes. Some cercariae may be intact (with a tail), whereas detached bodies may also be apparent. Cercarial tails will be numerous, but a count of the tails is an unreliable indicator of penetration success, since many of the detached tails may be caught up in the hair or tail skin of the mouse. Under the best of conditions, one can expect a penetration rate of at least 95%, or higher. A penetration rate of less than 90% may indicate a problem in the cercarial pool, or from some other source.

References

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