



BIOMEDICAL RESEARCH INSTITUTE

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Isolating *Schistosoma* spp. eggs from murine liver and gut

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Introduction

Schistosome eggs are collected for experimental use primarily from either livers or intestinal tissue of infected animal hosts. This procedure describes the isolation of eggs from the livers of *S. mansoni*-infected mice. To obtain the cleanest preparation of eggs, the mice should first be perfused of adult worms lest the final egg preparation contain unwanted adult worm fragments.

Equipment

Spray apparatus (Deck sprayer, 2-gallon, pump-type, typically found in hardware stores)
Stainless steel sieves of decreasing pore sizes (Newark Wire Cloth Company; mesh openings of 420 μm , 180 μm , 105 μm , and 45 μm)
Waring blender, with variable speed control
Glass petri dishes (100 mm diameter) with flat bottoms
Pasteur pipettes
Light box
15 mL conical test tubes

Materials and reagents

1.2% NaCl (2 liters)
S. mansoni-infected mice

Procedure

1. Dissect the livers from mice that have been perfused of adult worms.
2. Place the livers in cold 1.2% NaCl overnight.
3. Homogenize in a Waring blender for 30 seconds until liver is a smooth consistency.
4. Place the homogenate in the top tier (420 μm) of stainless-steel sieves and allow it to pass through the tier of stacked sieves, from the largest pore size on top to the smallest pore size on the bottom tier while rinsing the tissue continuously on the top sieve with a spray apparatus containing 1.2% NaCl. *Agitate the sieves throughout the entire process to ensure that most of the eggs will pass through to the lowest sieve.*
5. For best results, re-homogenize the homogenate trapped on the top sieve in the Waring blender and pass the homogenate through the sieves again, using the technique described.
6. Remove the upper three sieves; the fluid remaining in the lowest sieve (45 μm pore size) will contain the eggs.
7. Pour the suspension into a glass petri dish and add 1.2% cold NaCl so that it is about $\frac{1}{2}$ full. The egg suspension will contain eggs of several stages of maturation. *Cold NaCl will keep most of the eggs from hatching into miracidia, keep eggs on ice in between swirling.* To enrich the

population for mature eggs, gently swirl the dish over a light box (for better visibility). Mature eggs will concentrate in the center of the vortex. These can be withdrawn with a Pasteur pipette and placed in a 15 ml test tube on ice. Keeping the volume of the egg suspension in the petri dish constant by adding fresh cold 1.2% NaCl as needed, continue to swirl the dish and collect eggs from the center until no more can be seen concentrating in the center. After 3-4 complete cycles, the resulting egg population will be highly enriched in mature eggs.

8. Centrifuge eggs at 5 minutes at 100 x g. Pour off supernatant and freeze eggs as a dry pellet.

Comments

The greatest yield of eggs from mouse livers can be obtained from mice that were exposed to approximately 200 cercariae, 7-8 weeks post-infection (> 25,000 mature eggs per liver).

Harvesting eggs from the intestinal walls of the mice is possible, but only after the intestines are cleaned of feces and rinsed in copious amounts of 1.2% NaCl. Mice that were exposed to approximately 200 cercariae will yield ~10,000 mature eggs per mouse from the gut at 7-8 weeks post-infection.

References

1. 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., Nov 2013 in Wiley Online Library (wileyonlinelibrary.com). doi: 10.1002/0471142735.im1901s103.