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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 heavily infected animal hosts. This procedure describes the isolation of eggs from the livers of *S. mansoni*-infected mice. In order to obtain the cleanest preparation of eggs, the mice should first be perfused of adult worms, or the final egg preparation may contain many unwanted adult worm fragments.

Equipment

Spray apparatus (2-gallon, pump-type, typically found in hardware stores)
Stainless steel sieves of decreasing pore sizes (Newark Wire Cloth; mesh openings of 420 µm, 180 µm, 105 µm, and 45 µm)
Dissecting instruments
Waring blender, with variable speed control
Glass petri dishes (100 mm diameter) with flat bottoms
Falcon #2340 cell strainer, 40 µm nylon
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 previously been perfused of adult worms.
2. Place the livers in cold 1.2% NaCl
3. Mince the livers with scissors, then homogenize in a Waring blender, first for 10 seconds at low speed, then at intermediate and, finally, at high speed for an equal amount of time.
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 ½ full. The egg suspension will contain eggs of several stages of maturation. *Cold NaCl will keep most of the eggs from hatching into miracidia.*
8. To enrich the population for mature eggs, gently swirl the dish over a light box (for better visibility).
9. 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.
10. Using a Falcon (#2340) 40 µm nylon cell strainer (or similar device of that pore size) to concentrate the mature eggs collected with the Pasteur pipette significantly facilitates this procedure. Place the cell strainer in a second glass petri dish ½ full of cold 1.2% NaCl next to the plate you are swirling, and place each pipette-full of mature eggs into the strainer until no more eggs can be seen in the center of the petri dish.
11. Discard the waste contents (immature eggs, small pieces of liver, etc.) of the petri dish, add the collected suspension of enriched eggs from the strainer to its petri dish and repeat this procedure, using a fresh petri dish of 1.2% NaCl and the cleaned out strainer for the next cycle. *Use the pipette to (gently) rinse remaining eggs from the strainer into its petri dish, and continue to use the same strainer for all cycles.*
12. After 3-4 complete cycles, the resulting egg population will be highly enriched in mature eggs. Two photos show the "before" and "after" stages of egg purification by this method.
13. After the last cycle, gently pipette the clean, mature eggs from the strainer (while tipping it slightly onto its side to facilitate pipetting the eggs) into a 15ml conical centrifuge tube, on ice. Fill to the top with cold 1.2% NaCl and allow eggs to settle to the bottom of the tube. *Alternatively, one may gently rinse the strainer of eggs with a small volume (>10 ml) of cold 1.2% NaCl into a clean petri dish and then pipette into the 15 ml tube, rinsing the petri dish with another 5 ml of NaCl and adding this to the tube.*

Comments and Recommendations

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. With practice, one can obtain at least 20,000 mature eggs/liver at this level of infection.

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/mouse from the gut at 7-8 weeks post-infection.

References

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.