

## ***Schistosoma japonicum* cercariae collection from patent *Oncomelania* spp**

*Authors: Laksiri Karunaratne, MS, Fred Lewis PhD, Sarah Li, Yung-san Liang, PhD and Mei-Shei Su*

### **Equipment**

Dissecting microscope

### **Materials and Reagents**

Petri dishes

Pasteur pipettes

Artificial Pond Water (water)

Fine forceps or small hypodermic needle (e.g., 25 gauge) placed on a syringe

Hairloop or inoculating loop

### **Introduction**

- Primary sporocysts are difficult to see in the head-foot tissues of exposed *Oncomelania hupensis* ssp. snails; however, at about 3 ½ months post-exposure, the developing secondary sporocysts are visible through the shell (apex area), provided that the shell is not too eroded.
- Cercariae can be obtained by two methods: *crushing* or *shedding*. Currently, the SRC is mainly utilizing a shedding protocol. We have observed that the same patent *O. hupensis* snails can be shed at least six times over the course of 4-5 months. This shedding protocol conserves the patent *Oncomelania* population over time since it does not require that the snail be sacrificed in order to obtain cercariae.
- Time point: at about **3 ½ months** post- exposure (to *S.japonicum* miracidia), it is possible to obtain cercariae from *Oncomelania hupensis*.

### **Procedure: Shedding *Oncomelania* spp. for cercariae collection**

- For snails that are 3 ½ or more months post-exposure, single snails can be placed into the well of a 24well plate and covered with water. Put the plate under a light for a minimum of 2hr. Cercariae are liberated and can be collected from the well with a Pasteur pipette, hair loop or plastic pipette tip.

### **Procedure: Crushing *Oncomelania* spp. for cercariae collection**

- Place 3-4 snails on an inverted plastic petri dish top, and gently crush the shells by placing the bottom of the dish on top of the snails and pressing down. (The snail is killed by crushing). Add a small drop of water to the crushed snail, tease out the secondary sporocysts from the tissue, then place them in a 60 mm petri dish with water
- Using fine forceps and a small gauge needle, tease the sporocysts apart, releasing the cercariae. *The most infective (mature) cercariae will be those that swim to the top of the water and hang there.*

- With the aid of a dissecting microscope, collect the cercariae in the meniscus of a hairloop or inoculating loop. If you are performing percutaneous exposure via mouse tail, the cercariae can be placed into a 5cc tube containing water.

### Comments

To obtain *S. japonicum* cercariae in large numbers, investigators have dissected the secondary sporocysts from the tissues in order to release them. This means the snails cannot be used at a later time for cercarial production. Each positive *Oncomelania hupensis* ssp. snail may yield greater than 100 infective cercariae.

To obtain a sufficient mixed-sex population of *S. japonicum* cercariae, it is best to collect cercariae from a minimum of 10 patent snails. Collecting cercariae from less than 10 snails may not provide a good ratio of male to female cercariae, resulting in unpaired schistosomes and reduced egg production.

Mice infected with *S. japonicum* have a high tissue egg burden, due to the significant egg production from *S. japonicum* adult females. SRC technicians infect mice with 35-45 cercariae per mouse. At this infectious dose, mice can be sacrificed at 6-7WK post-infection to collect adult schistosomes; parasite eggs can be isolated from the liver and gut.

For additional technical information, contact Sarah Li at [sli@afbr-bri.com](mailto:sli@afbr-bri.com)

### References

Liang, Y-S., Bruce, J.I., and Boyd, D.A. 1987. Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles. Proceedings of the First Sino-American Symposium 1: 34-48.

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](http://wileyonlinelibrary.com)). doi: 10.1002/0471142735.im1901s103.

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](http://wileyonlinelibrary.com)). doi: 10.1002/0471142735.im1901s103.

*Schistosomiasis Resource Center, November 2016*