Schistosoma haematobium, Egyptian Strain, Exposed *Bulinus truncatus* subsp. *truncatus*

Catalog No. NR-21965

For research use only. Not for human use.

Contributor and Manufacturer:

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Product Description:

Flatworm Classification: *Schistosomatidae*, *Schistosoma*
Species: *Schistosoma haematobium*
Strain: Egyptian
Host: *Bulinus truncatus* subsp. *truncatus*
Original Source: The Egyptian strain of *Schistosoma haematobium* (*S. haematobium*) was originally isolated circa 1950 from an unknown location in Egypt. The laboratory stock of the Egyptian strain of *S. haematobium* was later mixed with an isolate that was thought to be obtained from Abrawash (Cairo) by the Naval Medical Research Unit III, in 1977. The current Egyptian strain of *S. haematobium* was produced when the 1977 stock was mixed with another Egyptian isolate obtained in the 1980s.[1] The *Bulinus truncatus* (*B. truncatus*) subsp. *truncatus* snail host was obtained from Egypt.

S. haematobium is a species of trematode worm which causes the chronic parasitic disease Schistosomiasis. Worldwide, more than 200 million people are infected and nearly 700 million are at risk, primarily in areas with poor sanitation that lack access to safe drinking water.[2]

Infection occurs through contact with larval-stage schistosomes (cercariae) that are released by freshwater snails. Upon exposure to infested water, these larvae penetrate human skin and travel through blood vessels to the liver where they mature. Mature *S. haematobium* parasites deposit eggs in the bladder. Some of these eggs are then passed through human urine into water to re-infect the snail host and continue the parasite's life cycle. Schistosome eggs that remain in the human body cause an immune response and damage to internal organs.[2]

Material Provided:

NR-21965 consists of up to 100 *B. truncatus* subsp. *truncatus* infected with the Egyptian strain of *S. haematobium* in a petri dish.[1]

Packaging/Storage:

NR-21965 is packaged in moist paper towels in a petri dish encased in bubble-wrap and shipped overnight in cardboard boxes (42.5 cm x 23 cm x 23 cm). To insure viability, snails should be placed in suitable aquaria at 26°C to 28°C. Note: Infected snails are exposed to miracidia only a few days before shipment. Laboratories should be aware and take caution of possible cercarial exposure following a 4 week prepatent period after exposure to miracidia (depending on the temperature and species). BEI Resources recommends consulting personnel in your institution's Occupational Safety and Health office for clearance to receive and maintain the infected snails.

Growth Conditions:[3,4]

Food Source:
Romaine lettuce or *Nostoc* blue-green algae grown on a layer of autoclaved mud may be used as food sources (see Appendix I for details).
Maintenance of *B. truncatus*:
Depending on the number of snails to be maintained, they may be kept in plastic trays (approximately 1.5 L volume) or larger aquaria (40 L to 110 L) in filtered tap water that has been aerated for 2 to 3 days (pH ~ 7.1). The ambient temperature should be 26°C to 28°C, and the aquaria should be under continuous illumination from a 40-watt fluorescent bulb. Note: *B. truncatus* adheres strongly to surfaces. In order to avoid damage, gently tilt the shell from right to left with a pair of forceps, then gently lift the snail to the left to remove.
Propagation of *B. truncatus*:

1. Place ten snails in plastic trays containing aerated tap water and Styrofoam (75 mm x 125 mm). Maintain trays at 26°C to 28°C under continuous illumination.
2. At the end of one week, remove egg masses from the trays and styrofoam and transfer to Petri dishes that contain aerated tap water.
3. After one to two weeks, about 20 newborn snails 0.6 mm to 0.8 mm in shell diameter are transferred with a pipette to each Petri dish that contains water, mud and blue-green algae.
4. Each week, transfer and maintain snails at a density of 25 snails per dish. After 14 days, 2 mm to 3 mm shell diameter snails may be infected with *S. haematobium* or used to initiate new cultures.

Collecting Cercariae:

1. Screen infected snails individually under a dissecting microscope in a Petri dish containing a small volume of aerated tap water for the presence of *Schistosoma* sporocysts, which appear as light colored flecks that are visible through the shell.
2. Place infected (patent) snails in a beaker where moisture is maintained but does not contain water. Let beaker stand for 15 to 30 minutes at 26°C to 28°C under ceiling illumination.
3. Rinse snails with a small volume of water. Add a second volume of water (100 mL per 100 snails). Place uncovered beaker under a 15-watt white fluorescent light, 46 cm above the beaker for 20 minutes. Decant water containing cercariae into another beaker. On average,

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300 to 500 cercariae may be obtained from each infected snail.

4. To estimate the cercarial density, stir the beaker that contains cercariae continuously with a magnetic stir bar, and remove 0.1 mL aliquots from the center of the suspension.
5. Place each aliquot in a 10-cell depression slide. Add one drop of Lugol's Iodine solution to each cell prior to counting under a dissecting microscope. Determine the cercariae density per volume. This suspension may subsequently be diluted, or concentrated by pipetting out the excess liquid.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Schistosoma haematobium*, Egyptian Strain, Exposed *Bulinus truncatus* subsp. *truncatus,* NR-21965."

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. F. A. Lewis, Personal Communication.
2. Roberts, L. and J. Janovy. Foundations of Parasitology. 5th ed. Dubuque, Iowa: Wm. C. Brown Publishers; 1996
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4. Bruce, J. I. and Y. S. Liang. "Cultivation of Schistosomes and Snails for Researchers in the United States of America and Other Countries." J. Med. Appl. Malacol. 4 (1992): 13-30.

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APPENDIX I: PREPARATION OF MUD-BASED MEDIA FOR GROWTH OF *NOSTOC* ALGAE[4]

Equipment
Autoclave
Spatula(s)

Materials
Mud or soil source
Chicken manure
Calcium carbonate (pulverized limestone)
Clay
0.06% sodium nitrate solution prepared with aged tap water
Nostoc (stock cultures can be obtained from Ward's Biological Supply, Rochester, NY)
Plastic Petri dishes (25 mm x 100 mm)
Stainless steel baking pan (250 mm x 380 mm x 75 mm)

Procedure
1. The proportions of dried mud, lime and chicken manure needed for good growth of *Nostoc* will likely vary, depending on the richness of the soil obtained. A small amount of clay may be necessary for cohesion of the mud mound that will be placed in the Petri dishes. The following describes the current proportions of each component for the soil. Trial and error will be the rule, rather than the exception, to accommodate the apparent richness (or lack thereof) of soils in different regions.
2. The soil and site chosen ideally should be one where there is considerable sedimentation (e.g. a stream bed bottom) or topsoil. Soil should be obtained where no known herbicides or pesticides have been used.
3. The mud or soil brought back to the laboratory from the field site should be strained through a series of crude screens to remove rocks and other large debris. Once it is a fine consistency, it should be completely dried before use.
4. Mix 3 kilograms of dried mud with 90 grams lime (pulverized limestone) and 30 grams dried chicken manure. To this mixture add enough tap water to make a paste. Place the mud mixture in a large stainless steel baking pan and cover with aluminum foil. The depth of the mud should be no more than about 100 mm. Autoclave for a continuous 2 hours.
5. Once the mud is autoclaved and cooled to room temperature, use a sterile spatula (spatulas should be wiped down periodically with gauze drenched in pure alcohol) and place about 40 grams of the still wet mud in the center of a Petri dish and form a smooth and solid mud mound about 15 mm high and 60 mm in diameter. If the mud has dried too much during autoclaving and needs some additional liquid to make it easier to spread, add a few mL of sterile 0.06% nitrate solution and mix thoroughly. To expedite the spreading process, one can use two curved sterile spatulas to stir a third to half of the mud in the steel pan (adding the sterile 0.06% nitrate solution as needed) before spreading it into the Petri dishes. This ensures consistency of the ingredients in the mud that is placed in each Petri dish.
6. Once the mud mounds have been formed in the Petri dishes, cover the mud mound with 0.06% nitrate solution and add about 2 mL of a suspension of *Nostoc* (in 0.06% nitrate solution) to seed the plate for new growth. Be sure not to flood the Petri dish with liquid, so that the lid does not become wet with the growth medium.
7. Cover and place under fluorescent lighting (40 watt, cool-white fluorescent) at 25°C to 27°C for 1 to 3 weeks. For best results the lights should be about 30 cm above the Petri dishes.
8. The preparation is suitable for feeding to the snails once a solid mat of the *Nostoc* has grown over the surface.