

Tel-Aviv University – Safety Unit

Standard Operating Procedure for Working with *Schistosoma* spp. in Animals

1. Health hazards

Schistosoma, commonly known as blood flukes, are parasitic flatworms responsible for a highly significant group of infections in humans termed schistosomiasis.

Schistosomiasis, also known as bilharzia, is a disease caused by the parasitic worms.

The parasites that cause schistosomiasis live in certain types of freshwater snails.

Schistosoma are dioecious, have a two host life cycle, and exhibit a high degree of sexual dimorphism.

Fertilized adult female releases eggs which are deposited in the environment in feces or urine of the definitive host (humans and other mammals).

Infection occurs when skin comes in contact with contaminated freshwater in which certain types of snails that carry the parasite are living. Freshwater becomes contaminated by *Schistosoma* eggs when infected people urinate or defecate in the water. The eggs hatch, and if the appropriate species of snails are present in the water, the parasites infect, develop and multiply inside the snails. The parasite leaves the snail and enters the water where it can survive for about 48 hours.

Schistosoma parasites can penetrate the skin of persons who come in contact with contaminated freshwater, typically when wading, swimming, bathing, or washing. Over several weeks, the parasites migrate through host tissue and develop into adult worms inside the blood vessels of the body. Once mature, the worms mate and females produce eggs. Some of these eggs travel to the bladder or intestine and are passed into the urine or stool.

Symptoms of schistosomiasis are caused not by the worms themselves but by the body's reaction to the eggs. Eggs shed by the adult worms that do not pass out of the body can become lodged in the intestine or bladder, causing inflammation or scarring. Most people have no symptoms when they are first infected. However, within days after becoming infected, they may develop a rash or itchy skin. Within 1-2 months of infection, symptoms may develop including fever, chills, cough, and muscle aches.

	<p>Without treatment, schistosomiasis can persist for years. Signs and symptoms of chronic schistosomiasis include: abdominal pain, enlarged liver, blood in the stool or blood in the urine, and problems passing urine. Chronic infection can also lead to increased risk of bladder cancer.</p> <p>Rarely, eggs are found in the brain or spinal cord and can cause seizures, paralysis, or spinal cord inflammation.</p> <p>Host range: Humans for <i>S. haematobium</i>, <i>S. mekongi</i>, <i>S. intercalatum</i> . <i>S. mansoni</i> primarily infects humans, but can infect mammals occasionally. <i>S. japonicum</i> infects humans and bovine species. Other <i>Schistosoma</i> species primarily infecting mammals only rarely infect humans.</p> <p>Mode of transmission: Transmission occurs in water contaminated with feces or urine. Free swimming cercariae directly penetrate through the skin to infect humans. There is not a direct transmission from person to person.</p> <p>Reservoir: Depending on the species, humans and animals, including cats, dogs, water buffaloes, pigs, cattle, horses, monkeys, rodents.</p> <p>Zoonosis: Reservoir animals may be responsible for indirect zoonosis, especially with <i>S. japonicum</i>.</p> <p>Vectors: Snails.</p>
<p>2. Housing and Biosafety consideration</p>	<p>ABSL-2</p>
<p>3. Training</p>	<p>Practical experience with animal care/maintenance, as well as general biosafety, is required.</p>
<p>4. Personal Protective Equipment (PPE)</p>	<p>Gloves, Eyes safety goggles, Lab coat, Disposable shoe covers and Animal handling gown.</p> <p>N-99 respirator mask covering the mouth and nose when not working in a Class II Biosafety Cabinet (BSC).</p> <p>Appropriate PPE recommended for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat.</p>

5. General Precautions for Animal Use	<p>Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity. Animals should be restrained or anesthetized during injection.</p> <p>Animals should be restrained or anesthetized during injection.</p>
6. Environmental / Ventilation Controls	<p>Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.</p>
7. Animal handling practices	<ol style="list-style-type: none"> 1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard). Handling the cages (including bedding) will be done only by the researchers. 2. Use a class II Biological Safety Cabinet at all times (especially during injection or any surgical procedure), when performing work on these animals and/or when moving animals from dirty to clean cages. 3. Infected animals shed <i>Schistosoma spp.</i> after treatment ;the schistosoma's eggs are eliminated with feces or urine, take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room. 4. Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious waste incineration. 5. All surfaces and racks that may be contaminated will be decontaminated with 0.5% bleach ASAP. 6. When changing cages, use a standard microisolator technique: <ul style="list-style-type: none"> • place the cage containing the animals, under the biological safety cabinet and transfer the animals into a clean cage. • spray the dirty cage with 0.70% ethanol, remove from the safety cabinet and place on a transfer rack . • when all cages have been changed, spray the dirty cages and rack again with 0.70% ethanol, and cover the rack. Put on a pair of new gloves and bring the rack directly to the autoclave in the dirty cage wash area. • immediately autoclave the dirty cages (1 hour at 121°C/250°F, 15psi of steam pressure). Once the autoclave cycle is completed, the cages can be emptied and the bedding disposed of in a normal fashion. <p>**In cases where the use of autoclave (within the animal facility) is not an option:</p>

	<ul style="list-style-type: none"> the cages (bedding) must be emptied inside the BSL-2 cabinet, directly to a double biohazard bags. Before closing the bags, carefully, add a small amount of water (250ml) to improve the sterilization process. <p><i>Do not close the bag completely/tightly (in order to avoid entering of steam during the sterilization process).</i></p> <ul style="list-style-type: none"> Spray the dirty bag with 0.70% ethanol or virusolve. Remove from the safety cabinet and place on a transfer rack/container. <p>Put on a pair of new gloves and bring the rack/container, directly to the collection point of your department.</p>
8.Decontamination	<p>** Decontaminate work areas with 0.5% bleach for 15 minutes. Follow with water.</p> <p>Survival outside host: Cercariae may survive in water for about 2 days.</p> <p>Disinfection: 2% glutaraldehyde, sodium hypochlorite, 70% ethanol</p>
9. Spill and Accident Procedures	<ol style="list-style-type: none"> Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure. Wearing protective clothing, gently cover the spill with absorbent material. Starting at the edges and work towards the center. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant. Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes: viscous spills require 30 minutes. Use paper towels to wipe up the spill, working from the edge to center. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves Discard absorbent material in Chemical waste bags. Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, allow to disinfect for 15-20 minutes longer, and wipe with towels. Discard all cleanup materials (soaked with disinfectant) in Chemical bag. Any contaminated PPE (pay special attention to gloves and shoe covers) in a biohazard bag. Close and secure the bags. Place bag in a second biohazard bag, secure and disinfect by autoclaving.

	<p><u>Exposure:</u></p> <p>**In case of skin contact or injection with Schistosoma spp., rinse thoroughly for 15 minutes, while pressing the area of injury to draw blood. Disinfect with of 70% ethanol solution.</p> <p>**For eye exposure, flush with water for at least 15 minutes.</p> <p>**Consult with Employee Health Center. Report incident to supervisor. Supervisor reports the accident/injury to the Biosafety Unit.</p>
10. Waste Disposal	Autoclave all waste (1 hour at 121°C/250°F, 15psi of steam pressure).
I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with Schistosoma spp. in Animals, and agree to follow these procedures.	
Name:	Title:
Signature:	Date:

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