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Schistosoma mansoni cercariae shedding

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Sarah Buddenborg¹, Geetha Sankaranarayanan¹, Magda E Lotkowska¹, Catherine McCarthy¹, Arporn Wangwiwatsin¹, Carmen L Dias Soria¹, Lisa Seymour¹, Gabriel Rinaldi¹, Matt Berriman¹

¹Wellcome Sanger Institute

Schistosoma mansoni



Sarah K Buddenborg

Wellcome Sanger Institute, University of Cambridge

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We use this protocol and it's working

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Abstract

Schistosoma mansoni cercariae can be obtained from infected *Biomphalaria glabrata* snails. Although infected snails will release (shed) cercariae constantly at low numbers, it is possible to trigger the release of larger quantities by keeping the snails in the dark for a few days before shedding.

Material obtained from these procedures can be mechanically transformed into schistosomula for further experimentation.

Materials

- ✕ MilliQ water
- ✕ 12-well Clear TC-treated Multiple Well Plates Individually Wrapped Sterile **Costar Catalog #3513**
- ✕ 24-well Clear TC-treated Multiple Well Plates Individually Wrapped Sterile **Costar Catalog #3524**
- ✕ Sterile Graduated Transfer Pipets **Fisher Scientific Catalog #13479108**
- ✕ Blunt featherweight forceps wide tip **BioQuip Catalog #4750**
- ✕ Lugol solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #32922-250ML**
- ✕ Nalgene®; PPCO Wash Bottles, 500mL **Thermo Fisher Catalog #2405-0500PK**

Equipment

Zoom stereomicroscope with diascope illumination stand		NAME
stereomicroscope		TYPE
Nikon		BRAND
SMZ800N		SKU

Lamp or other light source

1x Aquarium Water (diluted from 10X; see recipes)

10X AQUARIUM WATER

5.56g CaCl₂

12.28g MgSO₄·7H₂O

0.43g K₂SO₄

4.2g NaHCO₃

480µl FeCl₃·6H₂O (0.5g/100ml water)

Fill to 10L and store in 1L bottles

Dilute to 1x: 9L MQ water + 1L 10x

Safety warnings

! *Biomphalaria glabrata* snails are fresh water snails. In the laboratory, infected snails can be kept in the dark; however, infected snails will release small amounts of cercariae all the time, even in the dark.

Cercariae are infectious to humans. Please use proper PPE at all times, including a lab coat, waterproof over-gown, long-cuff gloves AQL ≤ 0.65 , and face shield.

- All disposable materials should be placed in biohazardous waste bins
- Glassware should be immersed in a klorsept solution of at least 50ppm for at least 2 hours, rinsed with diH₂O and then autoclaved
- Any liquids should be treated with klorsept solution of at least 50ppm for at least 2 hours
- Liquids treated with klorsept should be diluted further and disposed in the drain



Preparing snails for shedding

- 1 Place aquarium tank containing snails ≥ 4 weeks post-exposure to *S. mansoni* on a stable bench top

Note

Snails infected with one miracidium ("monomiracidium-infected") can take up 7 weeks to begin shedding. Repeat sheddings maximum of 1x/week until the 7th week post-exposure.

- 2 Using featherweight forceps, remove snails from the tank and rinse gently with 1x aquarium water in a wash bottle
 - This is critical in order eliminate false positives (snails with cercariae on them from a different shedding snail)
- 3 Place snails into labelled 12- or 24-well plates with one snail per well



Shedding snails

- 4 Place cover on plate and place snails under LED or fluorescent lighting for up to 2 hours to trigger release of cercariae from the infected snails.

Note

IMPORTANT - While shedding, cercariae are in high density and the water is highly hazardous!

- 5 Add enough 1x aquarium water to each well so that they are covered
- 6 Check snails for shedding by viewing each well under a zoom stereomicroscope with diasopic illumination stand

Collecting cercariae



- 7 Collect water containing cercariae every 30 minutes (alternatively, you can just collect cercariae at the end of 2 hrs shedding; this will give you the most concentrated cercariae suspension) using a transfer pipet and top up wells with new 1x aquarium water
 - If needed (i.e. for monomiracidium-infected snails where separate sexes are needed), keep cercariae from each snail separate and use new transfer pipet for each snail
- 8 Dispose of snails that did not shed by putting them in a container klorsept (See "Guidelines and Warnings") or place in new tank if you'd like to re-shed after an additional week.
- 9 Return shedding snails to a new tank with food and appropriate labels – make sure their air stones are under the water level and bubbling properly (See protocol "Snail husbandry for maintaining the *Schistosoma mansoni* life cycle")

Note

Snails should not be re-shed for one week minimum

Protocol references

Paul F. Basch. Cultivation of *Schistosoma mansoni* In vitro. I. Establishment of Cultures from Cercariae and Development until Pairing

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