Apr 07, 2023

Schistosoma mansoni cercariae transformation (with needle)

DOI
dx.doi.org/10.17504/protocols.io.x54v9d4wqg3e/v1

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Schistosoma mansoni

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Protocol Citation: Sarah K Buddenborg, Geetha Sankaranarayanan, Magda E Lotkowska, Arporn Wangwiwatsin, Carmen L Dias Soria, Anna V Protasio, Gabriel Rinaldi, Matt Berriman 2023. Schistosoma mansoni cercariae transformation (with needle). **protocols.io https://dx.doi.org/10.17504/protocols.io.x54v9d4wqg3e/v1**

Protocol status: Working
We use this protocol and it's working

Created: March 31, 2023

Last Modified: April 07, 2023

Protocol Integer ID: 79822

Keywords: schistosoma mansoni cercariae transformation, becoming schistosomula, schistosoma mansoni cercariae, schistosomula, called schistosomula, schistosoma, transformation of cercariae, mansoni cercariae transformation, loss of cercariae, number of cercariae, cercariae, epidermis to the dermi, mansoni cercariae, epidermis, triturating cercariae, host skin, first intramammalian stage, somule, eliminating tail, dermi, lymphatic vessel, somules in somule media, vasculature

Funders Acknowledgements:
Wellcome Trust
Grant ID: 206194
Wellcome Trust
Grant ID: 098051

Abstract

Free-living aquatic S. mansoni cercariae transform into the first intramammalian stage,called schistosomula or somules, by burrowing in the host skin. Upon contact, cercariae begin to enter the skin and lose their tails, becoming schistosomula. Somules migrate through the epidermis to the dermis to find a small venule or lymphatic vessel to enter the vasculature.

Transformation of cercariae to schistosomula can be mimicked in the laboratory by triturating cercariae to remove tails, eliminating tails in a percoll gradient, and then culturing the somules in somule media. This method is used when the number of cercariae is high as the percoll gradient results in loss of cercariae.

Somules can be cultured for several weeks with regular media changes.

Guidelines

Media changes and opening of transformed somules to take place in tissue culture hood using sterile techniques

Materials

- DMEM high glucose GlutaMAX **Gibco - Thermo Fisher Scientific** **Catalog #**31966021
- Lactalbumin Hydrolysate, powder (extra soluble) **Thermo Fisher** **Catalog #**11800042
- Hypoxanthine **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**H9636-1G
- Serotonin Hydrochloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**H9523-25MG
- Insulin solution from bovine pancreas **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**I0516-5ML
- 3′5-Triiodo-L-thyronine sodium salt **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T6397
- MEM Vitamin Solution (100×) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**M6895
- Schneiders Insect Medium **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S0146
- HEPES **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**Sigma H0887
- Fetal Bovine Serum **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**F4135
- Antibiotic-Antimycotic (100X) **Thermo Fisher Scientific** **Catalog #**15240062
- Dulbecco's Phosphate Buffered Saline 10X **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**D1283
- MilliQ water
- Sterile Graduated Transfer Pipets **Fisher Scientific** **Catalog #**13479108
- Falcon 15 mL Conical Centrifuge Tubes **Fisher Scientific** **Catalog #**10773501
- Falcon 50mL Conical Centrifuge Tubes **Fisher Scientific** **Catalog #**14-959-49A
- Nun Non-Treated 6-well plate **Thermo Scientific** **Catalog #**10396482
- 1000 mL Vacuum Filter/Storage Bottle System 0.22 µm Pore 54.5cm² PES Membrane Sterile 12 **Corning** **Catalog #**431098
- Percoll **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P1644-500ML
- Micro-Emulsifying Needles 22g x 2-7/8 With Bar **Catalog #**7975
- Laboratory pipetting needle with 90° blunt ends 22G L 1 1/2 in. **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**CAD7931-12EA
- BD PlastiPak Syringe with Luer Lock **Fisher Scientific** **Catalog #**15544835
- 5ml syringe luer lock **greiner bio-one** **Catalog #**SYR5LL
- 20ml syringe luer lock **greiner bio-one** **Catalog #**SYR20LL
- Corning Cell Culture Treated Flasks with Vent Cap **Fisher Scientific** **Catalog #**10288990
- 3ml Sterile Graduated Transfer Pipets individually wrapped **Fisher Scientific** **Catalog #**13469108

Lamp or other light source
Chilling benchtop centrifuge with 15ml swing bucket rotor

Incubator at 37°C and 5% CO2
Reciprocating water bath
Class 2 Microbiological Safety Cabinet

1X DPBS + 2% ANTI-ANTI

50ml 10x DPBS
10ml 100x Antibiotic-Antimycotic (-20°C)
Fill to 500ml in vacuum filter unit with MilliQ water
Sterilise media with vacuum filter unit and store at 4°C. Use within 2 weeks.

SOMULE MEDIUM – BASCH MODIFIED MEDIUM (BMM)

1. Mix the following reagents:

	A	B	C	D	E	F
	Reagent	**1L**	**500ml**	**250ml**	**[working]**	**Storage**
	1x DMEM high glucose	810.5 ml	405.25 ml	202.625 ml	1x	4°C
	1g/L Lactalbumin hydrolysate	1g	0.5g	0.25g	1g/L	4°C
	1mM Hypoxanthine	500µl	250µl	125µl	0.5µM	-20°C
	1mM Serotonin	1ml	500µl	250µl	1µM	-20°C
	Insulin	1ml	500µl	250µl	8 µg/ml	4°C
	1mM Hydrocortisone	1ml	500µl	250µl	1 µM	-20°C
	0.2mM Triiodo-L-thyronine	1ml	500µl	250µl	0.2 µM	-20°C
	100x MEM Vitamins	5ml	2.5ml	1.25ml	1x	4°C
	1x Schneider's medium	50ml	25ml	12.5ml	5%	4°C
	1M HEPES	10ml	5ml	2.5ml	10mM	4°C
	1x Fetal calf serum inactivated	100ml	50ml	25ml	10%	-20°C
	100x PSF (add just before use)	20ml	10ml	5ml	2%	-20°C

2. Sterilise media with vacuum filter unit and store at 4°C. Use within 2 weeks.

SOMULE WASH

5ml 1M Hepes
10ml antibiotic-antimycotic
To 500ml with 1x DMEM in vacuum filter unit
Sterilize media with vacuum filter unit and store at 4°C for up to 2 weeks

Percoll Gradient - 15ml

6.5ml percoll (shake before use)
1.2ml 1M NaCl
2.5ml Somule Wash
Keep percoll solutions on ice until use
Invert the mixture several times to establish a good percoll gradient
Avoid bubbles in the percoll (remove any if found)

SEROTONIN STOCK 80mM (17mg/ml)

25mg serotonin hydrochloride (Sigma H9523-25MG) (4°C)
1.47ml H2O
Vortex well and store at -20°C in 350µl aliquots

1mM SEROTONIN

312.5µl of 80mM stock solution
24.6875ml NFW
Filter sterilize and store at -20°C in 1ml aliquots

3,3'5-TRIIODO-L-THYRONINE STOCK 10mM

100mg T3 (Sigma T6397-100MG)
14.86ml 0.2N NaOH
Vortex well and store at -20°C in 2ml aliquots

0.2mM 3,3',5-TRIIODO-L-THYRONINE

5ml of 10mM stock solution
20ml NFW
Filter sterilize and store at -20°C in 1ml aliquots

HYPOXANTHINE STOCK 368mM (50mg/ml)

1g hypoxanthine (Sigma H9636-1G)

20ml 2:1 formic acid:H2O
Vortex well and store at -20°C in 1ml aliquots

1mM HYPOXANTHINE

34µl of 368mM stock solution
12.466ml sterile medium
Filter sterilize and store at -20°C in 500µl aliquots

HYDROCORTISONE STOCK 2.75mM (50µg/ml)

1mg hydrocortisone (H0135-1MG) (RT)
1ml absolute ethanol
Gently swirl to dissolve
19ml sterile medium
Swirl to mix and store in 9.5ml aliquots at -20°C

1mM HYDROCORTISONE

9.058ml of 2.76mM stock solution
15.942ml sterile medium
Filter sterilize and store at -20°C in 1ml aliquots

Troubleshooting

Safety warnings

Cercariae are infectious to humans. Please use proper PPE at all times, including a lab coat, waterproof over-gown, long-cuff gloves AQL <=1.5, 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 diH_2O 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

Schistosomula in suspension are not a risk to humans UNLESS they are injected directly into the blood stream.

Before start

Place 1 sterile 15ml falcon tube on ice per snail to be shed
Pre-chill benchtop centrifuge to 4°C
Prepare 1x DPBS+2% anti-anti and place in 37°C reciprocating water bath (see recipes in "MATERIALS" section)
Prepare somule media and place in 37°C reciprocating water bath (see recipes in "MATERIALS" section)
Prepare somule wash and place in 37°C reciprocating water bath (see recipes in "MATERIALS" section)

Cercariae collection

30m

1 Shed cercariae from snails in a 6-well plate (see protocol "Schistosoma mansoni cercariae shedding"). The snails can be shed for up to 2 hrs by collecting cercariae and replacing with fresh water every 30 min

2 Using a sterile transfer pipette, dispense cercariae into an autoclaved 50ml beaker

3 Fill the beaker containing cercariae to 45-50ml with diH_2O and incubate for 30m
00:30:00 On ice

4 Carefully transfer the cercariae to a 50ml Falcon tube and centrifuge at 20m
500 x g, 4°C, 00:20:00

5

Note

IMPORTANT. All the following steps are carried out in the tissue culture biosafety cabinet. Keep a beaker containing 70% ethanol in the cabinet and before discarding any aspirating pipette or serological pipette aspirate ethanol to kill any contaminating cercariae in the pipette.

Discard the supernatant and add 5ml 1x PBS + 2% Anti-Anti to the pellet. Gently resuspend by flicking the tube (avoid using pipette because the cercariae are quite sticky and get lost)

6 Add 45ml 1x PBS + 2% Anti-Anti and centrifuge at 500 x g, 4°C, 00:10:00 10m

7 Repeat this steps 6-7 once more with 1x PBS + 2% Anti-Anti and then twice with somule wash

8 During the centrifugations in Steps 7 and 8, prepare 2 percoll gradient tubes (2 tube for less than ~150K cercs, 4 tubes every ~150K cercs)

9 Following centrifugation of the cercariae, remove the supernatant and add 5ml of somule wash per percoll gradient you will use

10 Vortex for 1 min at max speed. This will start to break off the tails.

Cercariae tail removal with micro-emulsifying needle trituration

11 Open 8 × 5ml luer-lock syringes

12 Of 8 syringes, take out the syringe pump of 4 syringes. Place 4 syringe pumps on 100mm or 150mm petri dish

13 Lock one intact syringe and one syringe with the pump removed to a sterile 22G micro-emulsifying needle on each side

14 Aliquot cercariae equally into the connected needles. Each syringe should hold ~3ml of cercariae

15 Place syringe pumps back into the filled syringes and push the pump all the way. The cercariae should pass through the needle to the opposite syringe
** Check for any leakage from the locks. If there is a leak, try tightening the lock or do not use that needle*

16 Unlock the empty syringe from the connected needle, place the needle and syringe with cercariae in the 15ml tubes containing the Percoll solution

17 Push the cercariae through the needle, into the tube. Do this for all four needles with max ~5ml per percoll tube

18 Push the syringes back and forth 12-13 more times

19 Remove the empty syringe from the connected needle and place the needle and syringe with cercariae in the 15ml tubes containing the Percoll solution

Alternative tail removal: 20G needle trituration

20 Pass the cerariae suspension thought a 22G blunt-end needle in a luer lock syringe as follows:
If 5ml solution – 15 times using 10ml luer lock syringe
If 10 ml solution – 30 times using 20ml luer lock syringe

21 Take a drop of cercariae in a petri dish to check under the microscope if the passages have separated the tails. If there are still many cercariae (more than ~20%), continue

with ~5 more passages

Percoll gradient

22 Place now-headless cercariae carefully on top of Percoll solution tube using a plastic pasteur pipette (5 ml per Percoll solution tube) or directly into the tube of Percoll solution from the syringe

23 Centrifuge at 350 x g, 4°C, 00:05:00 , acceleration and deceleration =1 5m

24 Remove from centrifuge carefully. Discard supernatant and the white interface of cercaria tails

Washing somules

25 Take schistosomula “pellets” and pool them together in a 15ml falcon tube. Top to 15ml with somule wash

26 Centrifuge at 500 x g, 4°C, 00:05:00 5m

27 Discard supernatant and add 15ml somule wash

28 Repeat washing steps 3 times in total

29 After the last wash, remove as much supernatant as possible and add 6ml of somule media

Somules in culture

30 Dispense 2ml of somules into each well of a 6-well plate and add 4ml somule media to each

31 Incubate at 37°C in 5% CO_2 incubator

32 The following day observe the somules to look for contamination, and replace media daily

Protocol references

Citation

Paul F. Basch (1980)
. Cultivation of Schistosoma mansoni In vitro. I. Establishment of Cultures from Cercariae and Development until Pairing.
The Journal of Parasitology.

https://doi.org/10.2307/3280632 LINK

Citations

Paul F. Basch. Cultivation of Schistosoma mansoni In vitro. I. Establishment of Cultures from Cercariae and Development until Pairing
https://doi.org/10.2307/3280632