In vitro excystment of Juvenile Fasciola hepatica

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DEC 11, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.14egn212qg5d/v1

Protocol Citation: Paul McCusker, Rebecca Armstrong, Duncan Wells, Emily Robb, Paul McVeigh, Aaron Maule, Erin McCammick, Nathan Clarke, Erica Gardiner 2023. In vitro excystment of Juvenile Fasciola hepatica. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn212qg5d/v1

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

ABSTRACT

This protocol describes our excystment protocol for *Fasciola hepatica* supplied by Ridgeway Research Ltd. *F. hepatica* metacercariae (also known as mets or cysts) are supplied attached to Visking tubing with both inner and outer walls intact. This protocol builds upon methodology described by McVeigh et al. (2014) and McCusker et al. (2020) and has been tweaked by many lab members over the years. Our thanks to all who have contributed.

GUIDELINES

Storage of *F. hepatica* metacercariae

When mets are received from Ridgeway Research we move sheets from original sheets into 50 mL falcon tubes in fresh RO water. They are then stored at 4 °C until required and the 'sheet is use' is moved into a 15 mL falcon tube.

Washing watchglasses

Watchglasses are rinsed with [M] 10 % (v/v) bleach before rinsing in RO water and dried out before subsequent use.

Created: Mar 19, 2023

Last Modified: Dec 11, 2023

PROTOCOL integer ID: 79051

Keywords: Fasciola, Metacercariae, Excystment

Funders Acknowledgement:
Developing a 'validation portfolio' to exploit key virulence proteins in Fasciola species for parasite control
Grant ID: BB/H009477/1
LIVER FLUKE MOTOR FUNCTION AND PARASITE CONTROL: EXPLOITING A 'TARGET VALIDATION TOOLBOX' AS A DRUG SCREEN-INTERFACE FOR FLUKICIDE DISCOVERY
Grant ID: BB/K009583/1
Probing in vivo parasite biology in vitro
Grant ID: NC/N001486/1
Exploiting stem cell biology for liver fluke control
Grant ID: BB/T002727/1

MATERIALS

Excystment Salt Solution – 0.9% (450 mg) and 1.2% $NaHCO_3$ (600 mg) in 50 mL RO Water.

1/20 HCl – 125 µL 1 N HCl and 2375 µL RO Water

Bleach solution – 100 µL

Sodium hypochlorite solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1056142500**

with 900 µL RO water and vortex

SAFETY WARNINGS

Infectious Agent
F. hepatica is a category 2 pathogen. The metacercariae is the infectious stage of the life and if ingested can infect humans. Therefore all plasticware that comes into contact with metacercariae or NEJs is disposed of in [M] 10 % (v/v) bleach.

Outer metacercariae (met) wall removal

25m

1 Pipette 300 µL 50% chicken serum (CS50) onto large petri dish base and lid, spread with finger. 30s

2 Fill petri dish base with 10 mL RO water. 30s

3 Gently lift rolled-up met sheet out of tube, place in dish base and unwrap the sheet (ensure you know which way mets are facing). 1m

4 Add 100 µL of RO water to petri dish lid to ensure it stays damp. Lay the met sheet onto the lid w 30s
mets facing down. NB: avoid bubble formation.

5 Use a scalpel to gently pop required number of mets out of outer wall (assume ~70-75% excystment 10m
rate). Outer wall is coloured brown whereas 'popped' mets are translucent. **NB. only count viable mets (those with a bilobed appearance following popping).**

6 Add 10 mL water to petri dish lid before moving sheet back to petri dish base (mets facing up). 1m

7 Transfer mets from petri dish lid and base to a watchglass using a serum-lined tip. 10m

Solution Preparation 5m

8 Make the following solutions and warm to 37 °C: 5m

8.1 **Tube 1 –** Dissolve 20 mg sodium tauroglycocholate in 2.5 mL **Excystment Salt Solution** in a 15 mL falcon tube.

8.2 **Tube 2 –** Dissolve 20 mg L-cysteine in 2.5 mL **1/20 N HCl** in a 15 mL falcon tube.

Bleaching mets 1h 10m

9 Swirl watchglass to gather mets in centre. Remove as much water as possible without drying out met 1m

10 Add 1 mL bleach solution to mets and start timer (time varies between sheets/strains - typica 2m 30s
min 30 s)

10.1 While bleaching is ongoing add 1 mL RO water to a new watchglass and serum line a p100 tip.

11 As timer approaches 0 swirl watchglass to group mets. As timer hits 0 collect mets in serum-lined p1 30s
tip and move into watchglass with water.

12 Swirl mets to group, remove as much water as possible and add another 1 mL RO water to wash 1m

13 Repeat step 12 x5. 5m

14 After final wash move mets to fresh watchglass using a serum lined p100 tip in 50 µL. 30s

15 Mix tubes 1 and 2 from step 8 together (some effervesce should be visible). Briefly vortex and ensure 1m
that the resulting solution is not cloudy. **NB. if cloudy, do not add to worms and remake.**

16 Pour mixed solutions onto mets and place clean watchglass on top to reduce evaporation. 1m

17 Incubate in a tupperware lunchbox (line base with damp paper towel) for 1 h at 37 °C. 1h

Collection of new excysted juveniles (NEJs) 2h

18 Collect NEJs with a p10 and place into pre-warmed RPMI (glass watchglass is best). After collecting excysted worms continue incubation at 37 °C and collect newly excysted NEJs at 20 min intervals until 3 h after addition of excystment solutions. 2h