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In vitro excystment of Juvenile Fasciola hepatica

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We use this protocol and it's working

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Developing a 'validation portfolio' to exploit key virulence proteins in *Fasciola* species for parasite control

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LIVER FLUKE MOTOR FUNCTION AND PARASITE CONTROL: EXPLOITING A 'TARGET VALIDATION TOOLBOX' AS A DRUG SCREEN-INTERFACE FOR FLUKICIDE DISCOVERY

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Probing in vivo parasite biology in vitro

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Exploiting stem cell biology for liver fluke control

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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  10 % (v/v) bleach before rinsing in RO water and dried out before subsequent use.



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  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 with mets facing down. NB: avoid bubble formation. 30s
- 5 Use a scalpel to gently pop required number of mets out of outer wall (assume ~70-75% excystment rate). Outer wall is coloured brown whereas 'popped' mets are translucent. **NB. only count viable mets (those with a bilobed appearance following popping).** 10m
- 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 mets. 1m
- 10 Add  1 mL bleach solution to mets and start timer (time varies between sheets/strains - typically ~2 min 30 s) 2m 30s
- 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 p100 tip and move into watchglass with water. 30s
- 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 ×5. 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 that the resulting solution is not cloudy. **NB. if cloudy, do not add to worms and remake.** 1m
- 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



Protocol references

McCusker, P., Hussain, W., McVeigh, P., McCammick, E., Clarke, N. G., Robb, E., McKay, F. M., Brophy, P. M., Timson, D. J., Mousley, A., Marks, N. J., & Maule, A. G. (2020). RNA interference dynamics in juvenile *Fasciola hepatica* are altered during in vitro growth and development. *International Journal for Parasitology - drugs & drug resistance*, 14, 46-55. <https://doi.org/10.1016/j.ijpddr.2020.08.004>

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