LIFE CYCLE OF *HELIGMOSOMOIDES POLYGYRUS*

Heligmosomoides polygyrus (formerly known as *Nematospiroides dubius*, and also referred to by some as *H. bakeri*) is a gastrointestinal helminth that employs multiple immunomodulatory mechanisms to establish chronic infection in mice and closely resembles prevalent human helminth infections. *H. polygyrus* has been studied extensively in the field of helminth-derived immune regulation and has been found to potently suppress experimental models of allergy and autoimmunity (both with active infection and isolated secreted products). This protocol outlines management of the *H. polygyrus* life cycle for (a) consistent production of L3 larvae in faecal cultures; (b) recovery of larvae and infection of mice and (c) recovery of adult parasites. The culture of adult worms for Excretory-Secretory (HES) products is detailed in a separate protocol.

Reagents and Equipment

(a) For Faecal Cultures and Production of Infective Larvae

1. Forceps, scissors
2. Activated Charcoal NORIT GAC 1240 12-40 Mesh, Acros Organics Cat.No. 395712500 (washed and autoclaved, see *Note 1*)
3. Filter paper circles 55 mm diameter (Whatman, Cat.No. 10311807)
4. 60 mm with 2 mm Grid TC-treated Culture Dish (Corning, Cat.No. 430196)
5. Standard 90mm Petri Dishes, (Thermo Scientific, Cat.No. 101VR20)

(b) For Recovery of Larvae and Infection of Mice

6. Dissecting Microscope
7. Gavage needle

(c) For Recovery of Adult Worms

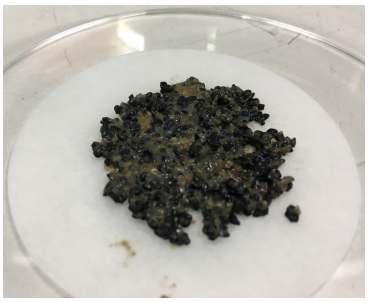
8. Round-ended scissors
9. Baermann apparatus (see below).
10. 37°C incubator.
11. HBSS, no calcium, no magnesium, Gibco Life Tech, Cat.No. 14170138
12. P/S: Penicillin (10,000 U/ml) and Streptomycin (10,000 µg/ml), Gibco Life Tech Cat.No. 15140122.
13. Muslin fabric, paperclips and stapler
14. Microscope Slides Clarity 76x26 1.0mm Silane Treated, DixonScience, Cat.No.N/C360
15. 20 ml Luer slip Syringes BD Plastipak, BD, Cat.No. 300613
16. 15 ml and 50 ml Falcon tubes, Greiner, Cat.Nos. 188271 and 227261

Protocols

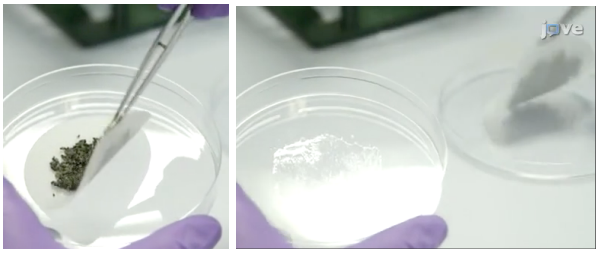
(a) Faecal Cultures and Production of Infective Larvae

1. Collect colonic contents and faeces by scraping out the lower gut with forceps and scissors. Do this in fume hood.
2. Mix the faeces with granulated charcoal at a ratio of at least a 1:1, until sticky consistency (not too wet or dry) and peanut butter colour. Smear a thin layer on the centre of some dampened filter paper in a petri dish.

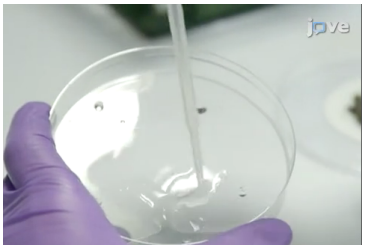
3. Store in a humid box (containing some damp blue roll and a dish of water) in the dark for 14 days. From day 7 onwards larvae can be collected, and again up to day 14 before discarding paper; the larvae form a ring around the edge of the filter paper.



4. Lift the filter paper out of the petri dish, on the open lid with tweezers ***(A)***.
5. Check for live (motile) larvae and for contamination (eg *Paramecium*, see notes below, or too any particles of charcoal) with dissecting microscope. If OK, harvest larvae fromn the plate, with a plastic pipette, add 5 ml of autoclaved water and flush several times to collect larvae ***(B)*** and transfer to a 50 ml Falcon tube.



(A)

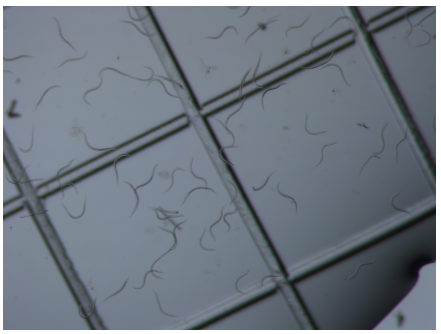


(B)

6. At day 14, harvest larvae and discard plates.
7. Leave to settle overnight. Take off water without removing pellet of larvae and add ~50 ml of fresh water. Wash the larvae 6 times (once a day). Keep in fridge, be sure to label tube with how many washes. Can be stored for about 6 months, or, if desperate 1 year.

(b) Recovery of Larvae and Infection of Mice

8. Before use, L3 larvae have to be washed at least 6 times in distilled water (see details above in step 7).
9. Dilute L3 larvae in a small volume of water and aspirate 2 samples of 25 µl and place them on the surface of a 60 mm culture dish.
10. Count the L3 larvae (usually motile, and best viewed under 50X magnification with a dissecting microscope). Add water to reach the concentration of 2,000 L3 larvae per ml. Take at least 8 samples to assure accurate count and avoid over-dosing mice with fatal results.



11. For life cycle production, infect 8 week-old male or female F1 (Female C57BL/6 x Male CBA) mice with 400 *H. polygyrus* L3 larvae in 200 µl of distilled water by oral gavage (restrain mice in the upright position by the scruff of the neck and gently pass the blunt gavage needle through the mouth and oesophagus into the stomach). Agitate the tube of larvae thoroughly prior to each infection (larvae settle quickly in water) and aspirate 200 µl in a 1 ml syringe; use a dedicated gavage needle with a rounded end.
    Note : Male F1s can be infected with 500 L3 larvae in 200 µl; for experimental infection of younger mice (6-8 weeks old), or inbred strains (e.g. C57BL/6 or BALB/c), infect mice with 200 L3 larvae.

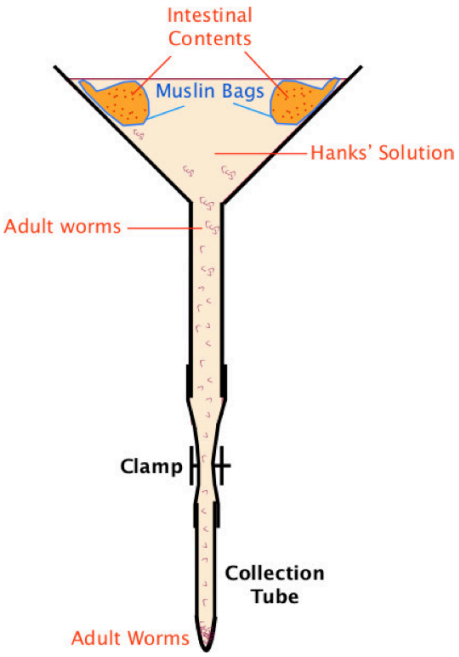
(c) Recovery of Adult Worms

At the animal house:

12. Cull mice 21 days after infection (Schedule 1 method routinely used – Exposure to CO_2 and confirmation by dislocation of the neck)
13. Wash the abdomen with 70% ethanol. Cut the skin over the abdomen and pull back to reveal the anterior abdominal wall. Make midline incision to enter the peritoneal cavity.
14. Remove complete gut, from bottom of stomach to end of colon. Place in dry petri dish.
15. Draw out gut to full length and remove caecum and colon into a 50ml Falcon tube for later egg preps.
16. Cut off top 2/3 of small intestine (20 cm) containing the worms – identified by the relatively thick wall of the duodenum and often a red appearance due to the intra-luminal worms. Place into a new 50 ml Falcon tube

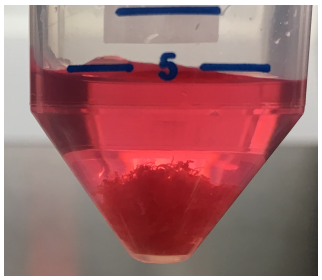
In the lab:

17. Into a dry (90 mm diameter) Petri dish, open the worm-filled proximal gut portion longitudinally with scissors (round-ended scissors are best for this)
18. Transfer into a new petri dish with HBSS (prewarmed to 37°C), scrape down inside of gut lining with a glass slide and forceps to dislodge the worms. Then discard the clean gut wall.
19. Tip worms into little muslin bags, staple closed and secure with paperclips around the edge of glass. The funnel should be attached to rubber tubing connected to a glass tube to collect the worms in and be sitting on a stand.



20. Fill funnel with water to detect eventual leaks, remove the water, then fill it with HBSS (prewarmed to 37°C) and add muslin bags of worms (4 bags maximum) into the funnel.
21. Place apparatus in 37°C incubator for 1-2 hours, gently agitating half way through to dislodge debris from the gut preparation that may occlude the muslin filter. Take care to avoid spillage of debris outside the muslin bag – this will cause contamination of the worm collection.
22. Adult worms should have slowly migrated through the muslin cloth and settled at the bottom of the glass test tube. Carefully detach the test tube from the connecting rubber hose over the sink (taking care to avoid losing worms at this point or splash yourself with media).
23. Use a plastic pipette to put worms in a 50 ml tube and wash 6 times with HBSS (prewarmed to 37°C), leaving to settle before removing media.
    NOTE: worm culture must be kept sterile from this point onwards.

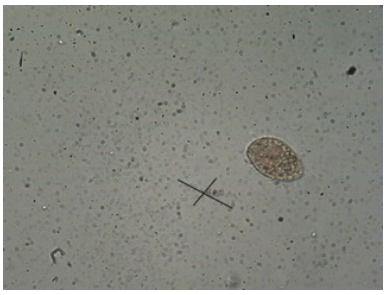
24. Move to a laminar flow hood (room B642) and wash another 6 times in HBSS (prewarmed to 37°C) supplemented with P/S (5 ml P/S per 500 ml of HBSS).



25. Soak worms in 10% Gentamycin (1 ml added to about 10 ml media left in tube) for 20 mins, leaving tube resting at an angle to ensure worms are fully covered.
26. Wash again 6 times with HBSS + P/S.
27. Count adult worms by taking replicate 20 µl volumes; use a pipette tip with a wide aperture (e.g. cut off the bottom 5 mm of a yellow tip).
28. For collection of HES, distribute into T25 flasks with approx. 1000 worms each in 15 ml *H. poly media* (Note 3) and place in 37°C incubator (5% CO2) for a total of 3 weeks as detailed in the *HES Protocol*

Notes

1. Charcoal must be washed well, dried and autoclaved before use with worms. Buy charcoal in active form. Deactivate in batches. Deactivate by filling large (5L) plastic beaker ½ way with charcoal, slowly add tap water. Air bubbles will come off. Run the water continuously for an hour. Pour off the water. Scrape out the wet charcoal onto paper towel inside a large rectangular tray. Spread into a thin layer and leave to dry at room temp. When the paper towel is dry the charcoal can be transferred in autoclavable storage jars (100 ml to 500 ml) and autoclaved. Always use a freshly autoclaved charcoal to avoid contamination (once jar opened, the charcoal needs to be autoclaved again before use).
2. Contamination of faecal cultures with *Paramecium*. Paramecia (ciliated protozoa) are widespread in freshwater, brackish, and marine environments and are often very abundant in stagnant basins and ponds. Very rarely, faecal cultures can be contaminated by *Paramecium* (round and very mobile). Discard plate(s) immediately and check the water source.



3. *H. poly media* (most important NEVER add FCS!!!!!!!) To 500 ml RPMI1640
   + 20 ml of 25% glucose solution, final concentration 1%
   + 5 ml P/S final concentration 100 U/ml penicillin, 100 µg/ml streptomycin;
   + 5 ml L-Glutamine, final concentration 2 ml
   + 5 ml Gentamicin, final concentration 100 µg/ml (optional)

References

[1] **Johnston, C. J. C., Robertson, E., Harcus, Y., Grainger, J. R., Coakley, G., Smyth, D. J., McSorley, H. J., Maizels, R.** 2015. Cultivation of *Heligmosomoides polygyrus*: An Immunomodulatory Nematode Parasite and its Secreted Products. *J. Vis. Exp.* (**98**), e52412.

[2] **Camberis, M., Le Gros, G. and Urban, J., Jr.** (2003). Animal model of *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*. *Current Protocols in Immunology.* 9.12.11-19.12.27.

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