Oral infection with *Trichuris muris*

Equipment

- Fridge (4 degrees celsius)
- Small magnet
- Magnetic stirrer
- Pipetteboy
- Micropipettes
- Microscope
- Centrifuge
- Discard container
- Anesthesia chamber
- Needle for oral gavage

Consumables

- 1 ml syringes
- 70% ethanol
- 75cm tissue culture flask non-vented
- 10mL pipettes
- Universal tubes
- Microscopy slides
- Micropipette tips

Reagents

- MilliQ water
- Isoflurane

PROCEDURE

Inoculum preparation (should be done right before the infection)

Eggs are kept at 4 degrees in a 75cm tissue culture flask non-vented in horizontal position.

1. Take an aliquot of 10mL from the stock flask and place it in a universal

tube containing a small magnet.
2. Put the universal tube on a magnetic stirrer. Switch the stirrer on and fix the speed at one in which the solution moves to keep the eggs in suspension but there is not a vortex.
3. To count, take 50μL from the same position in the tube where the inoculum for the infection will be taken. Do not change the stirrer speed as varying it will modify the count.
4. Put the 50μL aliquot on a clean slide. Do not put coverslide.
5. Count all the gas in the 50μL, going up and down the slide. Count 4-5 slides and get a similar number for it to be a good count. For the infection 0,2mL are given containing approximately 400 eggs, therefore the actual number that is given is 4 x the 50μL count.
6. If the count is way too high or low, it should be adjusted straight away. If the eggs are very concentrated, dilute them using MilliQ water and repeat the counting. On the other hand, if the eggs are too diluted, centrifuge at 720g for 15 min with a slow brake. Eggs should be seen as a dark, loose pellet. Pour off water and keep this if unsure, but leave about 3-5mL in the bottom of the universal tube. Add clean MilliQ water to a lower volume and stir so there is not a vortex in the tube, count again.

Infection of the mice by oral gavage

1. Bring the magnetic stirrer with universal tube to the animal facility. Connect the stirrer to electricity to keep the eggs in suspension.
2. Clean the needle for oral gavage with ethanol.
3. Anesthetize the mouse using isoflurane.
4. Take 0,2mL of the inoculum using the needle for oral gavage and a 1mL syringe. Before introducing the syringe in the mouse mouth, remember to give the syringe a little wiggle/tap as the eggs can settle quickly.
5. Infect one animal at the time to prevent the eggs to settle down.