MEASURING ANTHELMINTIC EFFICIENCY IN RUMINANT PARASITES*

Goal:
Screening of compounds for their effect on parasite motility assays, identifying anthelmintic candidates in a drug-repurposing library.

Parasites tested:
Exsheathed L3 stages (xL3s) of (see Note 1)

- *C. oncophora*
- *H. contortus*
- *O. ostertagi*
- *T. circumcincta*

What you need:

- wMicroTracker device
- 5% CO_2 incubator at 37°C
- Orbital shaker
- Sodium hypochlorate
- RPMI-1640
- Sterile 96-well flat-bottom microplates (Greiner)
- ~80 exsheathed larvae per well, 3 technical replicate wells per condition. Include positive and negative controls (see Note 2).
- Stock solutions (i.e., from drug library)

Protocol (originally developed for *C. oncophora*):

Prepare drugs and worms:

1. Collect ~800 larvae/mL in RPMI-1640.
2. Dilute stock solutions into RPMI-1640 in wells (100 µL total volume).
3. Transfer ~80 larvae (100 µL) to each well.
4. Agitate plate at 300 r/min for 10 min on an orbital shaker.
5. Incubate plate for 8 h at 37 °C in a humidified CO_2 incubator.

Measure activity:

6. Stimulate the worms by gently pipetting media up and down 5 times in each well.
7. Place the plate in the WMicroTracker and allow the worms to habituate for 5 min.
8. Add compounds to desired final concentrations and allow incubation time as necessary (see Note 3).
9. Record worm activity for a total of 3 h at 20 °C using wMicroTracker. To ensure reproducibility, stop (do not pause) the recording every 30 min and stimulate the worms by gently mixing the media in the wells by pipetting the media up and down 5 times before restarting the recording (see Note 4).

**From Liu et al. 2019 (KU Leuven, Switzerland).*

Protocol (cont'd):

Data export:

10. Export the average movement for each 30 min recording and combine data in Excel sheet to obtain the average movement over 3 h.

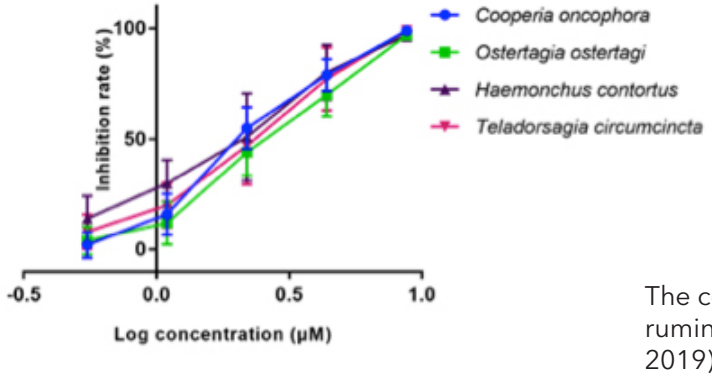
11. Use the percentage of the average movement over 3 h of exposure to test compounds, compared with the solvent controls, to estimate the relative anthelmintic activity.

12. Select the "hit" compounds with ≥70% inhibition of motility relative to the controls, for secondary confirmation assays.

Concentration-response curves:

13. Test compounds as previously described, at ~5 different concentrations to produce concentration-response curve and EC_{50} values.

14. Log10-transform the tested concentrations to establish their EC_{50} values.



The concentration-response curve of EVP4593 on four ruminant parasites (mean ± S.D., n = 3). (Liu et al. 2019)

Notes:

1. Adaptation to various species:
   "We tested whether the same approach could be applied to xL3s of other ruminant parasites, namely: *O. ostertagi, H. contortus, and T. circumcincta*. The protocol indeed also worked well for these other ruminant parasites (Z' factor > 0.5), suggesting that our assay can be highly adaptable to many other parasites." Liu et. al 2019

2. Make sure you use the proper controls:
   Solvent control: vehicle used to dissolve the compound/drug (e.g. DMSO)

   Positive control: 50 μM levamisole (four replicates)

3. The wMicroTracker can be placed in an incubator during data acquisition.

4. Pipette the media up and down manually using a multichannel pipette to stimulate the worms. This considerably improves reproducibly.

   "Worms kept in continuous darkness gradually decreased their spontaneous motility, making it hard to detect motility inhibition. The motility of worms in vitro decreased gradually and becomes undetectable after 30 min. This effect does not seem to be significantly affected by worm concentration, temperature changes or light/dark cycle."