Multivariate screening of *Brugia* spp. adults

Nicolas J. Wheeler
University of Wisconsin-Madison https://orcid.org/0000-0002-5909-4190
Kaetlyn T. Ryan
University of Wisconsin-Madison https://orcid.org/0000-0002-2051-7969
Kendra J. Gallo
University of Wisconsin-Madison https://orcid.org/0000-0002-3212-9226
Mostafa Zamanian (mzamanian@wisc.edu)
University of Wisconsin-Madison https://orcid.org/0000-0001-9233-1760

Method Article

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Abstract

The following protocol is for performing multivariate (motility, fecundity, metabolism, and viability) phenotypic drug screens against *Brugia* spp. adults.

Adults are allowed to recover in complete media after extraction and (if applicable) shipment in order to equilibrate. The assay is initiated the next day and terminated after 48 hrs, after which females will no longer release progeny.

Introduction

Reagents

24-well plates (Greiner Bio-One 662160)

96-well microtiter plates (Greiner Bio-One 655180)

96-well deep well plate (VWR 76210-518)

96-well plate sealing foil

1.5 mL tubes

RPMI (Sigma-Aldrich R8798)

100X Penicillin-Streptomycin solution (Sigma-Aldrich P4333)

FBS (Thermo 16000044)

DMSO (Santa Cruz Biotechnology sc-202581)

100X drug

Viability assay

CellTox Green (Promega G8742)

PBS

Equipment

37°C CO_2 incubator

Imaging setup (e.g., (2))

Microcentrifuge

Sterile BSC

Eppendorf M4 repeater pipette

ImageXpress Nano (or an alternative high-content imager, for quantifying microfilaria release)

Procedure

Day 1

1. On day of receipt, prepare 24-well plates with 750 µL complete media per well (RPMI + 10% FBS + P/S). Ensure media and plates are warmed prior to parasite arrival.

2. Upon receipt, place the tubes of parasites in the 37°C CO_2 incubator. Parasites typically arrive cold and sluggish but will resume normal motility after 30-60 min. of incubation.

3. After parasites have warmed, transfer a single parasite to each well of the prepared 24-well plates. Return the plate to the incubator immediately after it is filled with parasites and note the time.

Day 2

Time points 0, 0.1, & 1

1. Prepare new 24-well plates with incomplete media (no FBS).

- If drug treatments are planned, prepare wells with 750 µL minus the amount of drug to be added. For example, add 675 µL if a 10X drug dilution is planned.

2. Prepare drug stocks at 10X concentration in RPMI. Keep warm.

3. Transfer parasites to the new plates ~23 hr after the noted time from Day 1 and return the parasites to 37°C to recover for ~1 hr. Keep the old plates with conditioned media from females (for mf quantification); discard the conditioned media from males.

a. Transfer all the conditioned media from females to labelled 1.5 mL tubes.

b. Centrifuge the tubes for 5 min at 10,000 *x g*.

c. Discard 500 µL of the supernatant. Store the remainder (pellet of mf, should be ~200 µL) at 4°C.

4. Video record the plate immediately prior to adding drug.

a. We typically record the entire plate for 15 s. at 16 FPS using a setup similar to (1).

5. Immediately after recording, transfer to the BSC and add drug at the desired dilution.

6. Immediately record.

7. Return to the incubator.

8. Record videos again after 1 hr incubation.

Days 3-4

Time points 24 & 48

1. Prepare new 24-well plates with incomplete media + 1X drug. Warm plates at 37°C.

2. Record videos at the 24 hr mark.

3. Transfer parasites to new plates and return them to the incubator. Keep the plates with conditioned media.

4. Transfer all the conditioned media from females to labelled 1.5 mL tubes (**Note**: male conditioned media does not need to be centrifuged).

5. Centrifuge the tubes for 5 min at 10,000 *x g*.

6. Transfer 500 µL of the supernatant (or conditioned media from males) to a 96-well deep well plate, cover the plate with foil sealing film, and store at -80°C.

7. For female conditioned media, store the pellet at 4°C. Discard the remaining male conditioned media.

8. Repeat steps 1-7 on day 4.

Fecundity analysis

1. For mf pellets stored at 4°C, pipette to mix and transfer 50 µL to a 96-well plate used for the ImageXpress.

2. Image the plate with 4X, tiling a 2x2 grid to image the entire well. Merge the images after exportation.

3. Load the images from the entire plate into ImageJ (2) as a stack.

4. Draw circle ROI and make sure it doesn't contain the well rim for any images

5. Edit > Clear outside

6. Process > Find edges

7. Image > Adjust > Threshold (dark background, stack histogram) > Apply (unselect everything)

- Manually choose a percentage on the histogram that maximizes segmented objects and minimizes background.

8. Process > Binary > Make binary (unselect everything)

9. Analyze > Set measurements (Only select area & limit to threshold)

10. Image > Stacks > Measure stack

11. Save data as a CSV

Note: Worms that lay less than 200 mf (160,000 pixels) over the first day of recovery can be removed from all downstream data analysis.

Details for the motility, metabolism, and viability analyses can be found in the associated manuscript.

Troubleshooting

Time Taken

Anticipated Results

References

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