Bivariate, high-content screening of *Brugia malayi* microfilariae

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Method Article

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Abstract

The following protocol is for performing bivariate (motility and viability) phenotypic drug screens against *Brugia* spp. microfilariae (mf). It has been heavily optimized for utilization with an ImageXpress high-content imager, but only small adaptations will be necessary for usage with similar imaging platforms.

Mf are used immediately after receipt/extraction, and the experiment should not be run longer than 48 hours, after which mf health rapidly deteriorates. We have had success recording motility at 12 hours post-treatment (hpt) and again at 36 hpt, after which mf are immediately stained for viability, as well as 24/48 hpt time points.

The two phenotypes could be assayed separately, but we have found that not every compound that causes reduction in mf motility causes a concomitant loss of viability. Thus, the discriminatory power of the pipeline is increased by running both assays in parallel, using the same plates. We have run the experiment using 16 plates at a time which will necessitate several hours of imaging at the desired time points. If several plates are planned, drug addition will need to be staggered every 5-6 minutes in accordance with the time taken to completely image each plate.

Image data can be analyzed via a variety of software, but we have designed wrmXpress for this purpose (1).

Introduction

Reagents

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

RPMI (Sigma-Aldrich R8798)

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

DMSO (Santa Cruz Biotechnology sc-202581)

Troughs

Breathable plate covers (Diversified Biotech BERM-2000)

100X drug or pre-prepared assay-ready plates

Viability assay

CellTox Green (Promega G8742)

PBS

Equipment

Required

Incubator set to 60°C

ImageXpress Nano set to 37°C with 5% CO_2 (or an alternative high-content imager)

Orbital plate shaker

Optional

Plate spinner (VWR)

Plate washer (Molecular Devices AquaMax 2000)

Automatic multichannel pipette or other liquid handling

Procedure

Prepare microfilariae

1. Upon receipt, filter microfilariae (mf) using a PD-10 column, manually checking drops and beginning to collect when the filtered mixture is full of concentrated, pure mf. Periodically check the flow-through to ensure purity, and stop collecting when the concentration of mf in each drop begins to substantially reduce.

2. After filtration, calculate the mf concentration by counting at least 12 aliquots of 0.5 µL.

3. Aliquot the desired number of mf into a new 50 mL tube (100,000 mf are needed per 96-well plate, and it's best to take extra in case of loss during washing or imprecise counts).

4. Wash mf twice with 50 mL warm RPMI +penicillin-streptomycin (P/S) by adding media and spinning at 2000 RPM for 10 min.

5. After washing, resuspend mf in 25 mL RPMI + P/S.

6. Count 12 aliquots of 0.5 µL.

7. Pellet microfilariae, remove supernatant, add RPMI + P/S + 1% DMSO (or other solvent/vehicle) so that the final mf concentration is 10 mf/µL.

11. Transfer an aliquot of 10,000 mf per plate to a 15 or 50 mL tube for heat-killing; return the rest to 37°C.

12. Heat-kill the aliquoted mf by incubating for 1 hr at 60°C.

13. Return the killed mf to 37°C.

Prepare drug plates

Note: It takes ~6 min to image an entire plate using the established 10 frame ImageXpress protocol, so drug should be added to each plate at 6 min intervals.

1. If using pre-prepared assay-ready plates, move them from -20°C to 4°C to slowly warm. Move them to room temperature ~30 min before beginning the experiment.

2. If adding the drug manually, prepare V-bottom plates or PCR strip tubes with 100X drug.

3. Add 100 µL of heat-killed mf to wells of assay-ready plates (A/B/C/D12 and E/F/G/H01).

4. Add 100 µL (1000 mf) of the alive mf suspension to each well using an automated multichannel pipette and a 50 mL trough. Make sure that only 11 tips are used at a time, and do not add live mf to the wells with heat-killed mf.

5. If adding drug manually, add 1 µL of 100X drug to each well using a p10 multichannel pipette.

6. Cover each plate with a breathable film and gently shake on an orbital shaker for 15 sec.

7. Incubate plates at 37°C until ready to video.

MOTILITY MEASUREMENT

Note: The ImageXpress needs to be preheated 2 hours before imaging. Place the plate sealer in the machine during the preheat.

Note: The following procedure will need to be adapted for non-ImageXpress instrumentation.

1. Ensure the 5% CO_2 tank and water reservoir are pressurized/filled. The CO_2 tank regulator should adjust to 15-20 PSI. Check that the CO_2 pressure is being correctly sensed by clicking Control > ImageXpress > Environmental control… in MetaXpress.

2. Start MetaXpress.

3. In the Configure tab, update the date, plate name, and description. Set the protocol to image at 4X, with 10 frames per well at the highest FPS possible (~3.3 in our hands).

4. Remove the first plate from the 37°C incubator, taking care to not touch the bottom of the plate.

5. Remove the breathable film and set aside (it will be reused); place the lid on top.

6. Carefully place the plate in the orbital plate shaker, taking care not to splatter media on the lid. Shake for 15 sec.

7. Allow mf to settle at room temperature for ~3 min.

8. Load the plate by opening the loading door, removing the lid, and placing the plate on the stage. Securely seal the plate sealer on top of the plate and close the door.

9. Click Test to check that the default offset is suitable for focusing on your plate. Test several wells throughout the plate. If the offset needs to be adjusted, do so in the transmitted light tab.

10. View the Live stream to ensure all the mf have settled and the heat-killed mf are not floating.

11. When everything is prepared, click Run.

12. Prepare the subsequent plate for imaging.

a. Imaging an entire plate will take ~6 min. To avoid any delay between plates, the next plate will need to be prepared while the current plate is finishing.

b. When the current plate has just begun imaging well A06 (~3 min remaining), follow steps 5-8. This should allow for 2-3 minutes of settling time.

c. When the current plate has finished, open the loading door and remove the plate sealer while holding the current plate in place.

d. Remove the current plate and replace the lid.

e. Follow step 8-11. Testing and adjusting the offset should be unnecessary.

f. Replace the breathable film on the previous plate and return to the 37°C incubator.

VIABILITY MEASUREMENT

Note: If doing many plates, it is recommended to go through batches of 4 plates, which should take approximately 1 hour to finish.

1. Set 4 timers to 30 minutes.

2. Prepare the CellTox working solution (1 mL per plate):

a. Dilute the stain 1:200 (i.e., 100 µL in 20 mL) in the provided buffer in either a 15 or 50 mL conical tube. Wrap the tube in foil. Note: this is ½ the recommended concentration from the kit. It will work fine.

b. Fill a trough with the working solution.

4. Set the imaging parameters on MetaExpress. Click Acquisition Setup and configure a protocol to tile 4 GFP images at 4X across each well, with a 10% overlap.

5. Remove the first two plates from the 37°C incubator. Remove the breathable film and lid.

6. Using a p10 multichannel pipette, add 10 µL of working solution to each well of both plates.

7. Ensure the lids are on and gently shake the plates on an orbital shaker for 15 s.

8. Return the plates to the incubator and start a 30 minute timer.

9. When 20 minutes are left on the first timer, perform steps 5-10 on the third and fourth plates.

10. When 10 minutes are left on the first timer, perform steps 5-10 on the fifth and sixth plates.

11. When 1 minute is left on the first timer, perform steps 5-10 on the seven and eighth plates.

12. When the first timer is finished, remove the first two plates from the 37°C incubator.

13. Dispense 200 µL M9 to each well using a plate washer or multichannel pipette.

14. Spin the plates for 30 s.

15. After the spin has completed, carefully remove the plates.

16. Use the plate washer to aspirate to 5 mm and dispense 200 µL M9 (or aspirate 200 µL and dispense 200 µL M9 with a multichannel pipette).

17. Repeat steps 14-15.

18. Use the plate washer to aspirate to 5 mm or aspirate 200 µL with a multichannel pipette.

19. Make sure the lid is on the plate, and shake vigorously for 15 s.

20. Carefully set the plate on the top of the ImageXpress. Wait 2-3 minutes for the mf to settle.

21. In MetaExpress, click Eject Plate.

22. Carefully wipe the bottom of the plate with a Kimwipe; remove the lid from the plate, and place into the machine.

23. Click Load plate.

24. Right click a well at the center of the plate and click Preview.

25. Ensure that the mf are evenly dispersed throughout the well and have all settled to the bottom. If they are congregated at the well edge, redo steps 19-22. If they are not yet settled, remove the plate and repeat step 20-22.

26. Adjust the offset to focus on the fluorescent mf.

27. When the mf are evenly dispersed and have settled, and when the wavelengths are in focus, click Acquire Plate in the Run tab.

28. An entire plate should take 2.5 minutes to image.

29. When the first plate is finished imaging, remove the plate and repeat steps 22-28 with the next plate.

30. Repeat steps 13-30 for the the remaining plate couplets.

Use wrmXpress to analyze motility and viability data.

Troubleshooting

Time Taken

Anticipated Results

References

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