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## Infecting and mosquitoes with *Dirofilaria immitis* and *Brugia malayi* V.3

Michael Povelones<sup>1</sup>, Abigail McCrea<sup>2</sup>

<sup>1</sup>University of Pennsylvania School of Veterinary Medicine, <sup>2</sup>University of Pennsylvania

 Works for me

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povelab

Tech. support email: [mpove@vet.upenn.edu](mailto:mpove@vet.upenn.edu)



Michael Povelones

University of Pennsylvania School of Veterinary Medicine



### ABSTRACT

This protocol is for infecting *Aedes aegypti* and *Aedes albopictus* mosquitoes with the filarial nematodes *Dirofilaria immitis* or *Brugia malayi*. Although it is not necessary, it is strongly recommended to measure the number of ingested parasites, which indicates the exposure level for the mosquito and allows for better comparison between replicates. We use a post-feed treatment protocol, but if mosquito groups are treated prior to infection, then it is not just strongly recommended, but critical to measure ingested microfilariae in a group of mosquitoes immediately following the feed to confirm that treatment does not change the extent of blood feeding. Note that microfilariae are large (approximately 300 µm) and will quickly settle in blood samples. This should be considered throughout the protocol and this fact is pointed out in places in this protocol.

### GUIDELINES

Filariae are human and animal pathogens so all mosquito husbandry and infections should be performed in accordance to local safety and containment guidelines that meet or exceed published guidelines. We have performed this assay with *Brugia malayi* (a human pathogen) and *Dirofilaria immitis* (an animal pathogen) under the appropriate containment level.

American Committee Of Medical Entomology American Society Of Tropical Medicine And Hygiene. Arthropod Containment Guidelines, Version 3.2. *Vector Borne Zoonotic Dis.* 2019 Mar;19(3):152-173. doi: 10.1089/vbz.2018.2431. Epub 2019 Jan 29. PMID: 30694736; PMCID: PMC6396570.

### MATERIALS

NAME	CATALOG #	VENDOR
Sheep blood	SBH100	Hemostat

### STEPS MATERIALS

NAME	CATALOG #	VENDOR
Sheep blood	SBH100	Hemostat

### SAFETY WARNINGS

*Brugia malayi* is a human pathogen

*Dirofilaria immitis* is an animal pathogen

Safe blood handling procedures and a laboratory specific Exposure Control Plan in place.

### BEFORE STARTING

Secure all approvals. Establish standard protocols. Assemble all materials.

## Counting microfilariae

1 Disperse **5 µl** or **10 µl** microfilaremic blood in a **50 µl** spot of water on a microscope slide. This will lyse red blood cells and make the microfilariae more conspicuous and easy to count.

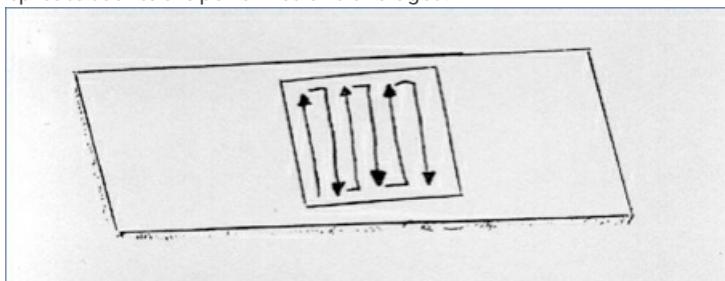


1. Water type is not important. We have used deionized water or ultrapure molecular biology grade water.
2. Before pipetting microfilaremic blood from the source container, mix evenly by gently inverting 10-15 times.  
Microfilariae are large and settle in the blood quickly.



*D. immitis* microfilaria in lysed blood sample

- 2 Immediately count the entire drop by scanning through the sample. Determine the concentration and express it as mf/ml by multiplying by the dilution factor. For example, if **5  $\mu$ l** was used, multiply by 200. If **10  $\mu$ l** was used, multiply by 100. Replicate counts are performed and averaged.



Scanning strategy for reading total microfilariae in an entire sample.



For best accuracy replicates counts are made. When counting more diluted blood, count **10  $\mu$ l**. If needed the water spot size could be increased and more blood can be dispersed. For example **40  $\mu$ l** of blood can be counted in a **200  $\mu$ l** spot of water.



From our infected dog, we typically count **5  $\mu$ l** of freshly drawn blood and count 200-300 microfilariae resulting in a concentration of between 40,000 - 60,000 mf/ml.

- 3 We typically set up infections at **[M]4000 microfilariae/mL** to **[M]5000 microfilariae/mL**. We use heparinized sheep blood to dilute. The infected blood and diluent blood are aliquoted under sterile conditions in a tissue culture hood. Once the desired dilution is created, recount the diluted to sample to confirm and then keep the sample warm at **at 37 °C** in a heat block or circulating water bath. For recounts we use **10 µl** of the dilution in **50 µl** drop of water. Replicate counts are performed and averaged.



Sheep blood

by Hemostat

Catalog #: SBH100



Because the microfilariae will settle during the course of feeding, the volume of blood and geometry and speed of blood feeding will all contribute to the number of microfilariae ingested per mosquito. Under the conditions described here, mosquitoes typically have 10-15 microfilariae ingested.

- 4 Once the water required for the feeder and the diluted blood are prewarmed to **at 37 °C** then fill a plastic baby bottle with a solid cap (see picture below) with **at 37 °C** water excluding as much air as possible without spillage. Then, invert the bottle and add **3.5 ml** of diluted blood to the depression in the bottom of the bottle and cover with stretched Parafilm.



Take care to not slosh water from the water bath onto the feeder as it might contaminate the sample and may also inhibit the mosquitoes from feeding. You better attract the mosquitoes for feeding, rub the side of the Parafilm that will be facing the mosquito cup/cage on a part of your body that does not have lotion or fragrance.



Baby bottle

300 mL bottle

Avent Phillips SCF693/59

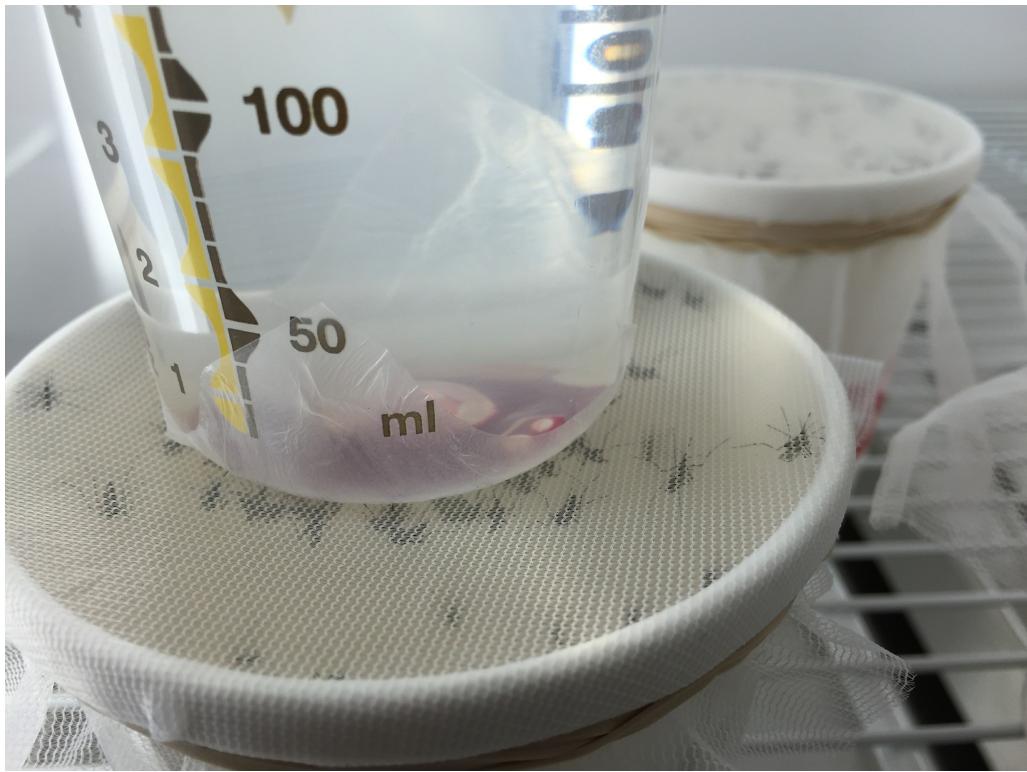
- 5 Invert the bottle and place on top of a cup/cage of mosquitoes. If required for safety reasons, perform this step in a secondary container in the incubator, such as a Large Bugdorm cage. To encourage mosquito blood feeding, exhale a warm breath into the cup or cage before placing the feeder on top. Feed for **00:15:00**. We feed at **at 27 °C** and **[M]80 %** relative humidity (our standard incubator conditions). If practical, turn off the light in the incubator.



Filled baby bottles ready to feed. They will be gently shaken before putting them on top of mosquito cups/cages parafilm side down.



Mosquitoes in a cup



Baby bottle inverted on a mosquito cup



For important comparisons, dilute enough blood, so that each cup gets the same volume of the same dilution and is fed for the same time. It is ok to reuse the feeders as described below, but it is best not to compare groups that are fed serially since the conditions will not be identical.



Large Bugdorm Cage

Large mosquito cage

Bugdorm BD4M3074 [🔗](#)



- 5.1 If required, you can refresh the water in the feeder to feed more mosquitoes. Take care not to spill water onto the Parafilm during this process. Hold the feeder, and remove the cap. Do not put it down on the Parafilm side or it will get damaged and leak. Quickly pour out the water. While the feeder is empty and inverted, give it a firm shake to disperse the blood again. Replace with fresh  $\text{at } 37\text{ }^{\circ}\text{C}$  water, cap, and put back on mosquito cup/cage for another round of feeding.

- 6 Sort blood fed mosquitoes. Mosquitoes are anesthetized using carbon dioxide and sorted on Flypad using a paint brush.



Blood fed *Aedes aegypti* (left) separated from unfed mosquitoes



Alternatively, blood fed mosquitoes can be separated on ice.



Flypad

CO<sub>2</sub> anesthetizing apparatus

Flystuff 59-114



Paint brush

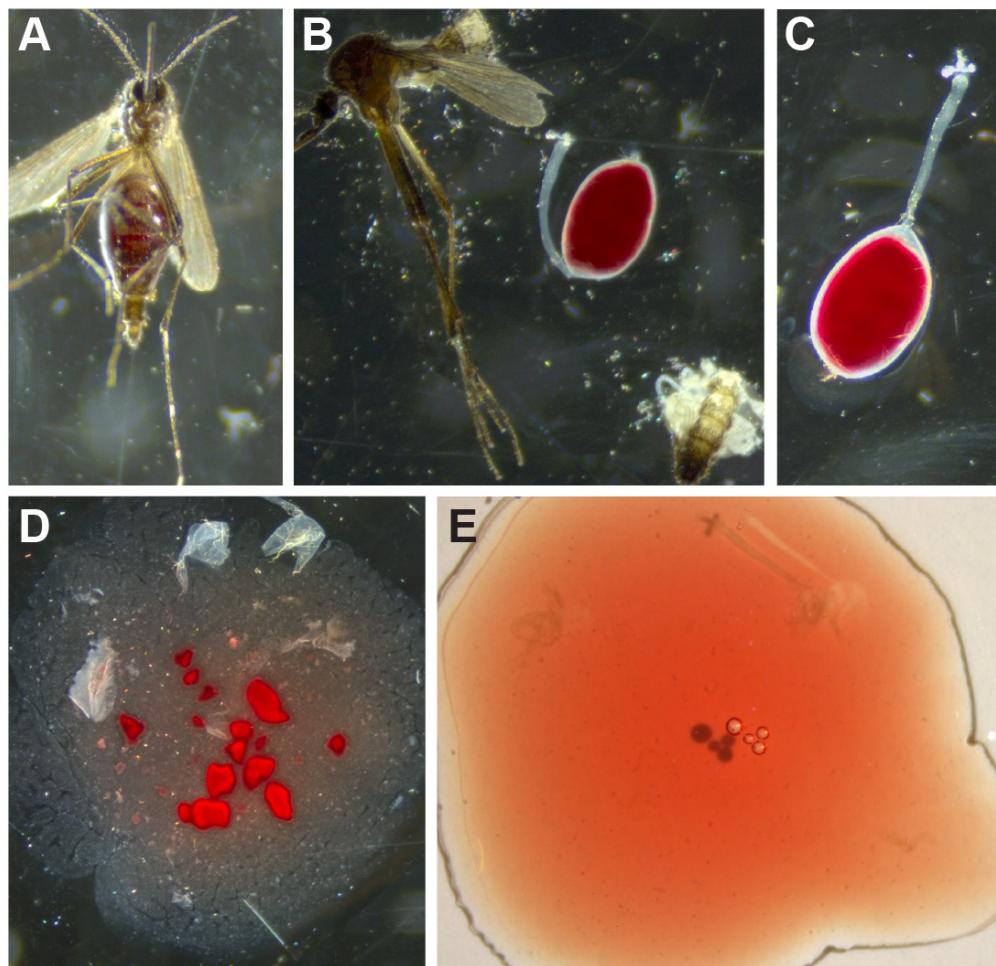
Paint brush

Generic Size 00

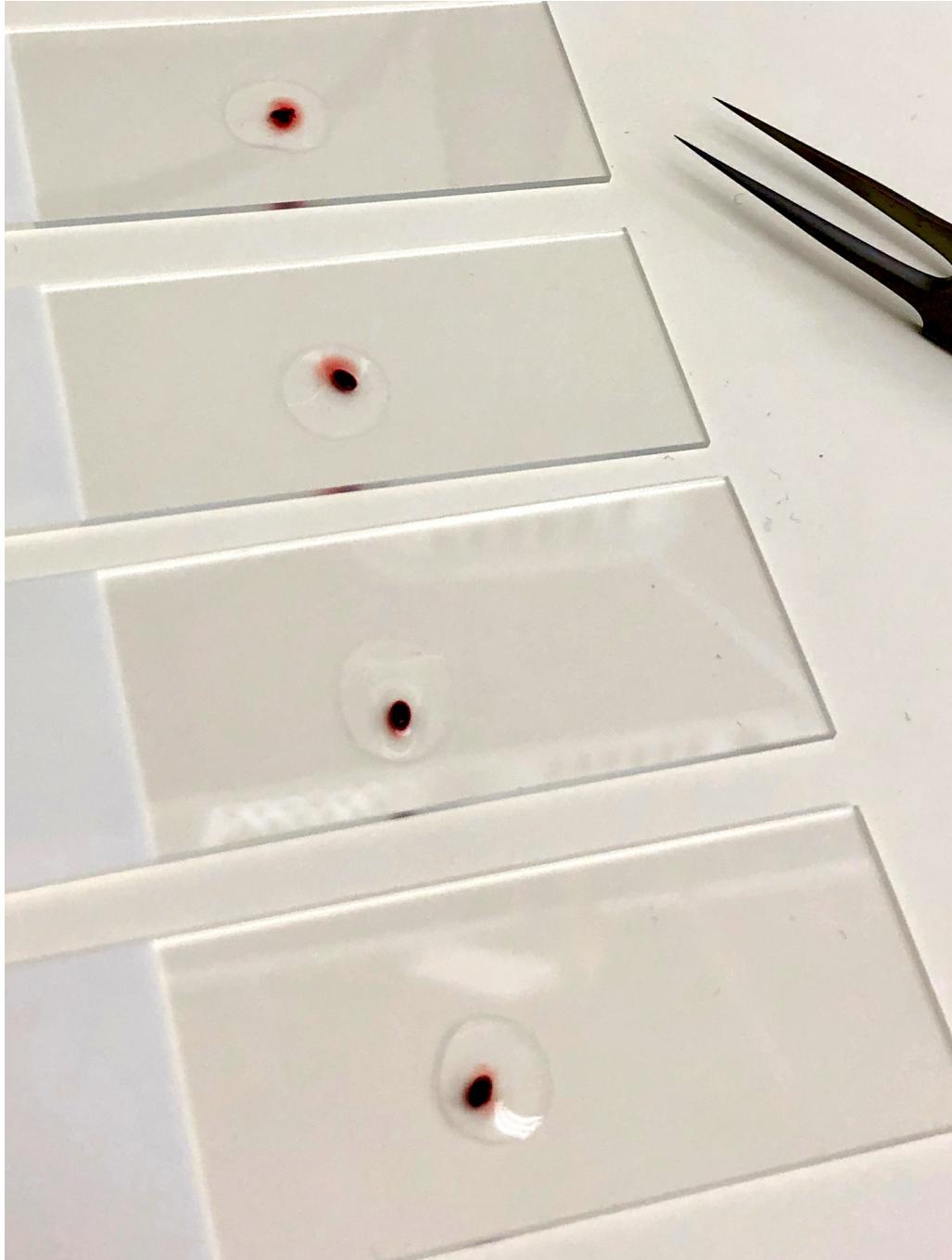
#### Checking uptake of microfilariae

- 7 Remove blood meal from some of the blood fed mosquitoes and count the microfilariae uptake. The objective is to count all microfilariae in the entire blood meal. Place the mosquito in a drop of water on a depression slide. Tear the abdominal cuticle where it meets the thorax with fine forceps. Tear in several places to facilitate gut removal without damaging it. Perform the dissections in ultrapure or deionized water in a depression slide. Discard any damaged guts that are leaking blood. Transfer the

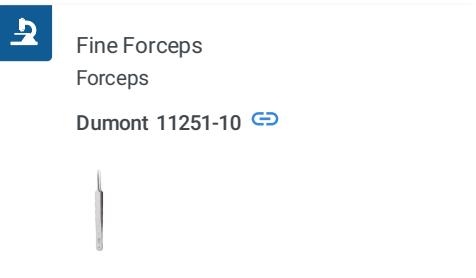
gut to a new slide with a  $\square$  50  $\mu$ l drop of ultrapure water. After transferring, remove the gut tissue by peeling it away from the blood bolus with fine forceps and "wash" through the water to remove residual blood, then discard the gut tissue. The peritrophic matrix typically stays intact. Disrupt the blood meal with the fine forceps and make sure to remove as much residual blood from the tines as possible. Disperse the blood meal fully using a P20 pipette.



Removing blood fed gut from *Aedes aegypti* mosquito. (A) Female mosquito in a drop of water. (B) Blood-filled gut removed. (C) Gut after dissection and transfer to new slide. (D) Blood meal following disruption with forceps. Pieces of peritrophic matrix are visible near the top of the image. (E) Blood meal dispersed in water using a P20 pipette.



Blood fed midguts on glass slides. Midguts were placed in a 50  $\mu$ L drop of ultrapure water and the gut tissue was removed from around the blood bolus. Several guts can be placed on slides prior to dispersing.



8 Count the entire drop as in step 2. [Go to step #2](#). Ideally, count 10 mosquitoes for all groups being compared.



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