

**Effective Date: 17 June 2022** 

SOP #: OVI-2021



# Scope

The purpose of this Standard Operating Procedure (SOP) is to outline the materials and processes required to assemble, deploy and service an ovitrap.

### **Overview**

<u>Description:</u> Ovitraps provide an attractive egg-laying habitat for gravid <u>Aedes</u> females that use containers for oviposition (egg-laying). Ovitraps were initially developed for detecting the presence of <u>Aedes aegypti</u> during the US <u>Aedes aegypti</u> Eradication Program which began in 1964 (Fay and Eliason 1965). This trap is a small, black, bucket or cup which holds water with organic infusion added. A wooden paddle acts as an ovistrip on which <u>Aedes</u> will lay eggs. This ovistrip is placed into the bucket for oviposition by <u>Ae. aegypti</u> and <u>Ae. albopictus</u>.

<u>Target species and physiological states:</u> Eggs of species that commonly oviposit in containers.

<u>Entomological surveillance indicators:</u> Ovitraps can be used to indicate the occurrence of <u>Aedes</u> vector species in an area, and eggs can be reared to adults and used as specimens for insecticide resistance bioassays.

Advantage: The benefits of using ovitraps are that they are c inexpensive and easy to use.

<u>Disadvantage:</u> Egg hatching and species identification requires laboratory space and trained staff. Additionally, ovitraps may not be useful as direct indicators of population sizes or disease risk due to confounding influences from competing larval habitats.

<u>Sample period</u>: Usually 5 - 7 days at a time; no longer or the ovitraps may become productive larval habitats to produce adult mosquitoes.

<u>Data:</u> The total number of traps with eggs, or the total number eggs per trap. When necessary, field data is merged with the results of subsequent laboratory analyses.

# **Materials**

$\bigcirc$	Black bucket/cup	$\bigcirc$	Rough sponge
0	Organic matter for infusion	0	Data device (phone or tablet)
0	Ovistrip/paddle	0	Paper towel
0	Water	0	Large rectangular tray
0	Bulldog clip	0	Cooler box
0	Permanent marker	0	Dissecting microscope
$\bigcirc$	Ziplock bag		

# **Trap preparation**

The smell of new plastic or paint might repel mosquitoes. Therefore if using brand new buckets, place the traps outside for two weeks for the smell to disappear before using them for surveillance.

# Trap assembly

### 1. Collect trap components.

a. Ovistrips made from clothe or paper should be cut to roughly 6 cm wide x 12 cm



2. Pour water into the bucket until it is about 2/3 full. For example, fill a 1 L bucket with ~660 ml of water.



- 3. Insert organic material (0.5 g/L) of such as alfalfa pellets (pictured) or dried grass into the water.
  - a. Use only a small amount of material to catch Aedes aegypti or Aedes albopictus mosquitoes. If the infusion (the water with the organic material) is too strong you may notice a powerful odour more attactive to high Culex quinquefasciatus and/or house flies than Aedes mosquitoes.
  - b. Alternatively, a hay infusion could be prepared in advance by adding 1 tablespoon of finely ground hay or grass to 4 L of water. Let sit overnight in a covered container and add directly to the ovitrap.



4. Label the ovistrip with the trap number and date.



- 5. Secure the ovistrip to the internal wall of the black bucket with the clip.
  - a. The clip can rust so place the ovistrip upside down (label away from clip) to prevent the labelling being marked by rust.
  - b. Expect the mosquitoes to lay the eggs on the ovistrips just above the water level.
  - c. As the water will evaporate over time, ensure that the water level is at least 3 cm above the bottom of the ovistrip.



### Additional notes:

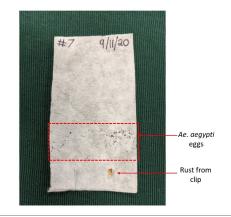
- Chlorinated (tap) water may not be attractive to female Aedes until it is left to stand for a couple of days. Either leave water to stand in containers prior to use or expect that the trap may not attract many gravid females in the first two days.
- Ovitraps are generally set for weekly periods. To ensure that the ovitrap does not become a productive larval habitat, empty, clean and refill the trap with fresh water and infusion weekly.
- The ovistrip can be many different materials such as filter paper, germination paper flannel cloth or wooden tongue depressors. The ovistrips should:
  - Be available and affordable in your location,
  - Be a light material so that eggs can easily be seen,
  - Not wick the water too quickly as the trap may dry out before being serviced, and
  - o Not hold too much water as eggs may hatch on the strip.

# **Trap location selection**

- 1. The specific location where the ovitrap is placed will greatly affect the mosquito capture rate.
  - a. Place the trap on, or close to, the ground.
  - b. Place the trap in locations sheltered from wind, water (rainfall or irrigation) and direct sunlight. Not only do these environmental factors negatively influence mosquito activity they also can impair trap effectiveness.
  - c. Place the trap within areas likely to be favourable mosquito resting locations (relatively dark or cool places which may include heavily shaded or bushy yards).
- 2. Talk with the householders about the location to place the trap. Ensure that the householders are happy with the location of the trap so they will be unlikely to move it. If no suitable trap location is identified in agreeance with the household, you must not place a trap in that property.
- 3. Ensure that trap is in a safe location where it is unlikely that children will play with it or that animals or passers-by will damage it.
- 4. When operating the trap always ensure that there is nothing within 50 cm above the trap.
- 5. If animals drink from the bucket a wide mesh (gaps > 2 cm, e.g. chicken wire) may be placed over the top.
- 6. To prevent the small containers from falling over, they can be wedged between structures, such as bricks.
- 7. Ensure the trap is placed in a location where hazards (electrical concerns, trip hazard, aggressive dog) to the occupants or staff servicing it are nearby.
- 8. Discuss permission to service the trap if the occupant is not home. If permission is granted, ensure the trap is in a location that is easily accessible when the occupant is absent.
- 9. Do not place the trap on an ant nest or touching the wall under a light where animals such as geckos may be active and interfere with mosquito samples. If ants attack the mosquito samples either move the trap or apply petroleum jelly or vaseline around the trap.

# Servicing the trap

- 1. Do not deploy the traps for more than 1 week (7 days) to prevent them from producing adults.
- 2. Check that the bucket has not been flooded, tipped over or that the ovistrips is missing. If any of these have occurred note that the trap has failed.
- 3. Carefully remove the ovistrip from the bucket and discard the water.
- 4. Check ovistrip for the presence of eggs and record positive catches.



5. Ensure the ovistrip's label is s clear and place the ovistrip onto 3 layers on paper towels on a clean surface.



6. Leave the ovistrip on the paper towel until it is no longer saturated, but still moist. This may take 5-10 minutes or longer depending on the temperature and ovistrip's material. Ensure that the ovistrips are not exposed to high temperatures (e.g. hot car left in sun) as the eggs will perish.

7. Place the moist ovistrip inside a ziplock bag and secure inside a storage container with the lid shut. Ensure only one strip is placed per zip-lock bag so that eggs don't mix from different ovitraps.



- 8. Clean the ovitrap with a rough sponge and water to remove any grime, eggs or larvae left inside the bucket. Reassemble with a new ovistrip and infusion if trapping is to continue
- 9. When back in the lab, it is vital that the eggs are kept in a humid environment so they do not dry out for 72 hours otherwise they will not hatch (either by pegging up each strip or placing them onto an open tray with paper towels as below). If you will leave the eggs in a laboratory or office with controlled temperature and reduced humidity, follow steps 10-12
- 10. Line the bottom of a plastic tray with paper towel one layer thick and place the ovistrip on top. Multiple ovistrips can be placed on large trays, but ensure the ovistrips do not touch each other.



11. Secure the lid on the tray and leave for 72 hours.

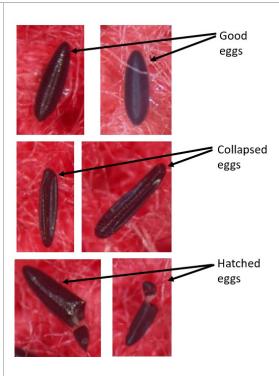


12. Loosen the lid on the tray to allow air to freely enter and the ovistrip to dry for another 24 hours.



### 13. Checking the condition of the eggs using a dissecting microscope.

- a. If the eggs have collapsed (look like a deflated football) then they are less likely to hatch. This is a good indication that the ovistrips were stored in conditions that were too dry.
- b. If the eggs are missing their tops and a very small white larvae is visible then they have hatched which means that the strips were too wet.



### 14. Now that the eggs have been properly conditioned they can either be:

- a. Stored for months in a dry sealed container
- b. Hatched and reared for different purposes

### Additional notes:

- The number of eggs per ovistrip can be recorded if this information is of value to your program.
- If the eggs are collected, stored and hatched properly you should expect a hatch rate better than 80% (at least 4 out of every 5 eggs laid should hatch)
- The ovistrips that return from the field may be contaminated by other invertebrates such as mites. Monitor and separate as necessary.
- Additionally, check that ants or other invertebrates are not interfering with the eggs during drying out and storage.
- Ovitrapping data may need to have analysed with caution because ovitraps compete with naturally occurring larval habitats. For example, oviposition indices may be skewed after source reduction campaigns when gravid females find fewer suitable habitats and lay a larger proportion of eggs in the ovitraps, confounding the evaluation of control efforts.

# **Videos**

To watch videos on how to assemble and deploy ovitraps go to:

• PacMOSSI How to assemble and deploy ovitraps - <a href="https://youtu.be/OmGaJgLtdSY">https://youtu.be/OmGaJgLtdSY</a>



# Safety/Risk assessment

Your workplace may require you to complete a risk assessment prior to conducting field work. There are a range of risks to which field workers could be exposed, and when sampling with ovitraps may include:

- Mosquito transmitted infections
- Dog bites
- Trip hazards

For further details on safety and risk assessments see SOP# MOS-2021.

## References

Fay R, Eliason DA. (1966) 'A preferred oviposition site as a surveillance method for *Aedes aegypti*.' *Mosquito News*.

https://www.biodiversitylibrary.org/content/part/JAMCA/MN\_V26\_N4\_P531-535.pdf

Whelan, P., Hayes, G. & Carter J. (1998), 'Exotic Aedes surveillance ovitrap servicing procedures.' *Bulletin of the Mosquito Control Association of Australia*. Vol 10:2.

https://www.dropbox.com/s/ui5v6z305vr88pd/Ovitrap%20servicing%20for%20Exotic%20Aedes %20surveillance.pdf?dl=0

### Citation

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https://www.protocols.io/view/standard-operating-procedure-for-assembly-and-depl-cbgpsjvn

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