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Standard Operating Procedure for assembly and deployment of ovitraps

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PacMOSSI

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This Standard Operating Procedure may be used for training and reference purposes. Users are responsible for ensuring any edits to this document are produced and approved in accordance with all relevant legal and ethical requirements governing the surveillance operation.

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

Description: Ovitrap provide an attractive egg-laying habitat for gravid *Aedes* females that use containers for oviposition (egg-laying). Ovitrap were initially developed for detecting the presence of *Aedes aegypti* during the US *Aedes aegypti* 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 ovistrap on which *Aedes* will lay eggs. This ovistrap is placed into the bucket for oviposition by *Ae. aegypti* and *Ae. albopictus*.

Target species and physiological states: Eggs of species that commonly oviposit in containers.

Entomological surveillance indicators: Ovitrap can be used to indicate the occurrence of *Aedes* 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 ovitrap are that they are inexpensive and easy to use.

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

Sample period: Usually 5 – 7 days at a time; no longer or the ovitrap may become productive larval habitats to produce adult mosquitoes.

Data: 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.

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Aedes, Eggs, Ovitrap

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Video

To watch a video on how to assemble and deploy ovitraps go to:

<https://youtu.be/ILJmjoT011w>

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>

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

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 pyrethrum spray catches may include:

- Mosquito transmitted infections
- Dog bites

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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. Ovislips made from cloth or paper should be cut to roughly 6 cm wide x 12 cm long.



- 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.

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 attractive to high *Culex quinquefasciatus* and/or house flies than *Aedes*

mosquitoes.

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.

The clip can rust so place the ovistrip upside down (label away from clip) to prevent the labelling being marked by rust.

Expect the mosquitoes to lay the eggs on the ovistraps just above the water level.

As the water will evaporate over time, ensure that the water level is at least 3 cm above the

bottom of the ovistrip.



Trap location selection

- 6 The specific location where the ovitrap is placed will greatly affect the mosquito capture rate.

Place the trap on, or close to, the ground.

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.

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).

- 7 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.

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.

- 8 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.

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.

When operating the trap always ensure that there is nothing within 50 cm above the trap.

If animals drink from the bucket a wide mesh (gaps > 2 cm, e.g. chicken wire) may be placed over the top.

To prevent the small containers from falling over, they can be wedged between structures, such as bricks.

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.

- 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

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



- 14 Ensure the ovistrap's label is clear and place the ovistrap onto 3 layers on paper towels on a clean surface.



- 15 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.
- 16 Place the moist ovistrip inside a zip-lock 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.



- 17 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.
- 18 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.
- 19 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.



- 20 Secure the lid on the tray and leave for 72 hours.



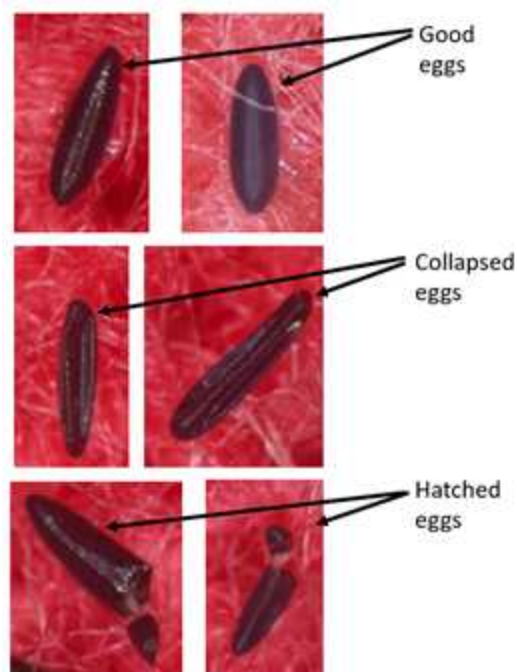
- 21 Loosen the lid on the tray to allow air to freely enter and the ovistrip to dry for another 24 hours.



- 22 Checking the condition of the eggs using a dissecting microscope.

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.

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.



23 Now that the eggs have been properly conditioned they can either be:

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

Additional notes

- 24
- 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.
 - Ovitrap 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.