

Standard Operating Procedure for vector surveillance, processing and storage

Effective Date: 17 October 2025

SOP #: MOS-2021



PACMOSSI
Pacific Mosquito Surveillance
Strengthening for Impact

Scope

The purpose of this Standard Operating Procedure (SOP) is to outline processes for the surveillance, processing and storage of mosquito samples in the field.

Target audience

This SOP has been developed for staff, researchers and collaborating partners responsible for planning, implementing and monitoring vector surveillance.

Vector surveillance work plan

A detailed vector surveillance plan is required to guide mosquito surveillance activities. The purpose and priority for vector surveillance should be outlined; as an example, routine surveillance may be conducted to understand:

- Adult vector occurrence, species composition and behaviour
- Vector insecticide resistance status
- Immature vector aquatic habitats

The work plan should map out the entomological indicators being monitored, timelines, milestones, staff requirements, sampling sites, sampling period, sampling methods, equipment, consumables, insectary maintenance, data management and dissemination, training and administrative requirements. The work plan will need to be fully costed within an approved budget.

As mosquito densities can vary greatly within even a limited area, a representative sample of the density of mosquitoes in a given surveillance site (i.e., a village or neighbourhood) requires collection of mosquitoes at multiple sampling stations (e.g., houses) to provide an accurate estimate of adult mosquito densities (Box 1). As an example, a minimum of 6 sampling stations (houses) per surveillance site (village or neighbourhood block) might be used to estimate adult mosquito densities. Depending on the resources available and size of the surveillance site, the number of sampling stations may be higher. This sampling effort is then replicated across multiple surveillance sites.

Box 1. Geographic hierarchy for designing mosquito sampling:

1. A **surveillance site** is a defined area for sampling, usually a village or a small cluster of neighbouring villages.
2. A **sampling station** is the precise location where mosquitoes are collected within the surveillance site.

Overview of surveillance procedures

Preparation for vector surveillance activities

- Develop vector surveillance work plan, with required standard operating protocols.
- Secure the required funding.
- Gain required research or ethical approvals.
- Perform a stock-take and then order required equipment and consumables.
- Plan for data capture.

Logistics

- Send advance notice of your arrival to the village.
- Travel to the village that has been selected for the survey.
- Hold community meetings.

Sampling processes

- During each sampling period, capture mosquitoes as outlined in the work plan.
- Process the mosquito samples captured. This may involve killing, sorting and identifying the mosquitoes to species and sex.
- When necessary, store the mosquito samples. Ensure all samples are appropriately stored and labelled. Make sure all tubes, boxes and bags are SEALED carefully.
- Record the data, including details about the sampling design and the number of mosquitoes that were captured (by species and sex).
- Every time you leave a village, pack up your workstation and ensure no rubbish is left in the village. Thank the community before leaving.

Surveillance methods

A wide variety of surveillance methods can be used to capture mosquitoes, and for each surveillance method a number of different collection tools may be used (Table 1). The surveillance methods selected generally depend on the particular life stage and behaviour of the mosquito species being targeted (Silver 2008), and the type of information needed about the mosquitoes.

The work plan will outline the surveillance methods and capture tools chosen for each vector surveillance site. The tools to capture mosquitoes vary depending on the purpose and targets of the surveillance activity, and the specific information to be collected. The methods to capture egg, larval and adult mosquitoes are outlined in separate SOPs, for example:

- [PacMOSSI SOP for assembly and deployment of ovitraps](#)
- [PacMOSSI SOP for conducting larval and pupal sampling for *Aedes*](#)
- [PacMOSSI SOP for conducting larval and pupal sampling for *Anopheles*](#)
- [PacMOSSI SOP for assembly and deployment of the BG-Gravid *Aedes* Trap](#)

- [PacMOSSI SOP for BG-Sentinel Assembly and Deployment](#)
- [PacMOSSI SOP for sweep net sampling of host-seeking mosquitoes](#)

These and other SOPs are accessible on the PacMOSSI website: <https://pacmossi.org/resource-category/sops/>

Table 1. The types of different surveillance methods for sampling mosquito vectors.

Life stage	Surveillance method category	Purpose
Adult	Human and animal baited traps	A human or an animal act as lures to attract host-seeking female mosquitoes to a capture tool such as a mosquito net or collection tube or chamber; includes human landing catch and a range of tent traps.
	Mechanical adult traps	A mechanical adult trap usually has a fan and collection chamber. The trap attracts adult male and female mosquitoes with various lures including different combinations of a light and synthetic host odours (such as CO ₂) or even visual cues.
	Interception traps	Interception traps provide a physical barrier such as a vertical surface to interrupt all flying mosquitoes that are moving throughout the village and enable their collection with a sampling tool such as a basin or mesh collection apparatus. These are useful for mosquitoes that are not attracted well with lures.
	Gravid traps	Gravid traps attract gravid (egg-carrying) females seeking a site for egg laying, as they contain water infused with organic matter such as hay, as a lure to attract females which are then captured.
	Natural resting site collections	Systematic visual searches for all resting mosquitoes are conducted indoors and/or outdoors (e.g., amongst vegetation or close to natural water bodies) and any resting mosquitoes collected, usually via an aspirator.
	Artificial resting site collections	Similar to natural resting sites, artificial resting structures such as human-made containers (e.g. plastic buckets or discarded car tyres) also provide attractive micro-habitats where mosquitoes can rest.
Immature	Ovitrap	Ovitrap provide an attractive egg-laying substrate for gravid <i>Aedes</i> females that use containers for oviposition. These facilitate egg collection from the field.
	Larval/pupal survey	Larval sampling involves capturing a sample of immature mosquitoes from aquatic habitats.

Mosquito sampling

There are some essential pieces of equipment that are helpful to use together with the selected capture tools or traps. For example, oral aspirators and collection cups are described below.

Oral aspirators

1. Using an oral aspirator:

- a. With the mouthpiece in your mouth, hold the aspirator tube with its opening 1-2 cm away from the mosquito.
- b. Move the end of the aspirator tube closer to the mosquito and, at the same time, suck gently but quickly to pull the mosquito into the aspirator tube.



2. Place your finger over the tube to prevent the mosquito from escaping but do not let the mosquito bite



3. Transfer the mosquitoes from the aspirator to the cup.

- a. Place the end of the tube, with your finger still in position, near the hole in the mesh covering the paper collection cup. Pull the cotton ball out of the hole in the middle of the cup. Remove your finger from the tube and quickly put the tube into the hole in the netting over the top of the collection cup.
- b. Blow gently into the mouthpiece so as to transfer the mosquito into the paper cup.



Collection cups

1. **Good collection cups may be ones which are paper with a waxy coating on the inside and are strong enough to not crumple when a rubber band is wrapped around them.**
 - a. On these cups you place a layer of mesh (10 cm x 10 cm or fit to size) and this mesh is held in place with a rubber band. Cut a 1 cm hole out of the middle of the mesh and place a cotton ball in this hole to prevent mosquitoes from escaping.
 - b. All collection cups should be clearly labelled with the details of the sampling effort.



2. **Keep the collection cups or trap bags organized and protected while in the field.**
 - a. Placing the collection cups or trap bags upright in an airtight container or an insulated cool box.
 - b. To keep mosquitoes alive and in good condition keep them in a cool place with high humidity and with access to a sugar source (i.e., cotton wool soaked in 10% sugar solution).



Sampling failure

It's important to document whether the sampling effort undertaken was a success or failure so that methods of sampling can be refined if necessary. If the sampling effort was a failure, the reason (if known) should be noted. Possible reasons why sampling efforts fail include:

- The mosquito collection apparatus or trap stopped working
- The lure stopped working e.g., a battery-powered fan
- The trap was broken
- The trap had fallen over
- There was a hole in the collection bag or chamber
- The trap was unable to be serviced as the occupant was not home
- The samples had been tampered with by an animal or human
- Human error in setting or monitoring traps

Processing adult mosquito samples

After the collection of the mosquito samples is completed, the next step is to process the mosquito samples. This may involve killing, sorting and identifying the mosquitoes to species and sex. These processes are outlined below.

Materials for processing mosquitoes include:

- ☐ Oral aspirator (1 per collector + spares)
- ☐ White tray
- ☐ Forceps
- ☐ Pencil/pen/markers
- ☐ Data collection form/digital device
- ☐ Dissecting microscope
- ☐ Petri dishes
- ☐ Chloroform (optional)
- ☐ Cotton wool
- ☐ Freezer (optional)
- ☐ Mosquito cage (optional)

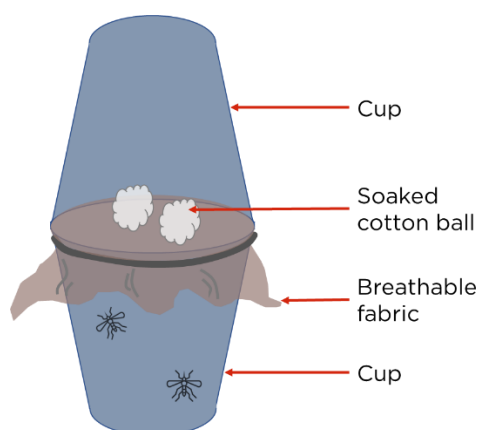
Killing adult mosquito samples

1. The first step to processing adult mosquito samples usually involves killing or knocking them down (anaesthetizing) by:

- a. Mosquitoes being held in cups or small containers could be placed in a freezer at -20°C for 60 mins.. They can be safely left in the freezer for an extended period before they are processed. If you do not have access to a freezer, place in a fridge at 4°C for 24 hours.



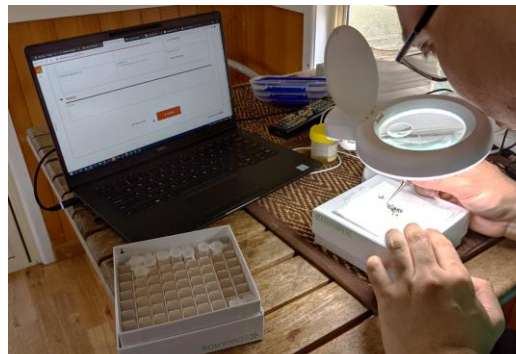
- b. A killing cup can be used to knock down the mosquitoes. A cotton ball moistened with ethyl acetate or chloroform could be placed on top of the cup mesh and covered to allow the vapours to concentrate and either knock down or kill the mosquitoes. Wait at least 1 – 2 minutes.



Morphological identification

Once all specimens are killed, taxonomic identification can occur in the field or back in the laboratory.

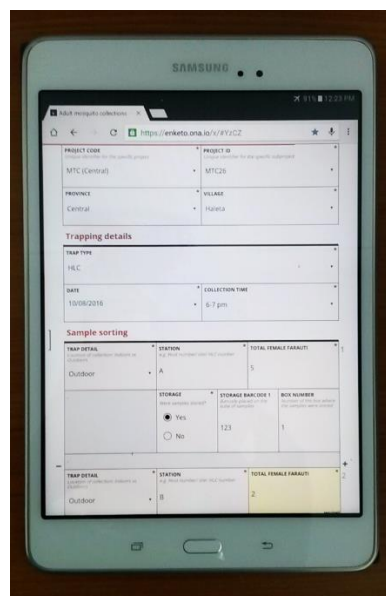
1. Tip the dead adults from one sampling effort onto a petri dish or paper towel.
 - a. Ensure you have adequate light to properly see the mosquitoes.



2. Use a microscope or magnifying glass and a taxonomic key to identify each mosquito.



3. Record the number of mosquitoes captured by taxonomic species and sex, also if specified by the work plan, record the physiological status (fed, unfed, gravid).
 - a. Always record zero counts



Storing samples

Depending on the work plan, mosquito samples may need long-term storage. Appropriate conditions are needed to protect the samples from degradation due to physical impact, bacteria and fungi.

Materials for storing adult mosquitoes include:

- ☐ Forceps
- ☐ Ethanol (optional)
- ☐ Silica gel (optional)
- ☐ Labels
- ☐ Microcentrifuge tubes
- ☐ Microcentrifuge tube storage boxes
- ☐ Data collection forms/digital device

Storage methods

1. Consider the intended purpose of the samples.

- a. For sequential molecular analyses, store the samples in small tubes containing enough 70 – 100% ethanol to cover the sample.
- b. If the mosquito morphology needs to be preserved, place the specimens in small tubes with desiccation beads separated by cotton wool. (Note that 1.5 ml specimen tubes can comfortably contain up to 10 samples in ethanol and 5 samples with cotton wool and beads.
- c. If beads are not available, use cotton wool on top and bottom of mosquitoes.

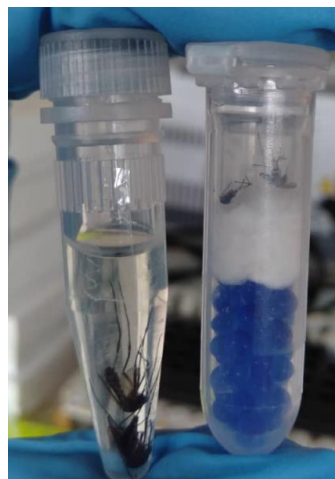
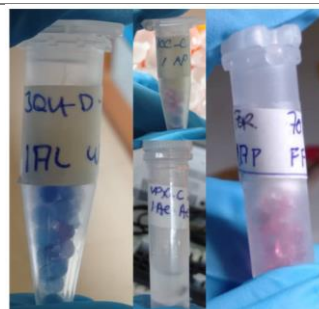


Photo by Joelyn Goi

2. It is vital that every tube is clearly and uniquely labelled.

- a. The label on each tube should contain the project code, date (DDMMYY), collection time and location. Ensure that sufficient detail is included so that each tube is uniquely labelled.



3. Store the tubes of mosquitoes in white storage boxes to keep the tubes upright.

- a. Uniquely label each box. Write the label on the side and lid of the box. Include the project code in the label.
- b. Record the details of each storage box.



Preserving and transporting samples

Mosquito samples may require preservation and transport to other locations such as specialised laboratories for further processing.

Preserving larvae for transport

1. Pipette larvae from the field sample container into a small specimen (e.g., 1.5 ml or 5.0 ml) tube.
2. Use the pipette to remove as much of the field water from the tube as you can.
3. Fill the tube with a 70-95% ethanol (EtOH) solution or isopropyl (rubbing alcohol). If using EtOH, a minimum concentration of 80% is preferred.
4. Close and label each tube with the project code (if available), date (DDMMYY) of collection, and location. Ensure that sufficient detail is included so that each tube is uniquely labelled.

Making Ethanol Stocks for preservation

If you have 100% (absolute) ethanol (EtOH) stock:

- To make 70%, add 70mL EtOH to 30mL water = 100mL 70% EtOH
- To make 80%, add 80mL EtOH to 20mL water = 100mL 80% EtOH
- To make 95%, add 95mL EtOH to 5mL water = 100mL 95% EtOH

Preserving adults for transport

1. After the mosquitoes have been killed, it is critical that they be dried thoroughly before transport. This will preserve the DNA as well as help prevent mold/fungus from growing on the samples.
 - Drying must be performed in an open or partially opened container, ideally at room temperature in an air-conditioned room to reduce environmental moisture.
 - It is best to dry for 3 days (72 hr). If time is short, dry for a minimum of 1 day (24 hr).
2. Once dried, the 1-10 adults can be added to a single 2.0 ml or 5.0 ml tube for transport.
 - Prior to adding the mosquitoes, place a piece of cotton (cotton ball, cotton pad) or similar in the bottom of the tube. If you do not have cotton, crumpled tissue or similar can be used.
 - Add adults to the tube. Adults may be fragile at this point, so it is important to be gentle and use a good pair of forceps if available. Alternatively, the adults can be gently 'tipped' into the tube.
3. Once adults have been added to the tube, place a second piece of cotton (or tissue) in the tube.
 - Push the cotton (or tissue) gently downwards until it is just above or barely touching the topmost mosquito. The idea is to keep the mosquitoes from bouncing around in transport, but do not push/squish the mosquitoes between the two pieces of cotton (or tissue).

4. Close and label the tube with an identification code that is also noted in your surveillance data records, along with the rest of the sample collection information. The date and location of the collection can also be written on the tube if there is space.

Community engagement

Community meetings should be held with the village elders and/or the entire village. Send advance notice of your intention to discuss the surveillance plan with the village before you travel to the village. The purpose of the meeting is to introduce yourselves and discuss the objective of the work as well as to receive permission to work in the village. Details include where the work will take place, and any advantages or disadvantages associated with the work. During the meeting allow questions to be asked and be sure to address any concerns that the village may have about the proposed work. If collectors are required, they should be recruited from each village when appropriate as this both provides employment and educates the community about mosquitoes.

Ethics approvals

Ethics approval for surveillance which involves using humans or animals as bait for mosquitoes may need to be obtained before starting any work, particularly if the mosquito collections are being performed for research purposes. Where approvals have been granted by your institution and/or your country's Ethics Review Board, subsequent collection of mosquito samples must comply with the requirements of the permit.

Human ethics

The first priority of any study is the safety, confidentiality and dignity of any participants involved in the surveillance program.

Participants must be over 18 years of age and not pregnant. The potential risks and benefits of the study will be discussed verbally in the local language with the aid of a participant information sheet detailing these issues in writing. The participants must provide written informed consent, and acknowledging their understanding that they are free to withdraw consent at any time.

Animal ethics

All activities relating to the care and use of animals for research should be conducted humanely. An animal is defined as any living non-human vertebrate (i.e. fish, amphibians, reptiles, birds and mammals).

Where animals are used as lures to attract mosquitoes, the animal should not be unduly stressed, managed by its owner and have access to food and water. Different individual animals should be rotated over sequential nights of sampling to allow rest periods. Animals showing signs of agitation will be immediately

released from the study. The owners will need to provide informed consent before the animal can be used.

Providing informed consent

Principles for informed consent:

- The adult >18yr recruited as a mosquito collector or animal owner must understand the language in which the informed consent material is presented.
- The research team must provide all relevant information to the participant.
- The participant must understand the information and their involvement in the study.
- The participant must agree to the proposed work in the research study.
- The participant's agreement must be voluntary and free from coercion.
- The participant must be informed that, even after voluntarily agreeing to take part, they may withdraw their agreement at any time without penalty.

The consenting process:

- Recruitment of participants will occur after consulting with the village and community leaders regarding the aims, risks and potential benefits of the study.
- Informed consent must be completed prior to collecting mosquitoes.
- Informed consent should be given in a language that the participant understands.
- Read out the information sheet word for word. **Do not paraphrase.**
- After agreeing to voluntarily participate in the study, the participant should sign and date **1 copy** of the consent form. A copy of the information sheet is for the household to keep, the signed copy of the consent form is for the office.
- The participant will be informed that even after voluntarily agreeing to take part in the study that he/she may withdraw at any time without penalty. Nothing further (such as signing anything, or collecting any personal information) should be requested from the participants.
- A copy of the signed informed consent form will be filed in the designated folder which will be kept locked in an office.

Signing the consent form:

The fieldworker is to print the date and the names of the participant/witness on the form.

If the consent giver can read there is no need for a witness

- The participant is asked to sign the informed consent form in the appropriate places.

If the consent giver cannot read then a witness is needed

- The witness is asked to sign the form confirming that the information has been provided and that the participant has fully understood the study.
- The participant should make their mark i.e. thumb print on the consent form. To make the thumb

print place the participants thumb gently, but firmly in a rolling manner on the inkpad. Then use a spare piece of paper for the first print to remove excess ink. Next, proceed to placing the thumbprint onto the proper place on the consent form in a rolling manner. Note: the thumbprint must be legible meaning that the lines present in the thumbprint must be visible.

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. Below are some examples of concerns for which risk assessments may apply to your situation.

Mosquito transmitted infections

There is a very small, but real risk of contracting malaria, dengue or other arboviruses from exposure to mosquito bites when conducting field work.

When working in the field one must:

- Within the scope of the work plan, take reasonable steps to minimise exposure to mosquito bites
- If working in a malarious area, provide access to malaria prophylaxis, rapid diagnostics and first-line malaria treatment.

What to do if a collector begins to feel sick:

- In case of illness, the participant should tell the supervisor who will contact the nearest health facility for diagnosis and appropriate health care.

Dog bites

There is a high risk of dog attack when entering a household's yard. Always talk with the occupant of the household and receive permission before entering the property. Ensure the dog is properly restrained before you enter the property. Ensure you can easily exit the property at all times. Ensure other staff members are aware that there is a dog at the house if they need to visit there.

Chloroform

Chloroform is an anaesthetic and a skin, eye and respiratory tract irritant and is suspected of causing cancer. Chloroform forms vapours and prolonged exposure of several minutes may cause dizziness and nausea. Continued exposure will produce persistent fatigue and headache. A face mask must be worn when handling Chloroform which should only occur in a well-ventilated room. This chemical should not be inhaled and should never be handled by pregnant staff members. Lastly, ensure that there are no open flames in a room where Chloroform is stored or used.

Trip hazards

Traps could be a trip hazard for staff and/or occupants. Ensure that the trap is not in a high-traffic location and communicate this potential hazard to occupants before leaving the household.

Battery hazards

Ensure that the battery is in proper working condition to avoid any irritations or burns from damaged units. Also ensure the connections are properly secured and power cords in good working condition to avoid any electrical shocks.

References

Silver, J.B. (2008) 'Mosquito ecology: field sampling methods.' 3rd edition; Springer: New York.

Acknowledgements

Content drafted by Tanya Russell, Kyran Staunton, Thomas Burkot (James Cook University), Amanda Murphy (World Health Organization), Brian Johnson and Elina Panahi (Queensland Institute of Medical Research Berghofer).

Citation

Tanya L Russell, Kyran Staunton, Thomas Burkot, Amanda Murphy, Brian Johnson, Elina Panahi, 2025. Mosquito vector surveillance, processing and storage. protocols.io

<https://protocols.io/view/mosquito-vector-surveillance-processing-and-storage-b9k7r4zn>

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