Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

GUIDELINES FOR COLONIZATION OF *AEDES* MOSQUITO SPECIES

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1- PURPOSE

This document aims to provide a description of procedures required for the establishment of *Aedes aegypti* and *Ae. albopictus* colonies in your insectary or laboratory. This guide is a summary of necessary steps such as collecting material from the field, species identification, and adapting your wild colony to laboratory conditions and artificial rearing procedures.

Before establishing a colony in the laboratory, *Aedes* material may be retrieved from an already established laboratory colony routinely maintained at another institute (possibly at the National Health Institute, Universities, Reference Reagent Resource Centers, or other research institutes), or from the field. Several guidelines have been published on the surveillance of *Aedes* mosquitoes, and include useful methods for collecting wild mosquitoes such as *Aedes aegypti* and *Ae. albopictus*:

http://ecdc.europa.eu/en/publications/Publications/TER-Mosquito-surveillance-guidelines.pdf;
http://ecdc.europa.eu/en/publications/Publications/surveillance-of%20native-mosquitoes%20-guidelines.pdf;
http://www.who.int/csr/resources/publications/dengue/048-59.pdf

2- FROM AN ESTABLISHED LABORATORY COLONY

Transferring material (such as eggs) from an already established laboratory colony is easier than to establish a colony from field-collected material as the mosquitoes have already been adapted to artificial rearing and insectary settings. The donor laboratory (sender) should ship 2 to 10-week-old eggs that have been collected preferably on germination paper or filter paper strips. The eggs should be matured and dried and sealed in zip lock bags within a sealed plastic box and properly labelled before shipment. Various types of Material Transfer Agreements exist and are recommended for donor and recipients of research materials
(see examples at: http://www.imperial.ac.uk/research-and-innovation/research-office/contracts/mta/ or https://spo.berkeley.edu/guide/mtaquick.html). It is also important for recipient to follow biosecurity rules (www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6.pdf; Arthropod Containment Guidelines).
Current international and modal requirements for the shipment should be carefully followed. Many countries have adopted the United Nations Model Regulations in their entirety (https://www.unece.org/trans/danger/publi/unrec/rev19/19files_e.html) to stand as their national dangerous goods legislation. Some countries apply variations of these.
National authorities should provide details of their own national requirements (http://apps.who.int/iris/bitstream/10665/254788/1/WHO-WHE-CPI-2017.8-eng.pdf?ua=1, July 12, 2017).
When the eggs arrive at the recipient's laboratory, they can be hatched by submerging them in water with or without additives for improved hatching. For a detailed hatching and larval rearing protocol follow the link (http://www-naweb.iaea.org/nafa/ipc/public/guidelines-for-routine-colony-maintenance-of-Aedes-mosquito-species-v1.0.pdf).

3- FROM FIELD COLLECTION

The uses of local strains are recommended for mosquito SIT programmes. *Aedes* mosquitoes can be collected directly from the field with tools and traps commonly used for entomological monitoring such as ovitraps (or "ovicups") which are traps used to collect eggs of *Aedes* spp in the areas where these mosquitoes are endemic. Alternatively, larvae and pupae can be collected directly from aquatic habitats which typically include natural and artificial containers, and other temporary water bodies (for example tyres). Larval and pupae collections can be done both in rural and urban areas using dippers or pipettes. Collected material can subsequently be reared to adulthood and bred in the insectary after species verification.

The Zootaxa *Aedes* identification keys (Rueda 2004) can help with mosquito identification to ensure correct identification of the mosquitoes (Figure 1) in an area.

Figure 1. ***Aedes aegypti*** (left) and ***Aedes albopictus*** (right).

Other useful keys and protocols are also readily available for species identification by morphology (http://www.wrbu.org/aors/aors_Keys.html) or by polymerase chain reaction (PCR) (Das et al. 2012).

Ae. aegypti and *Ae. albopictus* adults can be collected by aspiration (by backpack aspirator or a mouth aspirator) or by sweep netting (https://www.gemplers.com/tech/isweepnet.htm). Several tutorials are available on YouTube: e.g. https://www.youtube.com/watch?v=gqfFHLq_SdY. The BG-Sentinel™ trap, gravid trap and CDC light trap can also be used with varying efficacies in combination with several chemical or physical attractants (Le Goff et al. 2016).

Again, it is essential to identify and verify the species before introducing wild-caught mosquitoes into the insectary.

3.1- Ovitraps

Ovitraps for the collection of *Aedes* spp. eggs consist of a cup-like container partially filled with water and ideally dark in colour to provide shade and attract gravid females. Ovitraps should have one or two holes near the top of the cup to avoid over flooding (due to rainfall) and egg loss in the cases where the ovicup is placed in an unprotected location. A substrate protruding from the water allows gravid females to oviposit eggs thereon and for the eggs to be easily collected. Eggs can be oviposited and collected

on a wooden paddle (roughened on the side, hardboard for example) or on a paper strip (again rough is better, for example seed germination paper) (Figure 2). Using papers has the advantage in that they are easily handled, dried and stored. Egg counting and hatch rate scoring are also facilitated when eggs are collected on germination paper (see below). On the other hand, papers are subject to damage by snails or other creatures. Depending on your local wildlife, dog and cat populations, ovitraps may need to be protected by a wire mesh or secured by other means to prevent them being tipped over or carried away.

Figure 2. Example illustrations of ovitraps containing a piece of hardboard (left), wooden paddle (centre), and germination paper (right).

Ovitraps are best positioned near human dwellings, for example on porches or under the eaves of a house, and if practical and acceptable by the householders, ovitraps can also be placed within homes. Ovitraps should be placed in a shady place, sheltered from direct sunlight and rainfall, and protected from potential disturbance where possible. Where such locations are not available, some sort of cover or shelter structure may need to be added around the trap. Ovitraps, generally, are placed not more than 1.5 meters above ground for easier egg collection. A few drops of larval diet or larval rearing water (if available) can be added to the cup to increase attractiveness for gravid females. Ensure that the cups are collected or the water exchanged regularly (at least every 4-5 days) to prevent the cup from becoming a productive breeding site.

Collected eggs should be matured and slowly, progressively dried before storing them. Care should be taken to not keep egg papers too wet (with water) since this can induce unwanted hatching and not too dry to avoid excessive desiccation. Note that variations in drying/storage/hatching conditions such as temperature and relative humidity and populations can affect egg viability. Eggs are then hatched by submerging them in water containing a nutrient broth solution, larval diet, or using other methods to reduce oxygen levels in the water which stimulates hatching (see http://www-naweb.iaea.org/nafa/ipc/public/guidelines-for-routine-colony-maintenance-of-Aedes-mosquito-species-v1.0.pdf). Hatch rates are scored by counting all L1 larvae and dividing this number by the total number of eggs, or by counting hatched and unhatched eggs. The egg hatch rate equals the number of hatched eggs (or L1) out of the total number of eggs (hatch rate = hatched eggs/total eggs). Larvae can then be reared to adulthood for species identification.

3.2. Adult trapping

Adult *Aedes* mosquitoes can be collected either by actively catching them (for example by backpack aspirators, or sweep netting) or passively by using traps. Numerous adult traps currently exist in the market, and several reviews of these traps can be found online. A popular trap widely used for *Aedes* spp. adults is the BG-Sentinel™ trap (http://www.biogents.com/cms/website.php?id=/en/traps/mosquito_traps/bg_sentinel.htm), which uses a visually contrasting design and attractant air flow to lure mosquitoes into the trap where they are collected in a mesh bag and kept there by negative airflow without greatly injuring them. The trap can be used with or without an artificial lure or CO2 source (for example dry ice), depending on the catch rate in a given site. The trapping efficiency generally increases when the trap is used in combination with attractants (Le Goff et al. 2016). A battery or main power source is required to run the fan within the BG-Sentinel trap. Positioning of the trap is important for trapping efficiency and it should be placed in a sheltered location, in the shade and close to human dwellings (Figure 3), particularly if electrical power is being used.

BG-Sentinel traps can be run for an approximately 12-24-hour sampling period (depending on the battery age and capacity) before the battery requires replacement and charging. Once the battery (or power source) is depleted, the fan stops and the collected insect may escape the trap bag. Generally, airflow of 10-12 km/h is needed to retain mosquitoes within the trap bag. The airflow speed can be checked and monitored with a hand-held anemometer.

Figure 3. BG-Sentinel trap, showing the visual contrast and air flow, which attract adult mosquitoes (left) and a typical placement (centre), and a dark coloured BG-Sentinel trap close to human dwellings (right) (reference: https://www.biogents.com/)

It is recommended to add a trap # or sample ID # tag to the trap bag to keep track of information related to the trap position before pulling it closed and remove the bag while the fan is still running to avoid escapes.

Monitor battery lifespans carefully to ensure that the fan runs at the desired speed for the duration of your trapping activities.

The advantage of using field collected material is that the first generations after colonization are still behaviorally like the wild population. Enough individuals (preferably more than 500 couples) should be collected to start a genetically diverse strain. Mosquitoes that have been bred and inbred in the laboratory over many generations may experience changes in their genetic and symbiotic diversity which may significantly affect their life history traits including productivity and mating behavior. So, it's highly recommended that changes in the genetic and symbiotic diversity are being monitored during colonization to minimize any potential negative effects on the quality of the mosquito colony. *Aedes* mosquitoes collected from the field are relatively easier to colonise than other mosquito species, as they accept high density of adults in the cages and other insectarium factors that seem not to challenge the natural behavior of both males and females. However, artificial blood feeding in the laboratory is probably the most challenging step for colonization.

In arbovirus endemic regions, field collected mosquitoes can carry pathogens that can pass to the next generations through trans-ovarian transmission. Newly established strains must thus be carefully screened for the presence of human pathogens. Moreover, special precautions such as careful handling and containment should be taken to avoid insectary staff or visitors being bitten by these potentially infectious mosquitoes (see Arthropod Containment Guidelines), and additional care must be taken for the use and disposal of blood sources used for feeding the field-collected females.

4- LEARN MORE

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