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# GUIDELINES FOR ASSESSING THE EFFICACY OF INSECTICIDAL SPACE SPRAYS FOR CONTROL OF THE DENGUE VECTOR *Aedes aegypti*

By:

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## PREFACE

This document has been prepared in response to a recommendation of the Informal Consultation on 'Strengthening Implementation of the Global Strategy for Dengue Fever/Dengue Haemorrhagic Fever Prevention and Control', held at WHO/HQ, Geneva, 18-20 October 1999. It is intended to stimulate public health authorities to carry out entomological assessments of the impact of insecticidal space sprays on the main dengue vector, *Aedes aegypti*, and to guide them in that process. WHO will be pleased to assist countries in adapting the guidelines to their local needs. National authorities are encouraged to share the results of such studies and to assist the Organization to further develop the regional and global strategies for dengue fever prevention and control.

A complementary WHO document, 'Space Spray Application of Insecticides for Vector and Public Health Pest Control', will be published in 2002. This document describes the operational procedures and considerations needed to ensure correct use and application of insecticidal space sprays.

## ACKNOWLEDGEMENTS

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## 1. Introduction

*Aedes aegypti* is the principal vector of dengue in urban areas. The global strategy for dengue prevention and control calls for selective, integrated vector control, with community and intersectoral participation.

During epidemics and periods with a high risk of transmission, local authorities frequently use insecticidal space sprays in efforts to control the adult mosquito. These sprays are usually delivered from portable (backpack or hand held) or vehicle-mounted equipment, although helicopters and fixed wing aircraft have also been used. Their adulticidal effect is transitory and there is little or no effect on the aquatic stages of the vector.

The efficacy of space sprays is greatly influenced by a wide range of environmental and operational factors. For this reason, the World Health Organization recommends that public health authorities assess the impact of treatments on the vector mosquito population *under local conditions*. Such assessments should form an integral part of control operations and may also be considered as a first step in determining cost-effectiveness relative to other control strategies.

The present document is written to assist vector control managers and other public health specialists in carrying out such assessments. It does not attempt to quantify adult vector densities and other entomological parameters relating to thresholds required for interruption or reduction of virus transmission. This requires additional knowledge of factors that affect the dynamics of dengue transmission under local conditions, including seroconversion rates in the human population and viral virulence of the circulating dengue strains.

Factors that are critical to the effective use of space sprays include:

- Choice of insecticide compound and its formulation, and the susceptibility of the mosquito population to the insecticide[1]
- Dosage
- Droplet size of the aerosol

In addition, it is important that treatments are made:

- At the correct application rate
- Under optimal meteorological conditions
- During periods of maximum flight activity of the mosquito

Discussion of these factors is outside the scope of these guidelines; however, further details can be found among the relevant documents in the list of Selected References.

### *1.1 What is a space spray?*

A space spray—technically an aerosol—is a liquid insecticide launched into the air in the form of hundreds of millions of tiny droplets. Early equipment used insecticides diluted in large volumes of fuel oil or water. A heat-driven pressure system forced this solution through an atomizing nozzle into a powerful blast of hot air to produce a dense, white 'thermal fog'. Later studies showed that diluents were not critical to the efficacy of the method, and new, cold aerosol generators were developed to deliver small quantities of highly

[1] In many parts of the world, *Ae. aegypti* exhibits reduced susceptibility to several groups of insecticides, including those used most commonly for space spraying. For this reason, insecticide susceptibility tests should precede field evaluations. Standard test procedures are detailed in WHO/VBC/81.806.

concentrated insecticide, hence the term 'ultra low volume' (ULV). Both methods produce droplets ranging from 1-50 µm volume mean diameter (VMD). Larger droplets tend to settle under gravity whereas the smaller droplets do not impact readily on solid surfaces[2] and remain suspended in the air for extended periods of time. Modern ULV machines are designed to deliver the bulk of the insecticide at the optimum droplet size of 8-15 µm VMD.

### *1.2 How long does a space spray remain effective?*

After discharge from the machine, the drift of droplets is largely dependent on natural air movement. In open country, the drift distance is essentially a function of wind speed and wind direction, but for urban outdoor applications the situation is more complex. For outdoor applications, the orientation of streets and the layout, shape, thermal characteristics and juxtaposition of buildings and vegetation generate complex patterns of airflow, convection and turbulence that determine the drift path and ultimate fate of the droplets. Factors such as the size, design and placement of windows dictate penetration of droplets into buildings. Complex patterns of indoor airflow and ventilation govern their movement between and within rooms.

Indoor applications using portable equipment are less dependent on drift because the operator can direct the aerosol. Nevertheless, sites protected from air movement receive relatively few droplets; mosquitoes resting in such places are less likely to come into contact with the insecticide.

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[2] "Wet" sprays, or mists, with droplet sizes of 50-100 µm can produce an insecticidal deposit. However, such treatments are not strictly space sprays, and are not discussed here. Furthermore, their use is prohibited in some countries.

Drifting droplets tend to disperse, and this dispersal reduces the likelihood that enough droplets will impinge on the flying mosquito to deliver a lethal dose. The *effective swath width*—the distance from the point of delivery to which the aerosol droplets remain numerous enough to achieve kill—is a function of the performance of the machine, the type of insecticide, the rate of application, the droplet spectrum and all the airflow factors mentioned above. Ground aerosols from vehicle-mounted machines are usually applied to cover a swath of 60-90 m. Thus, in an open area with largely unimpeded airflow and an average wind speed of 5 km/hour (83 m/min), the droplets will drift beyond the standard swath in approximately one minute. In urban situations, where airflow is more complex, the rate of drift and dispersal may be slower.

Treatments are more effective at times when the insects are active because droplets are more likely to impinge on flying mosquitoes than on those that are at rest.

### *1.3 Why use space sprays?*

Space sprays are recommended in situations where source reduction has failed to limit the production of adult *Ae. aegypti*, and the risk of dengue transmission is high. The objective is to reduce the adult female population and its longevity as quickly as possible.

In practice, space sprays can only reduce dengue transmission if the vector population is reduced in density to below a hitherto undefined and variable threshold, or the mosquitoes are killed before they reach an age beyond which they can transmit the virus (a minimum of 8-10 days — the extrinsic incubation period). In theory, a minimum of three successive treatments must be made at maximum intervals of 7 days if virus is to be eliminated

maximum intervals of 7 days if virus is to be eliminated from both humans and mosquitoes[3]. In practice, treatments are unlikely to eliminate more than a portion of the wild *Ae. aegypti* population, so applications must continue for much longer to achieve optimum suppression of transmission. The main objectives of the entomological evaluation are to assess the degree of reduction of the adult female population and their survival that result from the space spray application(s).

## 2. Evaluation procedures: monitoring the *Aedes aegypti* population *in situ*

Because the insecticidal effect of space sprays is immediate and transient, the adult mosquito population must be monitored on a daily basis. The principle of evaluations described in these guidelines is that pre-treatment monitoring is carried out in two areas for at least three days; one area is then treated and daily monitoring continues in both areas for one week or until the population in the sprayed area recovers to pre-treatment levels. The impact of the treatment is assessed by comparing changes in the daily population count, and for

[3] It is important to consider the factors involved. When a person is infected by the bite of a mosquito, symptoms of illness generally appear after 4-7 days. This is the *intrinsic incubation period*. During incubation, the amount of virus in the blood rises until it is high enough to infect mosquitoes when they feed. The ensuing *period of infectivity* begins shortly before the onset of symptoms, and generally lasts 4-5 days. Thus, even if a spray application were to eliminate all the infected mosquitoes, virus would survive in the human population for a total of 7-12 days, and mosquitoes that emerged and fed during this period could become infected. Moreover, when a mosquito ingests virus in a blood meal, a minimum of 8-10 days must pass before the insect becomes capable of transmitting the virus - the *extrinsic incubation period*. Clearly, for effective control of dengue, the insects that emerge *after* the initial treatment but *within* the 7-12 day incubation/infectivity period must be eliminated at intervals that are less than the extrinsic incubation period, otherwise the virus will be passed on to new human hosts.

adult female resting collections, also changes in parity, in the treated area versus the untreated area.

The treated and untreated areas should be as similar as possible with respect to housing type, socio-economic characteristics, street layout and other factors likely to affect the target mosquito and the movement of the aerosol. To minimize the effect of mosquitoes entering from untreated neighbourhoods, the treated area should include a 'buffer zone', ideally extending 1000 m or more beyond the central entomological evaluation area. Natural barriers, such as rivers, large open spaces, or dense woodland can substitute for the 'buffer zone'.

In any assessment, it is preferable to use more than one monitoring method. The skill levels of workers and the availability of equipment and other resources will influence the choice of methods.

Two sampling methods are recommended for *Ae. aegypti*:

- Infusion-baited ovitraps to monitor egg-laying activity (oviposition)
- Backpack aspirator collections to monitor resting adults

Both methods have given useful results in several countries under a range of conditions. However, as with all field-sampling techniques, each has advantages and disadvantages that should be evaluated under local conditions before use.

Human landing collections of adult female *Ae. aegypti* have also been used for monitoring daily changes in abundance of the vector population. However, the method is very labour-intensive, inefficient and highly dependent on the skills of the collectors and their attractancy to blood-seeking mosquitoes. Moreover, in the absence of a

vaccine or prophylaxis, the risk of infection with dengue virus is of sufficient concern that in some countries this method of capture is considered ethically unacceptable. For such reasons, the method has not been included in the guidelines.

### *2.1 Infusion-baited ovitraps*

In its natural habitat in Africa, *Ae. aegypti* breeds in tree-holes, plant axils and other small natural sites where water accumulates. However, in villages and urban areas throughout the tropics and sub-tropics the species has adapted to a wide variety of man-made containers that serve as oviposition sites. Individual females generally distribute their eggs (oviposit) among a number of such sites, attaching them to solid surfaces just above the waterline.

Ovitraps are containers of convenient size that are placed in the field to collect the mosquito eggs. Glass jars, painted black, with clean water and a wood or 'masonite' paddle as an oviposition substrate were extensively used in the mid-20$^{th}$ century during the *Ae. aegypti* eradication campaign in the Region of the Americas. In situations where vector populations had been reduced to such low levels that larval searches were largely unproductive, they were a useful indicator of the presence or absence of the species. However, they are unsuitable for monitoring the *daily* oviposition activity because they must be left in the field for several days (usually one week).

Hay infusions are much more attractive to gravid females than clean water; ovitraps with hay infusion can therefore be deployed on a *daily* basis. The average number of eggs per trap per day is a function of the number of females present, and their oviposition activity.

### *2.1.1 Description of ovitraps*

Glass or plastic jars of around 500 ml, preferably dark in colour, are recommended. Dimensions are not critical, but all jars that are used in a particular study should be identical.

The infusion should be made by soaking a fixed amount of dry grass-hay[4] in a fixed quantity of clean tap water (e.g. 500 g in 120 l).

*Ae. aegypti* prefers to oviposit on a rough surface, so the jars are lined with a sheet of rough, absorbent paper of a type that remains strong when wet[5]. The paper is cut to a size that fits snugly, with an overlap so none of the interior surface of the jar is exposed.

Jars are set out in pairs, one containing the hay infusion and the other a 10% dilution (one part infusion plus nine parts water) of the same infusion (Fig. 1). The diluted infusion usually receives more eggs, but the presence of the undiluted infusion adds to the attractiveness of the pair.

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[4] Other dried plant material may be used, but should be field-tested for efficacy. Some workers have found that dried alfalfa is unsuitable.

[5] A satisfactory product is #76 seed germination paper, Extra Heavy Weight, Anchor Paper Co., Box 65648, St. Paul, Minn. 55165, USA. Strips of wood or masonite can be used instead of paper, but the jars must be kept scrupulously clean to discourage the females from laying eggs on the jar surface. Paper is also preferable because the eggs are distributed more evenly and are therefore easier to count.

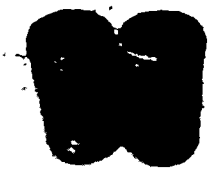

Fig. 1. Ovitrap jars in the field. The jars are always set out in pairs, one with undiluted and the other with diluted hay infusion.

### *2.1.2 Preparation of the ovitraps*

The collection procedure should be "standardized" as much as possible. Among the most important points:

- Infusions should be made in a shaded place.
- A new infusion should be set up seven days ahead of each collection day, so that the attractant is always the same age on its day of use (Figs. 2, 3).
- The same batch of hay should be used for an entire study (hay qualities can be quite variable).
- The infusion should be strained through a sieve before use to remove floating debris (Fig. 4).
- The diluted infusion should be prepared immediately before the jars are filled (Fig. 5).
- Jars should be arranged in stackable trays to prevent them from tipping during transport and two-thirds filled with infusion or diluted infusion, using a cup or other measure (Fig. 6).
- There should be a clear arrangement of trap-pairs in the trays, to avoid confusion.

- After adding the infusion, the labeled egg-papers, marked in pencil with the site-number and date, should be inserted to fit snugly against the jar walls (Fig. 7).
- The trays of jars should be protected from rain, direct sunlight and mosquitoes during transport, preferably in a covered vehicle.

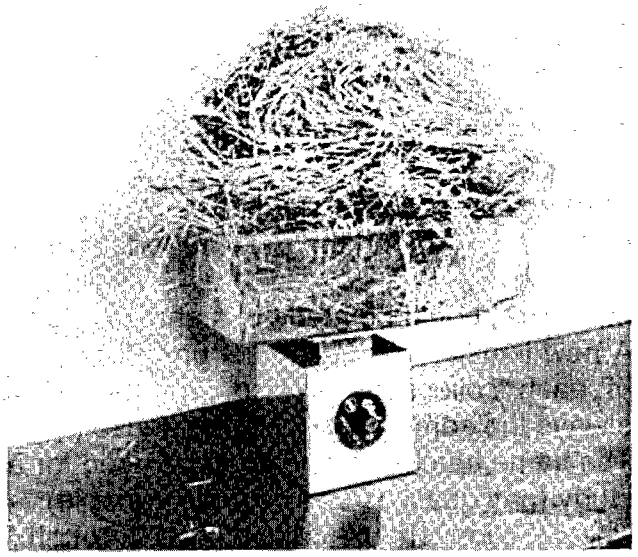

Fig. 2. Preparation of the infusion. Dry grass hay is weighed on a scale.

Fig. 3. The hay is immersed in tap water; in this case, 500 g of hay is added to 120 litres of water.

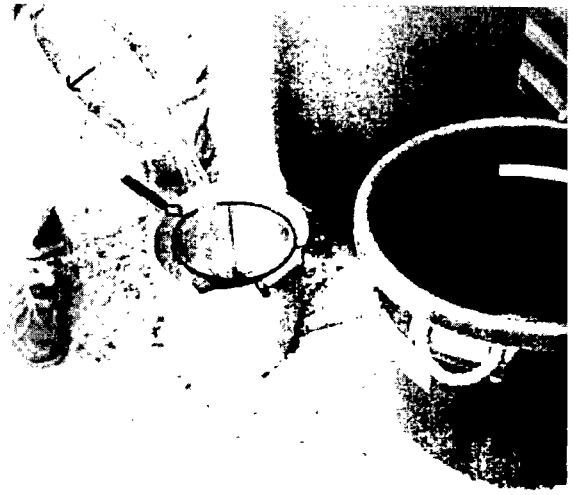

Fig. 4. After seven days the solid material is removed with a kitchen sieve.

Fig. 5. The diluted infusion (10%) is prepared immediately before filling the ovitrap jars.

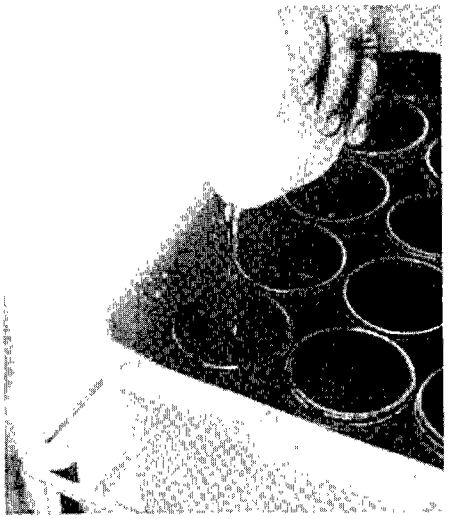

Fig. 6. Filling the ovitrap jars. Jars are filled to two-thirds with infusion, using a cup or other measure. They should be arranged in clearly marked alternate rows (undiluted/diluted) in stackable trays to prevent them from tipping during transport.

Fig. 7. Lining the ovitrap jars. Papers are pre-labeled in pencil with the site-number and date. The rough, absorbent paper is cut to a size that fits with an overlap so none of the interior surface of the jar is exposed.

### *2.1.3 Collection areas and sample sites*

The aim of the collections is to sample enough sites to obtain a statistically valid estimate of the mean number of eggs per site per 24-hour period. It is difficult to give specific guidance but experience suggests that at least 25-30 sites should be sampled in each area. In a collection area of 100 houses, roughly every 3$^{rd}$ or 4$^{th}$ house would be sampled. Practical considerations may affect selection of the actual houses where ovitraps are to be placed. For example, premises with locked gates or other obstacles can impede the work of the collectors. Sites with small children, domestic animals and birds should be avoided because the ovitraps may be disturbed.

*Ae. aegypti* uses visual cues to locate her oviposition sites, so ovitraps should be placed where they are highly visible. The base of a wall, sheltered from rainfall and direct sunlight, is ideal. A pale background, to contrast with the dark colour of the jars, is preferable.

Operators should explain the purpose of their work to the householder when soliciting permission to use a site for the study. They should clearly explain that the ovitrap pairs will be replaced at the same time of day, every day, that the jars should not be interfered with in any way, and that the infusion is completely natural and not a danger to people or pets.

### *2.1.4 Exchange of ovitrap pairs*

Two operators with one vehicle should be able to set out or service 100-150 ovitrap pairs each morning. The work should be restricted to the period of least oviposition activity, typically at least two hours after sunrise and at least five hours before sunset. The new trap-pair should be exchanged for the old one in the same position and at the same time of day as on the previous day. The infusion from the old pair should be discarded and not re-used. The operators should keep a record of their work (see examples of field and laboratory forms, Annex 1). If traps have been disturbed in any way, this must be noted and the egg-papers for that site discarded. At the vehicle, egg-papers should be removed, folded in half (eggs facing the inwards) and transferred to a covered container (Fig. 8). They should be stacked loosely to allow air circulation, and protected from sunlight and excessive heat.

### *2.1.5 Counting the eggs and interpreting the results*

The eggs are visible to the naked eye but, for accuracy, are best counted in the laboratory with the aid of a magnifying glass or binocular microscope at X10 magnification (Fig. 9). A tally counter is useful for the task. When many eggs are present, it is helpful to section off areas of the paper with pencil lines and count by section.

If there is need to hatch eggs so that larvae can be reared for species identification, the egg-papers should be kept moist for 2-3 days, then hung from lines for 1-2 days to dry at room temperature (Fig. 10). Then they can be stored in boxes or sealed bags until counting. Eggs will remain viable for at least two months provided they are kept in a cool, moderately humid environment and are protected from foraging cockroaches and ants.

The number of eggs per paper should be recorded on the field form. In regions where the eggs of other *Aedes* species are likely to be collected (e.g. *Ae. albopictus* or *Ae. polynesiensis*) samples of larvae hatched from the eggs should be reared to third or fourth instar and examined to determine which species are present.

Fig. 9. Counting the eggs. A tally counter is useful for the task.

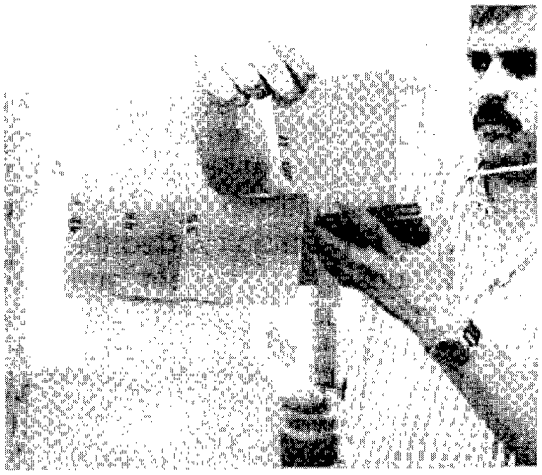

Fig. 10. Conditioning the eggs to enable hatching and species identification of larvae. Egg-papers are kept moist in a container for 2-3 days, then hung from lines to dry. After drying, they can be stored in a closed container for up to three months. A small wad of cotton soaked in water must be included to maintain humidity

## *2.2 Collection of adults by backpack aspirator from indoor resting sites*

*Ae. aegypti* is rarely found far from human habitation and readily enters buildings. Indeed, much of its activity occurs indoors or at sheltered outdoor sites. In many ecological settings and for much of the day, adults may rest indoors, in dark secluded places. Mosquitoes can be captured in such resting sites with a hand-held mouth or battery-powered aspirator or in flight with a hand net. However, both approaches are time-consuming, difficult to use in dark rooms, and are heavily dependent on the eyesight, skill and diligence of the operator.

### *2.2.1 Backpack aspirators*

A more effective device is the backpack aspirator (Fig. 11). The 12-volt battery-powered fan is powerful enough to capture mosquitoes that are 10-15 cm from the

mouth of the collection container but without damaging or killing the specimens. The flexible tube allows access to awkward sites. The components used in the construction of the aspirator can be purchased in many locations. Design and details for local assembly are given by Clark *et al.* (1994).

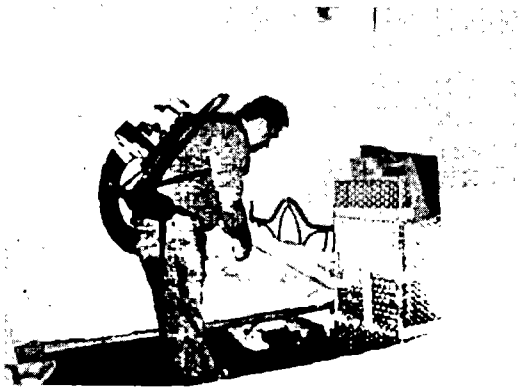

Fig. 11. Backpack aspirator. The device is easy to construct from a backpack frame, a car air-conditioner motor and fan, a length of flexible hose, and PVC piping.

Operators work best in pairs, one sweeping methodically over sites where the mosquitoes are likely to be resting or flying, while the other facilitates access by moving furniture, clothes and other articles. During this effort the assistant uses a hand-net to capture mosquitoes and adds them to those in the collection container of the backpack aspirator.

Sleeping areas and clothes closets, behind or between suspended clothes, are often very productive sites for collecting resting adults. Where dwellings comprise more than one room, it is useful to identify the most productive rooms before beginning an evaluation. By concentrating on two or three such rooms per house, a pair of operators can sample 10-15 houses per morning.

As an alternative to the CDC backpack aspirator, some workers have used modified household 'barrel-type' vacuum cleaners. These appliances must have a sufficiently powerful suction to be able to capture flying mosquitoes 10-15 cm from the mouth of the collection container and they require an easily accessible and reliable source of electricity in each house.

### *2.2.2 Field routine*

As with the ovitrap collections, the objective is to obtain a statistically valid sample of the adult female population within an area, expressed as the average number of mosquitoes per room or per house. A single collecting vessel can be used per room, or for all sampled rooms per house. As with ovitraps, maximum consistency is achieved by "standardizing" the procedure as much as possible. Among the most important points:

- Collections should be made during the period of minimum flight activity, usually from mid-morning to early afternoon.
- The same rooms should be sampled by the same operators at the same time each day.

Collection containers should be labeled with date and site of collection. They should be transported to the laboratory with their lids taped down, in a chill box to reduce the rate of physiological processes and to prevent losses due to captured predators (spiders, ants, etc.).

It is difficult to give specific guidance on the number of houses, or rooms within houses, that should be sampled. As a rough guide, if a trial run with two rooms per house and 25 houses yields a mean of at least 0.5-1.0 mosquitoes per room over several days, the sample will probably be adequate for revealing changes in the female

density and parous rate resulting from the insecticide treatment.

### *2.2.3 Processing the collection*

At the laboratory, the captured mosquitoes can be killed by placing the collection containers in a plastic bag with a small pad moistened with ethyl acetate, or in a freezer. Mosquitoes should be sorted and the female *Ae. aegypti* counted.

Where the technical skills and resources are available, unfed and freshly engorged females should be dissected to determine and compare the parous rates in the treated and untreated areas, using the method of ovarian tracheolar examination (Annex 2). A significant drop in the parous rate in the two-day period following treatment, which is not observed in the untreated area, is (all other factors being equal) indicative of the expected insecticidal killing effect; a greater proportion of the collected mosquitoes will have emerged post-treatment and hence will be nulliparous.

## 2.3 Advantages and disadvantages of the two mosquito sampling methods

Each of the mosquito sampling method has advantages and disadvantages. These are summarised in Table 1.

| Method | Advantages | Disadvantages |
|---|---|---|
| **(1) Infusion-baited ovitraps** | **Inexpensive, simple equipment**<br>**Minimal requirement for operator aptitudes, skills and diligence**<br><br>**Minimal subjective operator influence in collections**<br><br>**Efficient sampling: two operators can service 100-150 ovitraps daily**<br><br>**Entry indoors unnecessary; minimal intrusion of privacy**<br><br>**Minimal laboratory skills**<br><br>**Minimal risk of infection to operator during trap servicing**<br><br>**Monitors an epidemiologically important component of the population (gravid females)** | **Not a direct measure of mosquito abundance: results are a function of the number of gravid females present and their oviposition activity**<br><br>**If *Aedes* spp. other than *Ae. aegypti* are present, identification is time consuming and requires high level of skills** |

| Method | Advantages | Disadvantages |
|---|---|---|
| Indoor aspirator collections | Direct sample of the resting population<br><br>Collects females in all physiological stages; parous rate/longevity can be estimated | Collector aptitude, skills and diligence are highly variable<br><br>Labour intensive (two operators can sample a maximum of 10-20 rooms/day)<br><br>House entry necessary; presence and collaboration of homeowner is obligatory, and collections involve repeated intrusion of privacy<br><br>Equipment relatively expensive and requires skills for local construction<br><br>Batteries need careful charging and maintenance<br><br>Acid from batteries can be dangerous |

**Table 1. Advantages and disadvantages of infusion-baited ovitraps and indoor resting collections with backpack aspirators.**

Table 2 provides an example of the resources needed for a typical field assessment in which mosquito populations are monitored in one treated and one untreated area using infusion-baited ovitraps and backpack aspirators.

| | Ovitraps | Adult resting collections |
|---|---|---|
| **Operators** | 2 | 8 |
| **Vehicles** | 1 | 2 |
| **Basic Equipment** | 240 ovitraps<br>12 plastic tote trays<br>7 garbage bins<br>kitchen strainer (to remove hay debris)<br>3 buckets<br>seed germination papers<br>1 magnifying lens<br>hay (dry grass)<br>Shaded site for preparation of infusions<br>Water supply | 4 backpack aspirators<br>8 motor-cycle batteries (12v)<br>2 battery chargers<br>60 collection containers<br>4 butterfly nets<br>4 'styrofoam' cool boxes<br>8 ice-packs<br>Site for charging batteries |
| **Sites sampled per area** | 30 | 10 homes per team,<br>3 rooms per home |
| **Total samples** | 60 | 120 |

**Table 2. An example of the resources for a typical field assessment using infusion-baited ovitraps and backpack aspirators in one treated and one untreated area.**

## 3. Cage bioassays

Bioassays with caged mosquitoes have been widely used in field trials of space sprays against a range of mosquito species including *Ae. aegypti.* The location of cages greatly affects mortality, so they are not a measure of the overall impact of the spray on the vector mosquito population, e.g., large differences are observed between mortality rates in cages at open outdoor sites compared to

those in dense vegetation, and between cages suspended in the centre of rooms compared to those suspended in clothes closets. However, they do give an indication of the drift and penetration of droplets. They are also useful for comparing mortality of local *Ae. aegypti* strains with that of reference susceptible strains.

Cage design, materials and mesh size influence assay results. Wind tunnel tests indicate that a screen cylinder with the longitudinal axis perpendicular to the ground gives a consistent cage profile, regardless of wind direction. A simple cage design, constructed from 16-mesh nylon and two 2-cm lengths of 2.5-cm dowel rod, is shown in Fig. 12.

Fig. 12. Bioassay cage. The tube is sewn from 16-mesh nylon. The ends are plugged with 2-cm lengths of 2.5-cm dowel secured by rubber bands. Mosquitoes are introduced through a hole in the lower plug, which is then closed with a wad of cotton wool soaked in water.

Mosquitoes for bioassay should be obtained from eggs collected in the field. Larvae hatched from these eggs should be reared without crowding and with an adequate food supply to ensure uniformity of size. Shortly before the treatment, 20-25 females, 24-36 hours old and fed on a 2.5% sugar solution, should be transferred to each bioassay cage. The cages should be transported to and from the field in boxes protected from extreme heat. At least five houses in the treatment area should be used. As a minimum for evaluation of space sprays applied outdoors from vehicle-mounted machines or from aircraft, cages should be located at each house at the following sites: (a) outdoors in front; (b) outdoors at the rear; (c) indoors at an exposed site and (d) indoors in a sheltered site. The same number of cages should be exposed at similar sites in the untreated area.

Thirty minutes after exposure, the cages should be removed and returned to the laboratory in their transport boxes. They should be knocked down (immobilized) using carbon dioxide or by placing them for a few minutes in a refrigerator; transferred as quickly as possible to clearly marked, clean holding cages, each with a cotton pledget soaked with water; and held at ambient temperature (Fig. 13). Mortality in all cages should be determined 24 hours after the spray application.

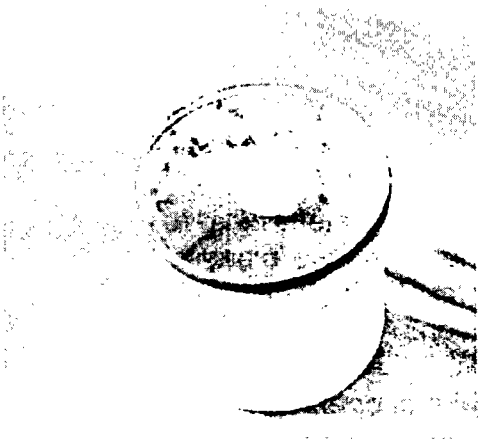

Fig. 13. Holding cage. After exposure in the bioassay cage, mosquitoes are transferred to a simple holding cage and maintained at ambient temperature. Mortality is determined after 24 hours.

## 4. Summary

All mosquito collection methods have practical limitations and introduce sampling bias. The two methods recommended in these guidelines, infusion-baited ovitraps and backpack aspirator collections, sample different portions of the adult mosquito population. The former selectively monitors the activity of gravid, egg-laying females, whereas the latter monitors indoor resting females in all stages of the gonotrophic cycle. Both methods were developed to enable public health authorities to assess the impact of space spray applications of insecticides by monitoring the *Ae. aegypti* population on a daily basis.

Infusion-enhanced ovitraps are cheap and simple to operate, use minimal manpower, and are less reliant on skills and diligence. Collections from resting sites by backpack aspirator monitor the adult population in a more direct way and provide material suitable for parous rate

determination. However, they are more labor intensive, require higher skills and dedication, and are more intrusive to local inhabitants. If resources are available, the simultaneous use of both methods will increase confidence in the results.

Insight into the movement of aerosols in the target area can be obtained by cage bioassays but these are not a substitute for monitoring effects of space sprays on the vector population.

The ultimate aim of operational evaluations is to determine whether space sprays are effective under local conditions, and if so, how often treatments must be applied in order to have an impact on dengue transmission. Local authorities should conduct such evaluations to determine whether space sprays are a useful public health intervention.

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## Annex 1

### *Field and laboratory forms*

Data are of no value unless they are accurately recorded. Each method yields data on a routine (24-hour) basis, so daily field and laboratory data forms should be used. The forms shown on the following pages are examples which can be adapted for local use.

If data are missing, this should be carefully recorded. In the case of ovitraps, the field operators should record the setting of a pair of jars and their collection on the following day. If either or both jars have been disturbed, data for the pair must be omitted from the record for that day and a note to the effect made in the margin of the field data sheet. Collectors should be encouraged to write down other relevant information and observations.

## OVITRAP FIELD & LABORATORY FORM

**District and Locality:**_____

| | | | Date of placement | Date of collection | Number of eggs | | |
|---|---|---|---|---|---|---|---|
| # | Street | Number | | | Jar A | Jar B | Jar A+B |
| 1 | | | | | | | |
| 2 | | | | | | | |
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| 27 | | | | | | | |
| 28 | | | | | | | |
| 29 | | | | | | | |
| 30 | | | | | | | |
| | | | | **TOTAL** | | | |

**Total no. of ovitrap pairs** ____

**Mean no. of eggs per ovitrap pair** ____

Notes: _____

## BACKPACK ASPIRATOR FIELD & LABORATORY FORM

**District and Locality:**_____ **Date:** _____

| | | | Room 1 | Room 2 | Room 3 | Total Number of females | Dissections | |
|---|---|---|---|---|---|---|---|---|
| | | | *Number of females* | *Number of females* | *Number of females* | | *Number nulli-parous* | *Number parous* |
| # | Street | Number | | | | | | |
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| | | | | | | | | |
| | | Total | | | | | | |

Total no. of houses sampled _____ Total no. of rooms sampled _____

Total no. of females collected _____ Parous rate (%) _____

Mean no. of females per house _____

Mean no. of females per room _____

Notes: _____

## Annex 2

### *Dissecting ovaries and determining parity*

### Purpose

To ascertain changes in the parous rate of the adult female population due to the killing effect of the insecticide.

### Essential equipment

Dissecting microscope, compound microscope, dissecting needles, fine forceps, slides, dropper, distilled water.

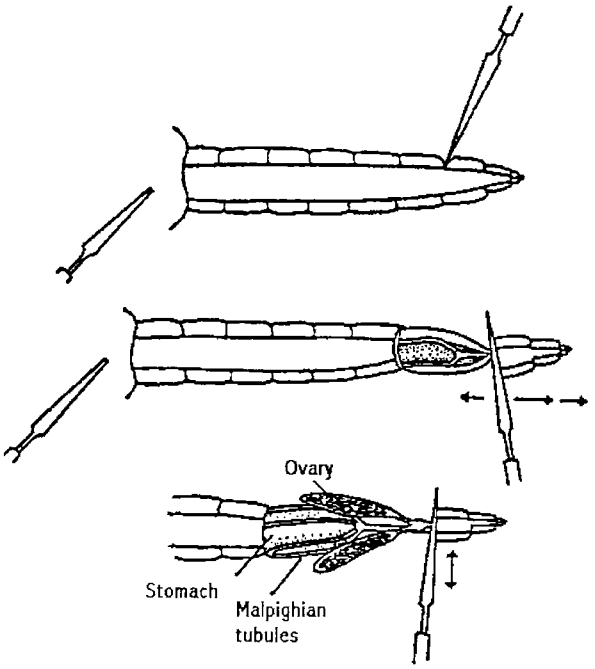
### Dissection of the female mosquito to obtain ovaries for parous rate determination

By examining the ovaries, one can tell if a mosquito is **parous** (has laid at least one batch of eggs) or **nulliparous** (has not laid any eggs).

Only females that are unfed or freshly fed are suitable for this method of determining parity. To dissect out ovaries, proceed as follows:

- Remove legs and wings from the freshly-killed female mosquito.
- Place the mosquito on a slide and add a drop of distilled water.
- While holding one needle on the thorax, pull the tip of the abdomen away from the rest of the body with another needle held in the other hand. The ovaries will then come out of the abdomen.
- Cut through the common oviduct and separate the ovaries from the rest of the specimen.

- Transfer ovaries to a drop of distilled water on another slide and allow them to dry.




**Differentiating between nulliparous and parous ovaries**

- Examine the dried ovaries under a compound microscope using the x10 objective, and if necessary, confirm using the x40 objective.
- Females in which the ovaries have coiled tracheolar skeins are nulliparous.
- Ovaries in which the tracheoles have become stretched out are parous.

- In some females not all developed eggs are laid; if some eggs (usually less than five) are retained in the ovaries, the female is parous.