GUIDELINES FOR DENGUE SURVEILLANCE AND MOSQUITO CONTROL

Second Edition

World Health Organization
Regional Office for the Western Pacific
Manila
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MOSQUITO CONTROL

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CONTENTS

FOREWORD .......................................................................................................................... vii

ACKNOWLEDGEMENT ........................................................................................................ viii

1. INTRODUCTION ............................................................................................................. 1

   Recognition and early management ................................................................. 1
   The role of mothers ......................................................................................... 2
   Basic treatment ............................................................................................... 2
   Clinical signs .................................................................................................... 2
   Activities on the prevention and control of dengue ..................................... 3

2. VECTOR IDENTIFICATION AND TRANSMISSION OF DF AND DHF .......................................................... 5

   Vector biology and breeding habitats .......................................................... 5
   Vector identification ....................................................................................... 6
   Identification of Ae. aegypti and Ae. albopictus ............................................. 9
   Basic vector biology ....................................................................................... 9
   Vector breeding habitats ............................................................................. 12

3. VECTOR SURVEY AND ITS APPLICATION ....................................................................... 17

   Larval surveys ............................................................................................... 17
   Pupal surveys ................................................................................................ 18
   Adult surveys ................................................................................................ 19
   Oviposition traps .......................................................................................... 19
   Priority areas for aedes surveillance and control ........................................ 20
   Disease surveillance ...................................................................................... 21

4. CONTROL: ENVIRONMENTAL MANAGEMENT ........................................................................ 23

   Container management .................................................................................. 23
   Elimination on alteration of breeding sites .................................................. 23
   Preventing breeding in water storage containers ........................................ 27
   Other control measures ............................................................................... 28

5. CONTROL: CHEMICAL AND BIOLOGICAL METHODS ................................................................. 29

   Chemical larviciding ...................................................................................... 29
   Biological control agents ............................................................................... 29
   Preventing spread of aedes immatures in tyre shipments ............................ 31

6. CONTROL: PERSONAL PROTECTION .................................................................................. 33

   Mosquito coils and aerosols ......................................................................... 33
   Insecticide impregnated curtains and mosquito nets .................................... 33
   Repellents ...................................................................................................... 34
7. CONTROL: SPACE SPRAY APPLICATIONS ........................................... 35
   Thermal fogs and ultra low volume aerosol sprays .............................. 35
   Insecticide formulation for space spraying ....................................... 36
   Spraying for epidemic control ....................................................... 36

8. SOCIAL MOBILIZATION AND COMMUNICATION FOR DENGUE PREVENTION AND CONTROL ........................................ 41
   COMBI ......................................................................................... 41
   COMBI planning ........................................................................... 42

9. LEGISLATION ............................................................................. 45
   Legislation and community awareness .............................................. 45

10. MANAGING OUTBREAKS ......................................................... 47
    Establishing an operation centre .................................................... 47
    Larval surveys ............................................................................. 47
    Larval control by households ......................................................... 48
    Adult control by space spray .......................................................... 48
    Health education ........................................................................... 48
    Summary of control action to take during large-scale DHF outbreaks .... 49
FIGURES

Figure 1  Differentiation of anopheline and culicine mosquitos at different stages of life cycle .......................................................... 7
Figure 2  Heads of male and female anopheline and culicine mosquitos .......................................................... 8
Figure 3  Key characters of *Ae. aegypti* and *Ae. albopictus* .................................................. 10
Figure 4  Potential breeding habitats of *Ae. aegypti* and *Ae. albopictus* in an indoor situation ........................................ 11
Figure 5  Potential breeding habitats of *Ae. aegypti* and *Ae. albopictus* in an outdoor situation ........................................... 15
Figure 6  Ovitraps for assessment of adult *Aedes* population ........................................................ 20
Figure 7  Community participation to eliminate larval breeding .......................................................... 24
Figure 8  Tyres and coconut shell breeding sites .......................................................... 25
Figure 9  Filling the tree holes .......................................................... 26
Figure 10  Cleaning roof gutter to remove leaves and debris .......................................................... 26
Figure 11  Covering water storage containers when not in use .......................................................... 27
Figure 12  Use of cloth or screen netting to cover containers used to collect rainwater .......................................................... 27
Figure 13  Woman cleaning water storage container .......................................................... 28
Figure 14  Application of larvicide in large water container .......................................................... 30
Figure 15  Infant protection under mosquito nets .......................................................... 34
Figure 16  Suggested route for space spray operation .......................................................... 39
Figure 17  Dipping mosquito nets in pyrethroid solution .......................................................... 73
# ANNEXES

<table>
<thead>
<tr>
<th>Annex</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annex 1</td>
<td>Laboratory and clinical diagnosis of dengue Haemorrhagic fever</td>
<td>51</td>
</tr>
<tr>
<td>Annex 2</td>
<td>Larval survey summary form showing house, container and breteau indices for <em>Ae. aegypti</em></td>
<td>63</td>
</tr>
<tr>
<td>Annex 3</td>
<td>Example of notification of dengue fever/dengue haemorrhagic fever by phone and written communication</td>
<td>65</td>
</tr>
<tr>
<td>Annex 4</td>
<td>Quantities of 1% temephos (Abate) sand granules required to treat different size of water containers to kill mosquito larvae</td>
<td>67</td>
</tr>
<tr>
<td>Annex 5</td>
<td>Procedure for treating mosquito nets and curtains</td>
<td>69</td>
</tr>
<tr>
<td>Annex 6</td>
<td>Selected insecticide suitable for use as cold aerosols and thermal fogs for mosquito control</td>
<td>75</td>
</tr>
<tr>
<td>Annex 7</td>
<td>Procedure, timing and frequency of thermal fogging and ULV space spray operations</td>
<td>77</td>
</tr>
<tr>
<td>Annex 8</td>
<td>Preparation of spray solutions to kill adult mosquitos during DHF outbreaks</td>
<td>83</td>
</tr>
<tr>
<td>Annex 9</td>
<td>Example of calculation of insecticide formulations for space spraying application</td>
<td>85</td>
</tr>
<tr>
<td>Annex 10</td>
<td>Behavioural objectives</td>
<td>87</td>
</tr>
<tr>
<td>Annex 11</td>
<td>Sample order form, backed by law enforcement, for distribution to house occupants to eliminate <em>Aedes</em> larvae</td>
<td>89</td>
</tr>
<tr>
<td>Annex 12</td>
<td>Procedures for collection of <em>Aedes</em> larvae with enforced legislation</td>
<td>91</td>
</tr>
<tr>
<td>Annex 13</td>
<td>Health education posters on DHF vector control</td>
<td>95</td>
</tr>
<tr>
<td>Annex 14</td>
<td>Safety measures for insecticide use</td>
<td>97</td>
</tr>
<tr>
<td>Annex 15</td>
<td>Selected references</td>
<td>103</td>
</tr>
</tbody>
</table>
FOREWORD

The main objective of these guidelines is to provide practical information on the steps for preventing and controlling outbreaks of dengue haemorrhagic fever. The main emphasis is on vector surveillance and control, and priority is given to simple environmental measures which individuals and communities can take to eliminate larval breeding. The strategy used to control adult *Aedes* mosquitoes before and during outbreaks is given within the framework of a comprehensive control approach which includes personal protection measures, space spraying, legislation, and the early recognition and treatment of dengue haemorrhagic fever cases.

These guidelines should be a valuable source of information for those engaged in controlling dengue haemorrhagic fever. Timely action by health personnel, teachers, vector control staff and members of the community, including mothers, can prevent serious illness and death, especially among infants and children.

It is hoped that these guidelines will contribute to the protection and implementation of improved dengue vector control programmes, resulting in better health of the communities at risk.

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1.

INTRODUCTION

Dengue is an arboviral disease complex which includes dengue fever (DF) and dengue haemorrhagic fever (DHF) and its subsequent dengue shock syndrome (DSS). It is caused by four serotypes of dengue virus. A dengue virus infection may be asymptomatic or it may lead to undifferentiated viral fever syndrome, dengue fever, dengue haemorrhagic fever or dengue shock syndrome.

Forty percent of the world's population (2.5 billion people) now live in areas where transmission occurs. The disease is endemic in the Americas, Southeast Asia, the Western Pacific, the Eastern Mediterranean and the tropical areas of Africa. An estimated of 50 million dengue infections occur each year, including 500 000 cases of DHF that require hospitalization.

The following information can be used to plan and implement preventive and emergency control measures against vectors of DHF. This disease is often, though not exclusively, closely associated with poor environmental sanitation, inferior housing and inadequate water supplies. Communities where such conditions prevail must be told what steps they should take to prevent and control DHF. The diagnosis and management of DHF may pose a problem for primary health care workers, as may the control of outbreaks. The disease tends to spread from large cities to smaller ones and to villages infested by vector mosquitoes, mainly *Aedes aegypti*. Transmission of the disease can be reduced by community participation in vector control. In addition, the case fatality rate of DHF can be considerably decreased if the appropriate replacement fluid therapy is given early in the course of the disease. Referral to a well-equipped hospital is not always possible, and health care workers should therefore be specially trained to cope with this situation. This applies particularly to rural areas, where *Ae. albopictus* also may be a vector.

Major vectors for DF and DHF are *Aedes aegypti* (Linnaeus) and *Ae. albopictus* (Skuse). In the Pacific islands, *Ae. polynesiensis* (Marks) under the *Ae. scutellaris* complex has also been incriminated as a vector, as well as several other minor species.

*Ae. albopictus* is an endemic species and has been associated with the transmission of DF in the Western Pacific Region since the early 19th century. Of the mosquito species imported into this area of the world, *Ae. aegypti* is often associated with the transmission of DHF. The most important vector of DHF in the Western Pacific Region is *Ae. aegypti*.

**Recognition and early management**

Basic signs:

An outbreak of DHF can be suspected in the community when:

- several children are found to be suffering from undiagnosed febrile diseases characterized by a high continuous fever of two to seven days' duration. Suspicion of DHF increases when such cases fail to respond to specific treatment for common diseases, such as malaria, meningitis, pneumonia and pharyngitis.
• unexplained deaths occur, with or without haemorrhage, within one week after the onset of an acute febrile illness.

• fever patients have petechiae (red spots on the skin), bleeding from the nose or gums, haematemesis (vomiting of blood) or melaena.

• fever patients remain ill despite a drop in temperature and the clinical situation deteriorates with the development of clammy skin, cold and sweaty extremities, drowsiness, and/or restlessness.

The role of mothers

The first step towards community involvement in DHF control is for mothers to learn that seeking early medical care for their sick children may prevent a serious outcome. Mothers must be trained to recognize the early features of DHF so that they can take their children promptly to a health centre for adequate treatment. They should be taught that the symptoms suggestive of DHF are a high continuous fever (lasting two to seven days) that may be accompanied by loss of appetite, nausea, vomiting, abdominal pain, and subsequent evidence of bleeding (persistent red spots on the skin, bleeding from the nose or gums, coffee-ground vomit, or dark stools). In particular, they should look for the early signs of shock - the patient remains ill despite a fall in temperature and develops cold clammy skin, restlessness, or drowsiness.

Basic treatment

High fever should be treated by sponging and appropriate use of paracetamol. Aspirin and other salicylates should not be given because they may lead to bleeding and cause gastric irritation and acidosis.

Oral rehydration must be attempted in the early stages of fever. Sugar and salt solution used for diarrhoeal diseases can be given in repeated small quantities. Fruit juice is preferable to plain water.

If there is any evidence of bleeding, the patient should be referred promptly to a hospital.

If the body temperature drops, the extremities become cold, and the patient becomes restless, prompt referral to a hospital or suitable health centre is necessary for intravenous fluid administration. If referral is not possible, oral rehydration should be continued until the child urinates and the skin becomes warm.

Timely fluid and electrolyte therapy can prevent mortality and facilitate complete recovery.

Clinical signs

The following clinical manifestations have been selected as indicating a clinical diagnosis of DHF. The use of these criteria may prevent overdiagnosis of the disease.

1. Fever: acute onset, high, continuous and lasting two to seven days.
2. Haemorrhagic manifestations, including at least a positive tourniquet test. Any of the following may be present:
   - petechiae, purpura, ecchymosis (red spots small surface haemorrhages);
   - epistaxis (nose bleed), gum bleeding; or
   - haematemesis (vomiting blood) and/or melaena (bloody stools).

3. Enlargement of liver: however, this is not always a constant feature in children with DHF.

4. Shock-manifested by rapid and weak pulse with lowering of pulse pressure (≤20 mmHg) or hypotension, with the presence of cold, clammy skin and restlessness.

The standard tourniquet test for capillary fragility can be carried out as follows:

1. Apply cuff of sphygmomanometer to an arm and take blood pressure.
2. Inflate cuff so that it registers a pressure midway between that of the systolic and diastolic blood pressure; hold for five minutes.
3. Examine the cubital fossa (depression in elbow) for petechiae.
   - if > 20 petechiae in a 3 cm diameter circle, the test is positive.
   - or if > 20 petechiae in a 2.5 cm (1 inch) square, the test is positive.

The test may be negative or mildly positive during the phase of profound shock. It usually becomes positive and even strongly positive, if the test is done after recovery from shock. A more detailed laboratory diagnosis and clinical case definition is described in Annex 1.

**Activities on the prevention and control of dengue**

Currently, the only methods for preventing and controlling DF/DHF are to ensure prompt diagnosis of fever cases and appropriate clinical management, to reduce human-vector contact, and to control larval habitats in and around domestic environments. A range of *Aedes* control under different situations are summarized below:

**Aedes control methods:**

- Environmental sanitation measures to reduce mosquito breeding sites, such as physical management of water containers, improvement of water supplies and solid waste management.
- Biological methods (e.g. fish, copepods, *Bacillus thuringiensis*).
- Chemical methods against the mosquito larvae (e.g. temephos sand granules, insect growth regulators).
• Chemical methods directed against adult mosquitoes, such as insecticide space sprays or residual application.
• Personal protection through use of repellents, vaporizers, mosquito coils, and insecticide-treated materials (screens, curtains, and bednets).
• Health education and communication to achieve behavioural change.

The Global Strategy

The Global Strategy was established in 1995 to focus and coordinate national efforts in the prevention and control of DF/DHF. It consists of the following five elements:

• Selective, integrated mosquito control with community and intersectoral participation, in which control is directed towards geographic areas of highest risk of transmission, integrating all appropriate methods in the most cost-effective and economic manner.
• Active disease surveillance based on strong health information systems, involving clinical and laboratory-based dengue surveillance for early detection of epidemics and vector surveillance for monitoring and evaluation of control programmes.
• Emergency preparedness, necessitating development of emergency and contingency plans, including education of the medical community, hospitalization plans, case management and emergency vector control.
• Capacity-building and training in surveillance, laboratory diagnosis, case management and vector control at professional, supervisory, technical and field levels.
• Vector control research including studies on vector biology and control, disease relationships, design and management of control programmes, including social and economic approaches, and cost-benefit analyses.
2. VECTOR IDENTIFICATION AND TRANSMISSION OF DF AND DHF

The vectors (carrier mosquitoes) of DF and DHF breed in and around houses and can be controlled by appropriate individual and community action. This approach should be adopted in extending vector control coverage to communities which do not routinely benefit from the activities of an organized vector control service.

The disease is transmitted by female *Aedes* mosquitoes. For practical purposes it may be assumed that the vector is *Ae. aegypti*, a mosquito that bites during early morning and late afternoon part of the day, rests in houses, and lays eggs in artificial and natural water containers. *Ae. albopictus* is also a vector and a species of the *Ae. scutellaris* complex such as *Ae. polynesiensis* can also lay eggs in both natural and artificial breeding sites such as tree holes and tyres.

The eggs of *Aedes* mosquitoes are laid singly above the water level of containers. They hatch after the containers become flooded with rain or filled with water by individuals up to six months later. The adult *Ae. aegypti* is easily recognized by distinctive lyre shaped markings on the thorax (see Figure 3). They fly short distances, usually less than 300 metres from the breeding sites, and transmit disease from person to person.

The duties of health inspectors and related vector control personnel should include health education activities aimed at increasing community participation in the control of DHF vectors. They should collaborate in providing technical guidance in simple and understandable language to community leaders, primary health care workers, schoolteachers and others who have day-to-day contact with the community. Special attention should be directed towards house occupants and especially parents in mosquito control methods. This may include pointing out breeding places and distributing information leaflets.

Radio broadcasts, press releases, posters and group talks are useful activities. Radio and television announcements, and pamphlet distribution can promote community clean-up campaigns. Environmental topics can be included in primary school curricula. Since DHF is an ever-present threat, health education should be built in progressively, beginning at school and continuing throughout life, based on simple but accurate information and using all available media, such as school books, lectures, radio, television, posters, pamphlets, plays and group discussions. Law enforcement measures can be used to strengthen community compliance and responsibility.

Vector biology and breeding habitats

To plan vector control, it is necessary to identify the DF and DHF vector and understand its basic biology and breeding habits.
Vector identification

The major differences between anophelines (subfamily Anopheinae malaria vectors) and culicines (subfamily Culicinae which have many genera of mosquitoes including Aedes) are shown in Figure 1.

The eggs of anophelines and some culicines (e.g. Culex species) float on the surface of water. However, for some of the culicines (e.g. Aedes), the eggs may be found on moist substrates and on the surface of water. The Aedes eggs can survive under dry conditions in a tropical environment for some time.

The eggs of anophelines are laid singly. In the culicines, they are clumped together in a raft (e.g. Culex) or are laid singly (Aedes). In addition, only anopheline eggs have "floats" (Figure 1).

At the larval stage, the culicine larva (e.g. Aedes and Culex) has an expanded breathing tube called a siphon. In the anopheline larva there is no siphon. With the aid of the siphon, the culicine larva hangs down some distance from the water surface, whereas the anopheline larva rests parallel to and immediately below the water surface (Figure 1).

For identification of the Aedes and Culex larvae within the culicine group, the diagnostic feature used is that the siphon of the Aedes is shorter with only one single tuft of setae. In contrast, the siphon of the Culex is longer with a few tufts of setae (Figure 1).

At the pupal stage, the anophelines and the culicines can be differentiated by the shape of the breathing trumpet. For the anophelines, the breathing trumpet is short with a wide opening. In contrast, the breathing trumpet of the culicine pupa is long and slender with a narrow opening (Figure 1).

For the adult stage, identification of anophelines and culicines can be done by differentiating between the length and shape of the (palpi) palps in the head region (Figure 2).

Females: Anophelines - palps as long as proboscis.
Culicines - palps very much shorter than proboscis.

Male: Anophelines - palps as long as proboscis, club-shaped at tip.
Culicines - palps longer than proboscis with tapered tips.
Figure 1. Differentiation of anopheline and culicine mosquitoes at different stages of life cycle
Figure 2. Heads of male and female anopheline and culicine mosquitoes
With live mosquitos, anopheline and culicine adults can be differentiated by observing their resting postures. Anophelines rest at an angle of between 50° and 90° to the surface whereas culicines rest more or less parallel to the surface (Figure 1).

**Identification of *Ae. aegypti* and *Ae. albopictus***

The common house-frequenting *Aedes* (Stegomyia) species are relatively small to medium size mosquitos. *Ae. aegypti* and *Ae. albopictus* adults are black in colour with distinctive white patches of scales distributed throughout the body.

The adults of *Ae. aegypti* and *Ae. albopictus* can easily be differentiated by the patterns of white scales on the dorsal side of the thorax. For *Ae. aegypti*, the pattern consists of two straight lines surrounded by curved lyre-shaped lines on the side. In contrast, *Ae. albopictus* has only a single broad line of white scales situated in the middle of the thorax (Figure 3).

At the larval stages, the two *Aedes* species can be differentiated by the following features (Figure 3):

- The shape of the comb scales (comb teeth) on the eighth segment of the abdomen:
  - for *Ae. aegypti*, the comb teeth have well-developed lateral denticles.
  - for *Ae. albopictus*, the comb teeth have no lateral denticles.

- The shape of the pecten teeth on the siphon:
  - for *Ae. aegypti*, the pecten teeth have less defined denticles.
  - for *Ae. albopictus*, the pecten teeth have three well-defined pointed denticles.

**Basic vector biology**

The act of laying eggs by mosquitoes is known as oviposition.

The eggs of *Aedes* mosquitoes are laid singly on substrates above the water surface of containers. They hatch after the containers become flooded naturally (rainfall) or artificially (human water storage).

When dried under natural conditions the eggs can retain their viability for up to six months or longer.

Flooding and submerging these dried eggs can induce partial hatching from the egg batches. Subsequent drying and flooding can induce further hatching from the remaining unhatched eggs of the same batch.

The *Aedes* larvae generally breed in clean and unpolluted water. However, they have been known to breed in septic tanks and other polluted water sources where polluted water breeding mosquitos such as *Culex quinquefasciatus* are commonly found.
Figure 3. Key characters of *Ae. aegypti* and *Ae. albopictus*
Generally, the immature stage of *Aedes* mosquitoes requires about seven days before adult emergence in a tropical environment. As such, any container, natural or artificial, that can accumulate water for that length of time can become a potential breeding habitat for *Aedes*.

The adult female *Aedes* mates and takes its first blood meal about 48 hours after emergence, and can take multiple blood meals between different gonotrophic/generational cycles. Engorgement to oviposition takes two to five days. Generally, a single female lays about 60-100 eggs in initial oviposition.

Laboratory studies on the survival potential of adults for both *Aedes* species indicate that the male and female mosquitoes survive an average of 20 and 30 days, respectively. Thus, each *Aedes* female can theoretically deposit up to four batches of eggs with subsequent blood meals.

*Aedes* are considered "day-biters" with two peaks of biting activities, one at dawn after sunrise and another at dusk before sunset. The major peak of biting occurs around one hour before sunset. Both *Aedes aegypti* and *Ae. albopictus* also appear to bite throughout the day and night, although at a very low density level.

After feeding on a person whose blood contains dengue virus, the female *Aedes* mosquitoes normally require an incubation period of 8 to 10 days, when the virus multiplies in the mosquito salivary gland. Thereafter, the mosquito becomes infected and will transmit the dengue virus to the next human host when feeding occurs again. In humans, the incubation period is from five to seven days. The female *Aedes* mosquito can also transmit the virus immediately from an infected person to another individual by a change of host when its blood meal is interrupted. This is termed "mechanical transmission."

The flight range of *Aedes* is rather short in comparison with other genera of vector mosquitoes such as *Anopheles*, *Culex* and *Mansonia*. Generally, the adults will be found around 50 metres from the breeding sources with maximum flight distance of around 200 metres.

Laboratory and field studies indicate that both *Ae. aegypti* and *Ae. albopictus* appear to prefer darker colour backgrounds for oviposition, with special preference for red and black over lighter colours.

*More Ae. aegypti* are found indoors than outdoors, unlike *Ae. albopictus*. Generally, *Ae. aegypti* prefer to rest indoors in shaded places, whereas *Ae. albopictus* prefer to rest outdoors in shrubs and trees.

For oviposition, *Ae. aegypti* lays eggs in practically all types of artificial containers, and in some natural containers, and *Ae. albopictus* oviposits in both natural and artificial containers.
Vector breeding habitats

In relation to their biology, both Aedes species breed in and around houses in close association with human habitations.

The main indoor breeding sites are:

- earthen jars used for water storage;
- ant traps for protection of food cabinets in kitchens;
- concrete water storage tanks for bathrooms;
- uncovered water storage tanks;
- flower vases;
- saucers for ornamental potted plants;
- softdrink bottles;
- water trays of refrigerators with automatic-defrosting and air conditioner trays;
- metal drums for water storage;
- plastic containers; and
- any other containers which can accumulate water for up to seven days.
Figure 4. Potential breeding habitats (indicated in dotted circle) of *Ae. aegypti* and *Ae. albopictus* in an indoor situation
Main outdoor breeding sites are:

- treeholes;
- bamboo stumps;
- leaf axils of various plant species (palm, banana, yam, etc.);
- earthen jars for water storage;
- bamboo pots and stumps;
- discarded bottles and tins;
- discarded tyres;
- metal drums for water storage;
- ram barrels for collecting rainwater;
- deficient and clogged up roof gutters;
- coconut shells and husks;
- latex collection cups in rubber plantations;
- cocoa husks and pods;
- canoes and small fishing boats; and
- all artificial containers which can breed mosquitoes.
Figure 5. Potential breeding habitats of *Ae. aegypti* and *Ae. albopictus* in an outdoor situation.
3. VECTOR SURVEY AND ITS APPLICATION

The main purpose of vector surveys for surveillance is to obtain information that can be used to control the *Aedes* vector which transmits dengue virus to humans.

Surveillance activities are to:

- determine the key containers in the domestic environments so that larval source reduction by community participation may be carried out through health education.
- pinpoint high-risk areas, especially those with high vector density, by plotting vector distribution and numbers of DHF cases on maps. These areas serve as priority areas for control during normal conditions especially during epidemics.
- determine seasonal population fluctuations for special emphasis on control and alertness during peak vector periods.
- monitor the impact of vector control interventions including community participation and insecticidal space spraying on vector population.
- recognize significant changes in vector density, distribution, insecticide susceptibility and vectorial capacity to plan control strategy.

Larval surveys

Three indices are commonly used to record *Ae. aegypti* and *Ae. albopictus* density levels:

1. The House (premises) Index (HI) or *Aedes* Index:
   
   Percentage of houses or premises positive for *Aedes* larvae. The HI is calculated as follows:

   \[
   HI = \frac{\text{No. of houses positive for } Aedes \text{ larvae}}{\text{No. of houses inspected}} \times 100\%
   \]

2. Container Index (CI): percentage of water-holding containers positive for *Aedes* larvae. CI is calculated as follows:

   \[
   CI = \frac{\text{No. of positive containers}}{\text{No. of containers inspected}} \times 100\%
   \]

3. Breteau Index (BI): number of positive containers per 100 houses in a specific location. BI is calculated as follows:

   \[
   BI = \frac{\text{No. of positive containers}}{\text{No. of houses inspected}} \times 100
   \]
Among the above three indices, the HI has been widely used to calculate the presence and distribution of *Aedes* populations in a given locality. However, the HI does not take into consideration the number of positive containers per house. Similarly, the CI only provides information on the proportion of waterholding containers that are positive. On the other hand, the BI establishes a relationship between positive containers and number of houses. Hence, the BI is considered the most useful single index for estimating *Aedes* density in a location. The BI and HI are commonly used for the determination of priority (risk) areas for control measures. Generally, a HI greater than 5% and/or a BI greater than 20 for any locality is an indication that the locality is dengue-sensitive. For epidemiological purposes, the HI is extremely important and indicates potential spread of virus through an area once an infected case becomes established.

On notification of a DHF case, all houses within a 300 m radius of the case house are surveyed and vector control measures are implemented as described in Section 10. A routine survey is carried out at least once every three months except in a priority area, where it should be carried out at least once a month. A sample *Aedes* larval survey form is shown in Annex 2.

**Pupal Survey**

It should be noted that larval indices are a poor indication of adult production. For example, adult emergence rates from rainwater jars are likely to differ markedly from those from discarded cans or natural habitats, yet the larval survey recorded them only as positive or negative. The implication is that for localities with similar larval indices but different container profiles, the adult densities and hence the transmission potential may be quite different.

The rates of recruitment of newly emerged adults to the adult mosquito population from different container types can vary widely. Estimates of relative adult production may be based on pupal counts (i.e., the counting of all pupae found in each container). The corresponding index is:

Pupal index (PI): number of pupal per 100 houses

\[
\text{PI} = \frac{\text{Number of pupae}}{\text{Houses inspected}} \times 100
\]

Given the practical difficulties and effort entailed in obtaining accurate pupal counts, especially from the large containers, this method need not be used in routine survey, but may be reserved for special studies or assessment.

**Sampling size in *Aedes* Larval Survey**

For *Aedes* larval surveys, the number of houses to inspect in each locality depends on the level of precision required, level of infestation, and available resources. Although the more houses inspected, the greater the precision, it is usually impractical to inspect a large percentage of the houses because of limited human resources.
Several sampling procedures that eliminate or minimize bias can be used for the selection of houses for larval survey. Systematic sampling of every "nth" house throughout a community or along linear transects through the community is the most widely accepted sampling method. For example, if a sample of 25% of the house is to be inspected, every 4th house (=100/25) would be inspected.

Simple random sampling methods, whereby houses to be selected are obtained from a list of random numbers, either from the random number tables or from the computer-generated list. This is a more accurate method and will require detailed house lists or location maps for identifying selected houses.

**Adult Survey**

Surveys of adult mosquitos are more time consuming (labour intensive) and the results are less satisfying than larval surveys. When the collection technique involving human bait collections, ethnical issue should be taken into consideration.

Human bare-leg catches (landing catches) of Aedes adults (both males and females) or indoor resting collections of adults are normally used to assess adult Aedes populations. Such catches are presently being discouraged because of risks of further spread of the disease. When collecting mosquitos landing on the body, every effort should be made to collect female mosquitos in tube before they begin to bite. The data collected are calculated to reflect the number of female Aedes mosquitos landing/biting on a single human bait per hour (e.g. number per human hour). The collectors should move from house to house and not collect in one place for more than 15 to 20 minutes. In a similar manner, indoor resting collections can be made and the data expressed as numbers collected per human hour or per house.

The adult collections can be used to determine the effectiveness of the control strategies used. Density levels are recorded before and after control. If epidemic space spraying is carried out, the dissection of adult female mosquitos to determine parous condition (young or old mosquitos) can also be done. Further details on evaluation of epidemic spraying are found in Annex 7.

**Oviposition traps**

"Ovitraps" provide a sensitive and economical method for detecting the presence of Ae. aegypti and Ae. albopictus in situations where the Aedes density is low and general larval surveys produce unsatisfactory results (e.g. when the BI is < 5).

The standard ovitrap is a wide-mouthed glass jar of approximately 250 ml which is painted black on the outside to attract the Aedes females to oviposit. A piece of hardboard or a wooden paddle is placed diagonally inside the glass as an oviposition substrate. In addition, the jar is partially filled with clean water to provide the right ovipositing medium for the female mosquito (Figure 6). Such jars in the absence of ovi-paddles can have white towelling strips placed inside attached by paper clips.

Generally, the ovitraps have proven useful for the early detection of new Aedes infestations in areas where the Aedes mosquitos have not been established previously. Hence, they are extensively used for surveillance at international ports of entry, (airports and seaports) which, according to international sanitary codes, should be maintained free of vector breeding.
Ovitraps can also be used to assess *Aedes* population fluctuation over a long-term period, especially in epidemiological studies of dengue infection.

A recently developed "enhanced CDC ovitrap" has proved much more attractive to gravid females than clean water; ovitraps with hay infusion can therefore be deployed on a daily basis. Unlike the original version, with which positivity rates and eggs counts are seldom sufficiently high, the enhanced ovitrap has proven suitable for monitoring changes in adult female populations following the adulticidal space spraying.

**Priority areas for *Aedes* surveillance and control**

Due to the widespread distribution of *Aedes* vectors as well as the expense of insecticide spray, judicious use of available resources to prevent or lessen DF/DHF outbreaks depends very much on setting up priority and high risk areas.

Priority areas for vectors surveillance and control are those having a concentration of cases and/or a high vector density. Special attention should be given to areas with high human concentrations, such as housing estates in urban areas, hospitals, schools and factories.

![Figure 6. Ovitraps for assessment of adult *Aedes* population](image)
Generally, priorities can be allocated as follows:

Priority I: Localities where an outbreak of DF/DHF has been recorded in the past.

Priority II: Localities in urban areas with high HI and/or BI e.g., HI≥5%, BI≥20.

Priority III: Localities in urban areas with relatively low larval indices. e.g. HI≤5%, BI≤20.

Priority IV: Rural areas where there are no dengue cases and low Aedes indices.

With respect to human bare-leg (landing) catches of Aedes adult females, areas with densities greater than two per human hour are considered high risk, whereas those less than 0.2 are low risk. However, outbreaks can occur at even lower vector densities in congested areas where isolated pockets of heavy breeding occur.

Because resistance to insecticide affects the effectiveness of the vector control measures applied, monitoring of insecticide resistance in conjunction with vector surveillance should also be carried out.

Disease surveillance

There are two types of DF/DHF surveillance: active and passive. Active surveillance implies a purposeful search for dengue infection, especially in situations where it might be attributed to other causes, such as influenza or rubella. Passive surveillance depends upon case reports from physicians or other health personnel who recognize dengue-like illness. In most countries where dengue transmission is reported, the surveillance system is of the passive type.

The main objectives of dengue surveillance are:

- to detect early warning signs of outbreaks in order to initiate timely and effective control measures;
- to monitor the trend of dengue incidence, including temporal and geographic distribution and severity of infections; and
- to monitor the case fatality rate of DHF.
Every dengue endemic country should have a surveillance system, mandated by law, making DF/DHF a notifiable disease. Health care units should report the following statistics as a minimum:

- Number of suspected DF cases.
- Number of suspected DHF cases.
- Number of confirmed dengue cases out of the total suspected DF/DHF cases.
- Number of deaths from DHF (suspected or confirmed).

Surveillance should require case reports, where each patient is recorded with age, sex, location, date of onset and major clinical characteristics from every clinic, private physician or health centre that provides medical attention to the population at risk.

Where case-based surveillance is not possible, it should be attempted to report cases by age-group and administrative unit. In dengue programmes, the age-group division has traditionally been less than 15 years and 15 years and above. For coordination with Integrated Management of Childhood Illness (IMCI) and other child health programmes, it is desirable to consider the age-groups less than 5 years and 5-14 years separately.

When and where there is a high risk of epidemics, reporting should be weekly or daily; in other situations, monthly reporting will be satisfactory.

The system should require that every suspected case of DF/DHF be communicated by telephone to the relevant authorities followed by a written notification and confirmation. See Annex 3 for guidelines on telephone notification of cases.

Case reports should be investigated to determine the time of onset and location of possible transmission (based on patient's movements for the past two weeks prior to date of onset) as well as the population at risk. The laboratory diagnostic data should be obtained and updated as they will be needed for follow-up vector control measures. Annex 1 shows a model of the investigation form.

**Periodicity of international reporting**

Member States are encouraged to report their dengue statistics to WHO's Western Pacific Regional Office every three months, through the WHO Representative Offices. The reports may be submitted electronically, and a breakdown by first administrative unit (e.g. province), month, age-group (e.g. 0-4 years, 5-14 years and 15 years and above) is welcome. WHO is trying to improve its own capability to provide early feedback at regional and global levels including the use of Geographical Information Systems. At least at the end of the calendar year, a hardcopy of the summary for the year should be submitted under an official, signed cover letter.

Special reports on suspected outbreaks should be submitted as early as possible, and preferably not only to WHO, but also directly to neighbouring countries and to such networks as the Mekong Basin Disease Surveillance (MBDS) Project.


4. CONTROL: ENVIRONMENTAL MANAGEMENT

*Aedes* larvae are container-breeders that thrive in both clean and organically rich water in natural and artificial containers. Hence, container management to reduce the sources of breeding habitats is one of the best approaches for controlling *Ae. aegypti* and *Ae. albopictus*.

**Container management**

Container management should take into consideration the household's use of the containers. If the population considers the containers to be useful or essential (such as earthen jars, rainwater drums, ornamental plant containers), then the strategy employed will be the prevention of *Aedes* breeding in containers rather than destruction or removal of the containers.

If the containers are considered to be useless or non-essential (such as discarded tyres, abandoned domestic containers), then removal and destruction is desirable.

Natural habitats that accumulate water (such as tree holes, plant axils, bamboo stumps) can also be either eliminated or appropriately modified to prevent the breeding of *Aedes*.

Examples of the possible breeding habitats of *Ae. aegypti* and *Ae. albopictus* have been described in Figures 4 and 5.

**Elimination or alteration of breeding sites**

Destruction or elimination of unwanted natural and artificial containers in and around human living premises definitely contributes to an overall reduction of the *Aedes* population. Examples of such source reduction are as follows:

- Rubbish, including artificial and natural containers, should be cleaned up, packed in disposable plastic bags and sent away through the local rubbish collection system. Municipal sanitation department collaboration should be obtained. If a local rubbish collection system is not available, then the discarded containers should be buried (Figure 7).

- Discarded tyres should be disposed of in such a way that they are kept away from occupied premises and not exposed to rain. In tyre collection and storage areas for the districts, they should be properly arranged, placed under shelters and covered to prevent breeding of *Aedes* (Figure 8). Tyres also can be shredded and placed in a landfill away from populated areas.
Figure 7. Community participation to eliminate larval breeding

Cleaning the environment and removing breeding sites

Tins and bottles in plastic bags for disposal

Burying of discarded tins and other rubbish
Proper arrangements of tyres by stacking and covering the top. Tyres should be placed in sheltered areas not exposed to rainfall.

Coconut shells and husks should be removed to prevent accumulation of water and larval breeding near houses.
Tree holes around housing compounds should be filled with sand or concrete to prevent breeding (Figure 9).

Structurally deficient roof gutters should be repaired. All roof gutters should be cleared of debris regularly (Figure 10).

Household and garden utensils (buckets, bowls and watering devices) kept outdoors should be turned upside down when not in use to prevent accumulation of rainwater and breeding of *Aedes*.

Leaf axils of various plant species within the house compound (palm, banana etc.) often contain rainwater that should be removed or treated with proper larvicides to prevent *Aedes* breeding.

Canoes and small fishing boats should be emptied of water and turned upside down when not in use.

Agricultural crop remains (coconut shells, cocoa husks) should be disposed of properly. For the extraction of cocoa seed, the fruit should be opened with three cuts, one at the middle and two at both ends to prevent accumulation of water.
Preventing breeding in water storage containers

Water storage containers in various shapes and sizes (earthen jars, drums, barrels, tanks and concrete storage vessels) are essential for daily use especially in areas where the water supply is unreliable. Collection of rainwater from the house roof through gutters into barrels is a common practice in such areas. Proper management of the essential water storage containers is shown below.

Figure 11. Covering water storage containers when not in use

Figure 12. Use of cloth or screen netting to cover containers used to collect rainwater
For large water containers that are frequently used or that need to be left opened, application of suitable larvicide (chemical or biological agents) at recommended dosage should be carried out. Fish or other biological agents can be placed in water tubs as described in Section 5.

Other control measures

Other types of control that can be used in certain situations are puncturing water-logged tree holes with a knife, leveling or filling in tops of bamboo fences to prevent accumulation of water and breeding sites, filtering water from one container to another through cloth in order to trap and dislodge larvae and pupae (this technique conserves water), and pouring boiling water down the sides of small earthenware jars to kill larvae and eggs when the water level is low.

For other household containers that inherently and regularly accumulate water, such as those of ant traps for food cabinets, flower vases, bottom saucers for ornamental pot plants, water trays of refrigerators for automatic defrosting, and condensed water collections from air conditioners, the following steps can be taken.

- Proper larviciding, such as addition of chemical or biological larvicides or table salts, should be done when appropriate.
- Water in containers should be changed or discarded at least once a week.
- The edges and sides of the containers should be scrubbed and cleaned to remove possible deposited *Aedes* eggs.

![Figure 13. Woman cleaning water storage container](image)
5.
CONTROL: CHEMICAL AND BIOLOGICAL METHODS

Chemical larviciding

Chemical larviciding including organic synthetic insecticides such as temephos (Abate) and insect growth regulators (IGRs) such as methoprene (Altosid, juvenile hormone mimic) have been shown to be effective against container breeding *Aedes* mosquitoes in clean water. The environmental impact of the above chemical larvicides is minimal if they are properly used in human premises.

Temephos (Abate) 1% sand core granules applied at a dosage of 1 ppm (10 grams of granules to 100 litres of water) can provide effective control for 8 to 12 weeks, especially in porous earthen jars, if the water is not changed. Resistance of *Ae. aegypti* and *Ae. albopictus* to temephos has not been reported in member countries of the Western Pacific Region. In countries using temephos, tests should be carried out, to determine the susceptibility level of the *Aedes* mosquito, to ensure the effective use of the insecticide. Annex 4 shows the quality of sand granules required to treat various sizes of water jars.

IGRs interfere with the development processes of the mosquito. Most IGRs have exceedingly low mammalian toxicity (LD50 value of acute oral toxicity for methoprene is 34 600 mg/kg). In general, IGRs provide long-term residual effects (three to six months) at relatively low dosages when used in porous earthen jars. However, the action of IGRs involves interference of chitin synthesis during the moulting process of immatures or disruption of pupal and adult transformation processes of mosquitoes. Thus, the use of IGRs will not cause immediate mortality of the immature. For countries with legislation stipulating that the breeding of *Aedes* larvae is an offence, the use of IGRs will require some alteration of the law so as not to penalize homeowners in whose residences these compounds are applied.

It is difficult and expensive to apply chemical larvicides on a long-term basis. They are best used in situations where epidemiological data and past experience indicate the existence of certain risk periods and localities where outbreaks might occur. Establishing the precise timing and location are essential for greatest effectiveness. Control personnel distributing larvicides should always continue to encourage house occupants to control larvae by environmental measures.

Large containers are not easy to clean or to mosquito proof and may require chemical larviciding until a more permanent answer is found.

Biological control agents

The bacteria *Bacillus thuringiensis* H-14 (*Bt*. H-14) has reached the stage of operational usage for control of mosquito immatures. *Bt* has exceedingly low mammalian toxicity (LD50 values for both acute oral and dermal toxicity are more than 30 000 mg/kg). Although not used extensively in the Western Pacific Region, its proper use in drinking water does not present a health hazard.

There is a whole range of formulated products of *Bt* produced by several major chemical companies for control of vector mosquitoes. Such products include wettable powders such as Bactimos,
corn-cob granules such as Vectobac, suspension concentrate such as Teknar, briquettes and pellets. These will vary in residual effectiveness and in most cases are less residual than temephos.

Larvivorous fish (top feeding minnows) can be used as complementary means of Aedes immatures control especially in large and permanent water storage containers. However, a rule in larvivorous fish usage is that only easily found indigenous species are used. Importation of exotic fish species for mosquito control is not recommended due to the potential adverse environmental impact on the local ecosystem.

Examples of common larvivorous fish include those in genera Poecilla (Lebistes), Apochelius, Panchax and Macropodus. Poecilia reticulatus is endemic and widespread in many tropical subtropical areas where DF/DHF endemic. Earlier attempts to introduce the so-called mosquito fish, Gambusia affinis, to tropical areas have not been successful in most places as the species does not breed well in tropical environments. Dragonfly nymphs also have been used to control larvae in containers.

Small copepod crustaceans of the genus Mesocyclops can be removed from ponds or lakes and placed in water containers to kill Aedes mosquito larvae. The technique is simple, cheap and can be very effective. A single copepod has been observed to kill 15 to 20 first and second instar larvae in a single day. A single introduction can produce effective larval control from several weeks to several years, depending on the larval habitat, species of Mesocyclops, climatic conditions and other factors. This technique should not be used in countries outside the Western Pacific Region where dracunculiasis occurs and Mesocyclops is the intermediate host of guinea worm infections.

Figure 14. Application of larvicide in large water container
Preventing spread of Aedes immatures in tyre shipments

The used-tyre trade has been an important factor in the spread of dengue vectors from one country to another. The following steps can minimize such spread:

1. Maintain routine surveillance and control activities in seaports and airports.
2. Inspect port areas and warehouses or supply depots of tyres bound for export.
3. Implement cost-effective methods of infestation control. This includes fumigation of tyres shipments with methyl bromide to kill eggs and larvae in infested cargoes at dockside before export.
4. Require the presentation of certificates of fumigation and other evidence of control to quarantine officials before tyre cargoes are off-loaded.
5. Report contaminated cargo to parties concerned and urge them to take appropriate action to avoid future occurrence.

To ensure vigilance concerning vector movement, mosquitoes should be regularly collected in and around airports and seaports, and staff should be trained in the identification of important species. A survey should also be maintained to monitor mosquitoes recovered from aircraft and ships, and ovitraps should be used to detect the presence of Aedes species, particularly *Ae.aegypti*, around terminals.

For larval mosquito control in and around airports and seaport terminals, source reduction and biological control (e.g. larvivorous fish) should be employed where possible. When insecticides are necessary, preference should be given to environmentally safe products such as temephos and *Bacillus thuringiensis*.

There is a risk to arriving, departing and transit passengers, where adult mosquitoes invade terminals. Adequate screening, air-conditioning and residual insecticide treatment of terminals, particularly transit lounges, should be employed to alleviate the problem.
6.

CONTROL: PERSONAL PROTECTION

For the control of adult mosquitos, personal protection measures involving household insecticide products, repellents and insecticide impregnated mosquito nets or curtains have been very much a part of active and sustainable community participation in the overall control of nuisance and disease-carrying mosquitos including *Aedes* vectors. It is also highly desirable that the house itself or at least the bedrooms be screened.

**Mosquito coils and aerosols**

The domestic use of household insecticide aerosols, mosquito coils, electric mats and more recently electric liquid vaporisers have been on the increase throughout the world including the developing countries. Industrial sources indicated that the annual consumption of aerosols, mosquito coils and electric fumigation mats as of 1989 was around 0.81, 12.50 and 3.65 million units (cans or pieces), respectively. Because of environmental concerns regarding ozone levels, industry is making efforts to replace the chlorofluorocarbon propellants in the aerosols with acceptable alternatives.

Among the major genera of vector mosquitos, comparative laboratory efficacy studies indicate the *Culex* species (e.g. *Culex quinquefasciatus*) appear to be more tolerant of household insecticide products containing synthetic pyrethroids as active ingredients than those species in the genera *Aedes*, *Anopheles* and *Mansonia*. Information from industrial sources indicates that the mosquito coil is the most commonly used households insecticide product in Asia and the Western Pacific Region. With its ease of use (no electricity requirement), economical pricing (cheapest among existing major household insecticide products) and high consumer acceptance (traditional and cultural practice of using smoke to get rid of mosquitos), the mosquito coil can be incorporated into overall *Aedes* control strategies. In addition to keeping mosquitos away, it is also possible that the smoke from coils normally burnt at night can kill *Aedes* mosquitos resting inside houses.

**Insecticide impregnated curtains and mosquito nets**

Although mainly used for malaria vector control, pyrethroid-treated mosquito nets can also used to control DHF vectors. Recently, long-lasting insecticide treated materials (fabrics, plastic sheets and curtains) have offered a potential for dengue vector control. Permethrin is the chemical of choice because of its proven residual effectiveness and safety under field conditions. Mosquitos are killed at night when they contact the net (malaria vectors), or during the day when the net becomes a toxic resting site (dengue vectors). Treated curtains, and other fabrics, even bamboo-impregnated curtains, can also be used inside the house to kill dengue vectors.
For bedridden patients, infants, the elderly and night shift workers, treated nets and curtains can provide a certain degree of protection especially in major outbreaks of DF and DHF. Hospital rooms with DHF patients should be mosquito-proof and/or utilize permethrin-treated mosquito nets. Families should be encouraged to use treated nets of curtains, spray their home once a day with insecticide aerosols to kill infected adult mosquitoes, or burn mosquito coils. Ideally, patients at home or in the hospital should be placed under the mosquito nets for at least five days or prevent daytime bites of *Ae. aegypti* and further spread of the disease.

The procedure and calculations required for treating mosquito nets are shown in Annex 5.

**Repellents**

Repellents applied to the exposed skin or impregnated clothing (including anklets, wristlets, headbands and detachable patches) can be used for preventing the landing and biting of mosquitoes. The most common active ingredient used in repellent treatment is DEET (N, N-diethyl-m-toluamide). It has a relatively low mammalian toxicity (acute oral = 2000 mg/kg and acute dermal = 10 000 mg/kg).

The use of skin-exposed repellent formulation such as lotion, cream, rub-on and aerosol spray have not been well accepted by the general public living in regions. Repellents applied to the skin are best used for short-term protection needs and should not be applied on a continuous daily basis especially to children. Repellents applied at night would have minimal effect against *Aedes* vectors which bite during the day.
7. CONTROL: SPACE SPRAY APPLICATIONS

The objective of space sprays (thermal fogging and ultra low volume aerosol sprays) in vector control is to achieve rapid knockdown and eventual mortality of the adult *Aedes* vectors especially under epidemic conditions. They should be employed in situations of emergency *Aedes* control to suppress and interrupt an ongoing dengue epidemic or to prevent an expected dengue outbreak from occurring. Adult *Aedes* vector densities, especially the older and potentially infected populations, should be reduced to sufficiently low levels to prevent or interrupt transmission. Desirable spray characteristics include a sufficient period of suspension in the air, suitable drift characteristics, and penetration into target areas with the ultimate aim of impact on adult mosquitoes.

**Thermal fogs and ultra low volume aerosol sprays**

Thermal fogs containing insecticides are normally produced when a suitable formulation condenses after being vapourized at a high temperature. Generally, a thermal fogging machine employs the resonant pulse principle to generate hot gas (over 200°C) at high velocity. These gases atomize the insecticide formulation instantly so that it is vapourized and condensed rapidly with only negligible formulation breakdown. Thermal fogging formulations can be oil-based. The oil (diesel)-based formulations produce dense clouds of white smoke whereas water-based formulations produce a colourless fine mist. The droplet (particle) size of a thermal fog is usually less than 15 microns in diameter, the exact droplet size depends on the type of machine and operational conditions. However, uniform droplet size is difficult to achieve in normal fogging operations.

There are many types and brands of thermal fogging machines (e.g. Pulsefog, Swingfog, Dynafog). Applications are made from a vehicle or by a walking sprayman. Small hand carried portable machines are meant for restricted outdoor use and for enclosed spaces (building) of not less than 14 m². They are suitable for areas inaccessible to vehicle-mounted foggers by road. Portable applications can be made in congested low-income housing areas, multi-storeyed buildings, go downs and warehouses, covered drains, sewer tanks and residential or commercial premises in dengue infected areas.

Cold aerosol ultra low volume (ULV) applications are mechanically generated by special ULV machines. ULV application requires a minimum volume of insecticide liquid concentrate to be sprayed over a wide and open area. The optimum droplet size for ULV spray usually falls within the range of 10 to 25 microns in diameter depending on the type of machine and operational conditions.

The ULV aerosol generators (e.g. Leco, Dynafog, Tifa, London Fog) are generally vehicle-mounted in order to cover a large area within a limited time frame. These machines have an engine driven air compressor system to produce a flow or air into which insecticide formulation is released and shared into fine aerosol droplets. The average droplet size is normally less than 25 microns but a few droplets can be as large as 40 microns. The droplet size spectrum of ULV spray is generally more uniform than that of a thermal fog. Backpack sprayers with ULV attachments or nozzles (e.g. Fontan, Solo) can spray around houses and areas where vehicle mounted machines cannot reach. Depending on the type of machine, the droplets can be relatively fine (20 microns) or coarse (50 microns).
Insecticide formulations for space spraying

The organophosphate insecticides such as malathion, fenitrothion and pirimiphos-methyl have been used for the control of adult *Aedes* vectors. Undiluted technical grade malathion (active ingredient 95%+) or one part technical grade diluted with 24 parts of diesel have been used for ULV spray and thermal fogging, respectively. For diluted technical grade ULV malathion applications from vehicles, the dosage on an area basis is 0.5 litres per hectare.

More recent insecticide formulations of permethrin (e.g. Resigen) and fenitrothion (e.g. Sumithion L-40S) have been shown to be effective for adult control. *Aedes* larvae breeding in exposed situations can also be killed by these applications. Sumithion L-40S (active ingredients consists of fenitrothion 40% and tetramethrin 1%) is an oil-based formulation that produces thick smoke as in malathion thermal fogging operations. The dilution factor is one part of the formulation with 19 parts diesel. Fenitrothion is more toxic than malathion, and suitable protective clothing and other precautions should be used for both insecticides. Safety measures for insecticides usage are shown in Annex 12.

Resigen (active ingredients consists of S-bioallethrin 0.75%, permethrin 17%, and piperonyl butoxide 17%) in a water-based formulation produces a fine mist in a thermal fogging operations. The dilution factor for thermal fogging spray is one part of Resigen diluted with 300 parts water. This low dosage is likely to be effective under less harmful environmental impact are more acceptable by the community and should be the trend for future space spray formulations.

Aqua Resigen contains S bioallethrin 0.15%, permethrin 10%, and piperonyl butoxide 11%, a well-known synergist. The unpleasant smell and at times difficult handling of malathion can be eliminated by the use of Aqua Resigen. It can be diluted with nine parts water and one part formulation to form a 1% solution for outdoor spraying close to and around houses with open doors and windows. Since the effective dispersal of diluted pyrethroid solutions in oil or water based solutions over large open areas can difficult, ULV malathion should be given strong consideration for vehicle mounted applications.

Apart from the above-mentioned formulations, a number of companies produce pyrethroid formulations containing either permethrin, deltamethrin, lambda-cyhalothrin or other compounds that can be used for space spray applications (see Annex 6). It is important not to under dose under operational conditions. Low dosages of pyrethroid insecticides are usually more effective indoors than outdoors. Also, low dosage are usually more effective when applied with portable equipment (close to or inside houses) than with vehicle mounted equipment, even if wind and climatic conditions are favourable for outdoor applications. Outdoor permethrin applications without synergist should be applied at concentrations ranging from 0.5% to 1.0%, particularly in countries with limited resources and a lack of staff experienced in routine spray operations. Regardless of the type equipment, spray formulation and concentrations used, an evaluation should be made from time to time to ensure that effective vector control is being achieved.

Spraying for epidemic control

In an emergency, once the first adulticidal treatment is completed, it is normally followed by a second application, seven to 10 days later. The time required to complete an application will depend upon the type of equipment used and size of the area. In general, the smaller the area, the easier it is to increase the number of applications.
a. Aerial spraying is seldom used because of high costs, difficulties with application in hilly terrain and problems with non-target arthropods such as domestically reared bees. However, good results can be achieved rapidly over a large area if suitable equipped aircraft and the appropriate insecticide are available. Sometimes aircraft used for agricultural spraying can be used for public health purposes. A large aircraft (e.g. the C-47) is capable of covering 6000 hectares with general flights in one day. With ULV applications, the aircraft flies at a speed of 240 kilometers per hour at a height of 62 metres above the ground with a swath spacing of 182 metres. Small fixed wing aircraft fly at 160 kilometers per hour at a height of 30 meters above the ground with a swath spacing of 50-100 meters.

b. A vehicle-mounted ULV sprayer can treat up to 320 hectares (about more square mile) in one day if applications are made on an adequate road system. An advantage of local health personnel owning only one such machine, although several might theoretically be preferred, is that control operations can be quickly launched and easily managed during epidemics. A vehicle-mounted thermal fogger is also effective and can cover about 150 hectares per day. For vehicle-mounted applications, the vehicle is driven cross wind so that the fog or mist moves at right angles to the line of travel. Vehicle speed is normally 5 to 15 kilometers per hour. A swath of 60 to 90 meters is normally covered. The discharge rate varies greatly from a few liters to several hundred liters per hour depending on the speed of the spray vehicle, the dosage of active ingredient required, and the strength of the formulation applied. Adequate control with a vehicle mounted Leco sprayer can be achieved by using a ULV malathion discharge rate of 45 ml per minute and a vehicle speed of 5 kilometers per hour. Figure 16 shows vehicle routes and spraying in relation to wind direction.

c. A backpack aerosol or mist blower sprayer with ULV attachments can be effective for spraying without the need to enter houses. One man is capable of covering 20 hectares per day. The spray nozzle is directed towards open doors and windows of houses and the particles can drift from 8 to 15 meters from the point of discharge to kill mosquitos. Discharge rates of various formulation strengths can range from 10 ml to 100 ml per minute. A hand carried thermal fogger requires more time to spray and can cover about 5 to 10 hectares per day. Annex 7 provides details on house spraying with backpack sprayers.

d. Moderate-sized cities should have at least 1 vehicle-mounted ULV sprayer or thermal fogger, 10 backpack aerosol or mist blower sprayers with ULV attachments, 5 swing fog machines, and also 1000 liters of ULV insecticide including malathion and pyrethroid formulations, in order to be prepared to carry out adulticidal operations rapidly over an area of 20 kilometers square. The coverage potential of ground equipment should be multiplied by the number of spraying machines available.

e. An inventory should be made of the location, quantity, and availability of insecticides and spray equipment for use against adult mosquitos. This also should include trucks of aircraft that can be covered for use in emergency spraying operations.

**Note:** at the time of spraying residents are requested to leave doors and windows open.

Information procedure, timing and frequency of space spray applications are shown in Annex 7. Calculations for preparing spraying formulations are shown in Annex 8 and Annex 9.


<table>
<thead>
<tr>
<th>Operational Considerations</th>
<th>Vehicle Mounted ULV</th>
<th>Backpack Mist Blower</th>
</tr>
</thead>
</table>
| Performance per day of 4 hours | 16 km/hr or 16,000 m per hr  
64,000 m per 4 hours per day | 3 minutes per house or  
20 houses/hour/team of  
3 sprayers  
80 per 4 hours per day per machine per team |
| Swath                      | 150 meters swath width | 10 meters horizontal |
| Optimum droplet size       | 20 µ  
More droplet density | Same as vehicle mounted ULV equipment |
| Safety to the operators   | Only 2-3 operators, less handling of concentrate | More work  
40 machines x 3 man team |
| Cost                       | Equipment cost  
2-3 spraymen to operate  
Vehicle cost | Cost of 40 machines and  
120 spraymen |
| Insecticide droplet        | Poor penetration to reach most indoor resting sites | Better penetration  
Likely to reach indoor target sites |
Figure 16. Suggested route for space spray operation
8. SOCIAL MOBILIZATION AND COMMUNICATION FOR DENGUE PREVENTION AND CONTROL

(Communication for Behavioural Impact – COMBI)

The control and prevention of DF and DHF depends ultimately on the behavioural responses of a broad range of individuals and institutions at the country-level, ranging from behaviours of individuals affected by or at risk of dengue to the behaviours of a host of others: community leaders, government officials, politicians, policy makers, government health care providers, private physicians, and middle management administrators in health systems. Every outcome is a behavioural outcome: someone has to do something. It is not enough that they are aware, or are knowledgeable, or are even convinced; they need to act.

A continuing dilemma for dengue control professionals is finding effective ways to encourage new behaviours and achieve behavioural results. Many different approaches have been useful in the past. While there have been some successes, there has also been enormous frustration at not being able to achieve more at a faster rate. Conventional “Information Education and Communication” (IEC) programmes and for DF/DHF control have been able to increase awareness and knowledge but have not been as successful at achieving behavioural results. There are abundant examples of people knowing that dengue is transmitted by mosquitos and that mosquitos breed in water containers; nevertheless people leave these containers unprotected. It is clear that informing and educating people are not sufficient bases for behavioural responses. Behavioural change will result only with effective social mobilization and communication programmes, carefully planned and purposefully directed at behavioural goals, and not directed just at awareness creation, advocacy or public education.

COMBI

There are many planning models for social mobilization and communication. Recently, the World Health Organization’s Social Mobilization and Training Team (SMT) began applying an approach known as COMBI (Communication for Behavioural Impact) in the design and implementation of behaviourally-focused social mobilization and communication plans for the adoption of healthy behaviours. COMBI is social mobilization directed at the task of mobilizing all societal and personal influences on an individual and family to prompt individual and family action. It is a process that strategically blends a variety of communication interventions intended to engage individuals and groups in considering recommended healthy behaviours and to encourage the adoption and maintenance of those behaviours (COMBI's integrated actions). COMBI incorporates the many lessons of the past 50 years of health education and communication in a behaviourally focused, people-centred strategy. COMBI also draws substantially from the experience of the private sector in consumer communication. It is an approach well suited for achieving behavioural impact in dengue control and has been use with success in Malaysia and is being implemented in Lao People’s Democratic Republic and Cambodia.
COMBI’s integrated actions

- **Public Relations/Advocacy/Administrative Mobilization**: used to put a particular healthy behaviour on the business sector's and administrative/programme management's agenda via the mass media (news coverage, talk shows, soap operas, celebrity spokespersons, discussion programmes) and through meetings/discussions with various categories of government and community leadership, service providers, administrators, business managers, official memoranda, and partnership meetings.

- **Sustained Appropriate Advertising and Promotion**: in M-RIP fashion – Massive, Repetitive, Intense, Persistent – via radio, television, newspapers and other available media, engaging people in reviewing the merits of the recommended behaviour vis-à-vis the “cost” of carrying it out.

- **Community Mobilization**: including use of participatory research, group meetings, partnership sessions, school activities, traditional media, music, song and dance, road shows, community drama, leaflets, posters, pamphlets, videos and home visits.

- **Personal Selling/Interpersonal Communication/Counselling**: involving volunteers, school children, social development workers, other field staff, at the community level, in homes and particularly at service points, with appropriate informational literature and additional incentives, and allowing for careful listening to people’s concerns and addressing them.

- **Point-of-Service Promotion**: emphasizing easily accessible and readily available DF/DHF diagnosis and treatment services, and DF/DHF prevention products such as insecticide-impregnated netting materials and personal repellents.

COMBI Planning

To develop a COMBI plan you need to consider the following major tasks:

1. Identify specific behavioural goals/objectives

   The development of a COMBI plan begins with the potential “clients/consumers” and their health needs/desires with a sharp focus on the behavioural result anticipated in relation to these needs/desires. The first COMBI principle is: “Do nothing… produce no posters, no T-shirts, no pamphlets, no videos, etc. … do nothing until you have set out specific, precise behavioural objectives.” This is an important but difficult task. To help you, Annex 10 lists possible behavioural objectives that you may want to consider during your planning meetings.

   It is crucial to target a few behaviours that are simple and cheap, and preferably fun to put into practice. Too many behavioural expectations are as bad as none at all. Even the largest programme budgets are too small to support more than one or two major initiatives at the same time. Entomologists and epidemiologists should also be consulted on whether selected behavioural objectives will have meaningful entomological and epidemiological impact.
2. **Conduct a situational market analysis**

   A second COMBI principle is: “Do nothing… produce no posters, no T-shirts, no pamphlets, no videos, etc. … until you have conducted a situational market analysis in relation to the stated behavioural objectives.” Situational market analysis (also known as formative, intervention or communication research) includes all research that helps to establish whether the stated behavioural objectives are feasible both from the public and programme’s current needs, beliefs, behaviours, and available resources. The term “market” is purposefully used here. It draws on the over 100 years of experience in market research and consumer communication. It locates the individual within their “market” environment (even in the poorest of communities) where they make “consumer” decisions ranging from, for example, whether to go to their rice fields straight away or to spend a little more time at home checking their yard for potential water containers.

   The situational market analysis includes a range of research activities: reviews of the scientific literature; entomological assessments of key containers; social assessments of community organizations and structures; ethno-entomology (study of local or folk taxonomies of insects); ethnographic investigations of health beliefs and practices; baseline Knowledge, Attitudes, Practices, and Behaviours (KAPB) surveys; Strengths, Weaknesses, Opportunities, Threats (SWOT) analysis of the dengue programme itself; media consumption surveys; research to determine appropriate communication channels and strategies; pre-testing materials or messages; and pre-testing specific behaviours (behavioural trials).

3. **Develop the communication strategy**

   The third major task is to develop an overall strategy for achieving the stated behavioural objectives. The strategy points to the broad approach that the COMBI programme will take to achieve its objectives. It is made up of a judicious, integrated blend of specific social mobilization and communication activities directed towards the expected behavioural results. The COMBI strategy should include key messages, their sequencing, the overall tone for the strategy, the blend of communication actions (COMBI’s integrated actions), and the relationships between these different communication actions, and an overview of how the plan will be managed, monitored and evaluated.

4. **Specify implementation, monitoring, evaluation and budget**

   The fourth major task in COMBI planning includes development of a structure for managing, monitoring and evaluating the implementation of the plan, and a description of how behavioural impact will be assessed. Finally, a detailed work-plan with time schedule for the preparation and implementation activities should be composed as well as a budget with detailed lists of costs for the various activities.

   For further information on planning social mobilization and communication for DF/DHF prevention and control see: *Planning Social Mobilization and Communication for Dengue Prevention and Control: A Step-by-Step Guide*. WHO (forthcoming)

9.

LEGISLATION

Within the framework of dengue vector control in most countries, sooner or later, legislation will be necessary to ensure the full compliance of the public with the control measures promoted by health authorities. Without legal backing, the implementation of these control measures in the prevention and control of DHF will never be completely satisfactory or successful. Public cooperation, whether voluntary or compulsory, is absolutely necessary to achieve the goal of DHF control and prevention.

Legislation and community awareness

Legislation must play role within the larger context of community involvement in dengue vector control programmes. For legislation to be effective and to provide a level of monitoring it should include the following principles:

1. Campaigns to create public awareness of the benefits arising from dengue vector control programmes as well as the role of the public in these programmes should be launched before and during enforcement of the legislation. Such action promotes community support and compliance with the environmental measures undertaken without the intervention of the law.

2. Vector control staff with or without assistants can enter premises between 6 a.m. and 6 p.m. to search for mosquitos. Depending on the circumstances, a 12- or 24-hour notice can be given before entry; usually, entry is made straight away.

3. No person shall perform any act which is liable to create conditions favourable for the breeding of harbouring of dengue vector mosquitos.

4. Health education pamphlets should be distributed during routine house inspections to reduce and eliminate *Aedes* breeding sources and harbourages.

5. Every person shall comply with all reasonable directions given by the Medical Officer of Health or other responsible health officer to eliminate vector-breeding conditions. Directions can be given to the owner or occupier of any premises and to any person therein, including his relatives and domestic house employees.

6. Any person who does not comply with the directions to prevent mosquito breeding on his premises is liable to a fine and/or prison term. A suggested fine is US$5.00 or more for first offenders eventually exceeding US$500 depending on the circumstances.

7. An uncooperative person can arrested and brought to court.

8. A person charged with an offence will be given notice to appear in court. If he fails to appear, a warrant can be issued and the person arrested. The court may commit him to prison for a term not exceeding two months.
9. A report on premises breeding is to be signed by a Medical Officer of Health, Entomologist or Public Health Engineer and submitted as evidence to the court. A copy is to be provided to the accused. The contents of the documents are presumed to be correct until the contrary is proven.

An example of a law enforcement form is shown in Annex 11 and the procedure for collection of *Aedes* larvae with enforced legislation in Annex 12. A sample health education poster on DHF vector control is shown in Annex 13.
10. MANAGING OUTBREAKS

Immediate control measures at an early stage during an outbreak are essential to prevent an outbreak from expanding. All senior health officers at districts and provincial levels should be involved in supervision either directly or indirectly. Officers at the district level need to supervise more closely and more frequently compared with provincial level officers to ensure that all control and prevention activities are carried out completely and effectively.

All provinces and districts must have a contingency plan to increase the manpower needs and equipment for immediate action when an outbreak occurs. The location of suspected and confirmed cases should be plotted on maps to determine the size of area or areas for emergency interventions. To plan accordingly, the number of vehicles mounted and portable sprayers available should be recorded, as well as the quantity of insecticide. For the control of epidemics, space spraying to kill the infected adult mosquito is considered an integral part of emergency vector strategy to reduce or interrupt transmission. It should be emphasized, however, that rapid and effective community source reduction and larviciding should be undertaken to achieve a more sustainable control.

Establishing an operations centre

1. The setting up of an operations room at different levels (such as district/provincial/national level) is based on the following criteria:

   a. District level; when, at any one time, an outbreak occurs in two localities, or there are four or more cases in any one outbreak locality;
   b. State or provincial level; when, at any one time, there are more districts are having an outbreak;
   c. National level; if, at any one time, there are three or more provinces experiencing a dengue outbreaks.

During an outbreak, the operations room must be open, even on weekends and public holidays.

2. To close an operations room, the following criteria should be applied:

   a. District level; when the number of outbreak localities is less than two or when there are less than four cases in an outbreak locality, at any one time.
   b. State or provincial level; when the number of districts with an outbreak is less than three at any one time.
   c. National level; when the number of states with an outbreak is less than three at any one time.

Larval surveys

a. All houses within 400 meters radius of the case house must be totally surveyed for *Aedes* breeding and the quality of the surveys should be ensured. Ideally such *Aedes* surveys (related to reported cases) must be carried out within 24 hours of notification of the first case from an outbreak locality.
b. The duties of each dengue control sub-team are also:

i. to carry out contact tracing and case detection;
ii. to issue warning of the outbreak to the community;
iii. to give technical advice and distribute information pamphlets.

**Larval control by households**

The Health Department is required to demonstrate as well as to educate the public on the use of appropriate measures to eliminate *Aedes* breeding in and around houses. Obtain collaboration of municipal sanitation department and community organizations in community mobilization for source reduction activity. And self-protection are essential.

**Adult control by space spray**

Space spray operations (thermal fogging or ULV aerosols) in the event of a dengue case must be carried out immediately following the notification of the case. Spraying should be done within a radius of 400 meters of the case house.

As and when a dengue serology test is requested by a hospital on any case of viral infection, the hospital authority is required to report the case to the health department so that control measures can be taken immediately as if it is a dengue case.

**Health education**

When an operations room is set up, the province or state health director's office should immediately initiate health education activities to the public through the mass media such as local radio stations, newspapers and other methods.

A special team for carrying out health education activities must be formed to ensure that such activities can be stepped up (during an outbreak) to obtain cooperation from the public in controlling the situation. Each special team should include a health inspector, public health assistant and a labourer.

A mobile health education unit should be used in all health education activities including announcements of fogging operations.

**Post-outbreak activities:**

a. Outbreak situational analysis

The relevant health authorities should carry out an analysis on any outbreak that has occurred. This feedback assists in identifying the factors causing the outbreak and may provide solutions for control of future outbreaks.

b. Larval surveys

*Aedes* survey in a locally following an outbreak should be carried out based on the priority classification of the locality.
i. Localities in priority I and II. (High risk).

*Aedes* surveys ideally should be carried out in all houses (100%) in every outbreak locality falling into this category. This survey should be carried out at least once every three months. Time constraints and manpower availability will limit coverage in densely populated areas.

ii. Localities in Priority III and IV

*Aedes* surveys should be carried out at least once every three months at a rate of at least 20% of the total number of houses in each outbreak locality.

**Summary of control action to take during large-scale DHF outbreaks**

1. Recognition of DHF cases; fever, red spots on skin, bleeding from gums, etc.
2. Mothers should take children to clinic or hospital for treatment and case management.
3. Cases should be confirmed by laboratory based (serological) diagnosis.
4. Confirm *Aedes* vector in epidemic area.
5. Set up an operations room.
6. Plot suspected and confirmed cases on map.
7. Immediately commence space spray operations in infected areas.
8. Intensify health education and community clean-up campaigns supported by legislation to eliminate larval breeding. Use radio and television broadcasts, newspapers, etc. to inform public.
9. Encourage municipal sanitation department to intensify refuse collection and environmental clean-up.
10. Intensify long-term larval control measures in permanent water storage containers that are difficult to clean and empty. These measures include deposit of fish and/or a safe larvicide.
11. Promote greater homeowner use of aerosol sprays and mosquito coils inside houses to kill infected adult mosquitoes.
12. Promote mosquito net use, preferably treated with deltamethrin, for DHF patients hospitalised or residing at home.
13. Determine size of area or areas to be sprayed to kill mosquitoes with vector control equipment.
14. Determine number of spray machines, vehicle-mounted and backpack sprayers available.
15. Determine quantity average coverage potential. As a general rule, 100 litres of ULV malathion can cover 200 hectares, or 2 square kilometres.
16. Determine daily average coverage potential. As a general rule, one ULV back-pack aerosol or mist blower sprayer can cover 20 hectares in one day and one vehicle-mounted ULV sprayer can cover 250 hectares a day.
17. Launch space spray operations in localities with the most cases and in crowded areas such as schools and hospitals. Repeat applications after 7 to 10 days if sufficient insecticide is available.
The main activities and steps to be taken in response to any notification of DF/DHF cases are highlighted as below:

### MAIN ACTIVITIES AND PROCEDURES ON THE CONTROL OF DF/DHF

- **Notification of dengue cases**
  - 1 case
    - 1. Case investigation
    - 2. Contact tracing
  - 1 or >1 case
  - >1 case
    - 1. Outbreak investigation
    - 2. Establishment of emergency control teams
    - 3. Establishment of operational centre

- **Notification of cases**
  - 1 case
    - 1. Focal spraying/fogging
    - 2. Larval source reduction
    - 3. Larviciding
    - 4. Health education
    - 5. Personal protection
  - 1 or >1 case
  - >1 case
    - 1. Mass space spraying
    - 2. Larval source reduction
    - 3. Larviciding
    - 4. Health education
    - 5. Community participation
    - 6. Personal protection
    - 7. Law enforcement if any

- **Notification of outbreak**
  - 1. Publicity/mass media
  - 2. Anti dengue campaign

- **Follow-up and investigation report**
  - 1. Public awareness campaign
  - 2. Inter-agencies collaboration
  - 1. Public awareness campaign
  - 2. Inter-agencies collaboration
  - 1. Follow-up activities
  - 2. Epidemic report
LABORATORY AND CLINICAL DIAGNOSIS OF DENGUE HAEMORRHAGIC FEVER

An essential aspect of the laboratory diagnosis of dengue is proper collection, storage and shipment of specimens. Health workers should be informed of the appropriate procedures for collecting specimens. Blood samples are drawn from suspected DHF cases promptly after hospital admission or attendance at a clinic, shortly before discharge from the hospital and, if possible, 14-21 days after onset of disease. It is normally important to have an interval of 10 to 14 days between the first two samples to serologically diagnose primary dengue infections. This is not necessary for monoclonal antibody techniques or DOT enzyme immunoassay. An abbreviated case history should accompany each specimen to include the minimum information of name, address, age, sex, date of onset of illness, date of hospitalisation and brief clinical findings.

Proof that the outbreak is caused by a dengue virus must be obtained as soon as possible after the first suspected cases have been recognized. Blood specimens can be collected in tubes, vials or on filter-paper discs or strips and sent with clinical data to a specialized laboratory. In addition, blood films should be made and sent to the nearest health center laboratory to be stained for differential white-blood-cell and platelet estimation. Severe cases have a leakage of plasma manifested by a rising haematocrit value. Institutions providing care for DHF patients should have microhaematocrit equipment and microscopes for platelet estimation.

It is essential for health workers making a diagnosis by means of viral isolation to make arrangements with the appropriate virology laboratory prior to the collection of specimens. A factor favouring successful isolation is to take blood samples from patients early in the course of the disease. The virus can also be isolated from vector mosquitoes and from autopsy material. Methods to confirm the presence of dengue virus include the inoculation of serum or plasma into mosquitoes or mosquito cell cultures. The four different dengue virus serotypes can be identified by using immunofluorescence and type specific monoclonal antibody methods.

The objective of a laboratory-based surveillance system is to provide early and precise information to public health officials on four aspects of increased dengue activity: time, location, virus serotype and disease severity. The system allows early detection of dengue cases and thus improves the capacity of health officials to prevent and control the spread of dengue transmission.

**Thrombocytopenia and haemoconcentration**

Important laboratory findings are as follows:

Thrombocytopenia (blood platelets equal to or less than 100 000/mm³).
Haemoconcentration – Haematocrit increases by 20% or more of recovery/normal value. This provides evidence of plasma leakage.
The presence of fever, positive tourniquet test, thrombocytopenia, of DHF. Anaemia or severe haemorrhagic, pleural effusion (chest X-ray) and/or hypoalbuminemia serve as supporting evidence of plasma leakage.

The haematocrit/haemoglobin ration has been proposed by some researchers as a useful tool to measure haemoconcentration. Normal figures fluctuate from 2.9 to 3.1; values of 3.2 are suspicious and those of 3.5 or more are considered conclusive for DHF.

Shock with a high haematocrit (except in patients with severe bleeding) and marked thrombocytopenia support a diagnosis of DHF/DSS.

Thrombocytopenia I established as follows:

In the laboratory a direct count phase contrast microscope may be used per platelet count (normal: 200 000 – 500 000/mm³).

In practice, for outpatients, an approximate count from a peripheral blood smear is acceptable.

Normal persons: 4-10 platelets per oil-immersion field (an average reading from 10 fields is recommended) indicates an adequate platelet count.

Low: 2-3 platelets per oil-immersion field or less (i.e., < 100 000/mm³).

### Haemagglutination inhibition test

For the diagnosis of dengue infection, the HI test requires the collection of paired sera 10 to 14 days apart and endpoint titres against the four dengue serotypes to establish a fourfold seroconversion. While still very much the reference test for confirmation of dengue c\'ivirus infections, the HI test is time consuming and requires careful optimisation with each batch of haemagglutinins and erythrocytes used. This is related to the exquisite pH sensitivity of the dengue haemagglutinin binding reaction to the goose erythrocytes which are now commonly used in this assay. The requirement for fresh reagents which may no be stored for too long, makes this a difficult test to use in a decentralized system.

Guidelines for interpretation of results obtained by the HI test have been described by a WHO Technical Advisory Committee on Dengue Haemorrhagic Fever and are reproduced here:

<table>
<thead>
<tr>
<th>First specimen</th>
<th>Second specimen after 1-4 weeks</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 4\textsuperscript{th} day &lt; 1:20</td>
<td>≥ 4x and less than 1:2560</td>
<td>Primary response</td>
</tr>
<tr>
<td>Before 5\textsuperscript{th} day &lt; 1:20</td>
<td>≥ 1:2560</td>
<td>Secondary response</td>
</tr>
<tr>
<td>Before 7\textsuperscript{th} day ≥ 1:1280</td>
<td>&lt; 4x</td>
<td>Presumptive recent secondary response</td>
</tr>
</tbody>
</table>

To interpret high-fixed titres, laboratories should establish baseline titres for the local population taken at a time of little or no dengue transmission. Titres more than twice the standard deviation of the geometric mean may be presumed to indicate recent dengue infections.
Complement-fixation test

The CF test may also be used in serological diagnosis wherever facilities for the test exist. This test is less sensitive than the HI test. Blood taken on filter-type is unsuitable for the CF test because it is haemolysed.

The CF test is useful since anti-dengue IgG fixes complement with dengue antigens. A fourfold rise in CF antibody where the interval between the two sera samples is less than two weeks signifies a secondary seroresponse pattern.

Primary antibody response

The primary antibody response to dengue infection is characterized by slow evolution of HI antibody which is often relatively monotypic, with the absence of CF antibody until at least two weeks after onset of illness. Definitive characterization of primary response is established by demonstrating rising titres of anti-dengue IgM.

In practice, dengue HI antibody titre is generally less than 1:20 in serum obtained before the fourth day after onset of illness. There is a fourfold or greater increase in titre in convalescent specimens (1-4 weeks after onset), with antibody titre not greater than 1:1280.

Secondary antibody response

Secondary antibody response is characterized by a rapid evolution of HI and CF antibody. All antibodies are broadly reactive. Definite characterization of a secondary response is established by demonstrating rising titres of anti-dengue IgG.

Evidence of recent infection

In practice, HI antibody to dengue antigen(s) is less than 1:20 in serum obtained before the fifth day of illness with response equal to or higher than 1:2560 in convalescent serum, or HI antibody at least 1:20 in serum obtained before the fifth day after onset of illness, with rise to ≥1:2560 in convalescent serum.

Regarding presumptive recent infection, the HI antibody is 1:1280 or greater in acute specimen without fourfold or greater antibody rise in convalescent specimen.

Other tests

Although the HI test has been successfully used for over 30 years for diagnosis of dengue, access to high titre haemagglutinin prepared from sucking mouse brain has been limited mainly to central, reference and research laboratories. This has created a dependence of peripheral health and vector control units upon diagnostic services offered by usually overloaded central laboratory services.

There are more than 20 rapid diagnostic tests for dengue on the market some of which detect both IgG and IgM antibodies. The sensitivity varies between the various brands of test and WHO currently has no system of evaluation or quality control. The rapid tests have been most useful in field situations where
access to serological confirmation is difficult or often delayed and also as part of an early warning system whereby the tests are run on a regular basis in order to detect possible introduction of dengue virus into an area. Standard confirmation using HI should be done whenever the rapid tests are used.

It is important to remember that there are four dengue serotypes which may give rise to a primary dengue infection if a patient is infected for the first time by any one of the four serotypes, or to a secondary dengue infection if the patient has been infected by a different serotype at an earlier time. This complicates both diagnosis and interpretation of results of serological tests not specifically identifying IgM antibodies.

Dengue virus may be propagated in mosquitos. Intrathoracic inoculation of adult mosquitos or intracranial inoculation of Toxorhynchites mosquito larvae have been successfully used to detect the presence of dengue virus in patient sera. The growth of virus in these mosquitos is usually confirmed by detection of viral antigen in head squashes, using immunoflourescent antibody techniques. Mosquito cell lines derived from Aedes albopictus, Aedes pseudoscutellaris and Toxorhynchites may also be used to isolate virus from specimens of patients. The normal method of confirming the presence of virus is by using monoclonal antibodies in the immunoflourescence technique.
CASE INVESTIGATION REPORT ON
DENGUE FEVER (DF) AND DENGUE HAEMORRHAGIC FEVER (DHF)

Case Ref No.: ______________________ Date of investigation: _________________
District/Provincial Health Department: ___________________________________________
Provincial/State: ________________ Name of Medical Officer: ____________________

PART I: TO BE FILLED BY HOSPITAL

1. PATIENT

Name of patient: ________________________________________ Age: ________________
Sex: ____________ Occupation: ______________________________
Home address: __________________________________________________________________
Occupational address/school: __________________________________________________________________
Patient's activities for the last 14 days before onset of sickness:
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________

2. NOTIFICATION

Hospital: ____________________________________ Date on onset: _________________
Registration No.: ______________________ Date of admission: ____________________
Date of diagnosis: ______________________ Date of notification: _________________
Initial clinical diagnosis: DF/DHF/DSS: ________________________________

<table>
<thead>
<tr>
<th>Main clinical findings</th>
<th>Yes/No</th>
<th>Laboratory findings</th>
<th>Serology (ELISA) Results</th>
<th>Serology (HI tests if any) Results</th>
<th>Virology (if any)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td></td>
<td>Tourniquet</td>
<td>1st sample (date)</td>
<td>1st sample (date)</td>
<td></td>
</tr>
<tr>
<td>Ache/pain</td>
<td></td>
<td>Platelet count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petechiae</td>
<td></td>
<td>Bleeding time</td>
<td>2nd sample (date)</td>
<td>2nd sample (date)</td>
<td></td>
</tr>
<tr>
<td>Haemorrhagic</td>
<td></td>
<td>Clotting time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spots</td>
<td></td>
<td>Blood for serology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frank bleeding</td>
<td></td>
<td>Haematocrit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shock</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Final diagnosis: ______________________ Date: __________________
Name of Physician: ______________________
PART II: TO BE FILLED BY HEALTH OFFICE

Case Ref No.: ___________________________  Notified from: ___________________
Date of receiving notified: _________________  Date of investigation: _______________

INVESTIGATION:

1. Patient home address: ________________________________________________________
   (urban/semi-urban/rural town/village)
2. Occupational address (school): ________________________________
3. Movement/travel history of the patient for the last 14 days before onset of sickness:
   ____________________________________________________________________________
   ____________________________________________________________________________
   ____________________________________________________________________________
   ____________________________________________________________________________

4. Environmental sanitation (patient house address)
   Type of house: ____________________________ water supply ____________________
   (detached/semi-detached/terrace/high-rise flat/rural house)
   Latrine _____________________________ garbage disposal _____________________
   Surrounding cleanliness _________________________________________________________

5. Contact tracing (give name, age, sex, home address and occupation)
   i. __________________________________
   ii. __________________________________
   iii. __________________________________

6. Number refer for admission: ____________________________________________________________________

7. Vector situation

   7.1 Locality (specify patient home address/places of work/place of visit etc.) ____________

   7.2 No. of houses surveyed: _______________  Date of survey: _______________

   7.3 Number of houses positive with *Ae. aegypti* larvae/pupae: ______________________

   7.4 No. of houses positive with *Ae. albopictus* larvae/pupae: _________________________

<table>
<thead>
<tr>
<th>Aedes species</th>
<th>House index (%)</th>
<th>Breteau index</th>
<th>Container index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ae. Aegypti</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ae. albopictus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

   7.5 Describe the major breeding found: _____________________________________________

   7.6 Action taken:
   ____________________________________________________________________________
   ____________________________________________________________________________
   ____________________________________________________________________________


8. Vector Control (patient home address)

8.1 Date of first space spraying and type of spray (ULV/fogging etc.) ______________________

8.2 Locality sprayed: _________________________________

8.3 Insecticide use and quantity (litre): ______________________

8.4 No. of premise sprayed: ______________________________

8.5 Dosage rate per house: _____________________________

9. Health education campaign

9.1 Date of health education organized: ______________________

9.2 Health education approaches:

_______________________________________________________________________________

_______________________________________________________________________________

_______________________________________________________________________________

_______________________________________________________________________________

9.3 Source reduction

_______________________________________________________________________________

_______________________________________________________________________________

10. Legislation (if applicable) _______________________________

11. Vector Control (occupation address/place of visit if applicable)

11.1 Date of 1st space spraying and type of spray (ULV/fogging etc.) ______________________

11.2 Locality sprayed: _________________________________

11.3 Insecticide use and quantity (litre): ______________________

11.4 No. of premise sprayed: ______________________________

11.5 Dosage per house: ________________________________

12. Health education campaign

12.1 Date of health education organized: ______________________

12.2 Health education approaches:

_______________________________________________________________________________

_______________________________________________________________________________

_______________________________________________________________________________

12.3 Source reduction:

_______________________________________________________________________________

_______________________________________________________________________________

13. Legislation (if applicable) _______________________________
Comments by field investigator i/c:

_______________________________________________________________________________
_______________________________________________________________________________
_______________________________________________________________________________
_______________________________________________________________________________

Date: ________________________

Comments by Medical Officer of Health i/c:

_______________________________________________________________________________
_______________________________________________________________________________
_______________________________________________________________________________
_______________________________________________________________________________

Date: ________________________
Clinical case definition for dengue haemorrhagic fever

All must be present:

1. Fever or recent history of acute fever.
2. Thrombocytopenia (100,000 mm$^3$ or less)
3. Haemorrhagic tendencies, as evidenced by at least one of the following:
   a. Positive tourniquet test.
   b. Petechiae, ecchymoses or purpura.
   c. Bleeding from mucosa, gastrointestinal tract, injection sites, or others.
4. Plasma leakage due to increased capillary permeability as manifested by at least one of the following:
   a. Hematocrit on presentation that is $\geq 20\%$ above average for that age and population.
   b. $> 20\%$ drop in hematocrit following treatment
   c. Commonly associated signs of plasma leakage: Pleural effusion
      Ascites
      Hypoproteinemia

Clinical case definition for dengue shock syndrome

All four criteria above plus evidence of circulatory failure manifested by all of the following:

- rapid and weak pulse,
- narrow pulse pressure (20 mmHg or less) or hypotension for age; and
- cold clammy skin and altered mental status.

Reportable cases of DHF or DSS will have the above, plus

One of the following:
1. Virological or serological evidence of acute dengue infection; or
2. A history of exposure in dengue endemic or epidemic areas (recognizing that during epidemic or significant levels of endemic transmission it is unlikely that many cases will have laboratory confirmation.

Clinical case definitions

Dengue cases are classified in two mutually exclusive categories based on clinical criteria:

- Dengue Fever (DF); or
- Dengue Haemorrhagic Fever (DHF).

Dengue Shock Syndrome (DSS) is clinically well-defined, but for surveillance purposes it is classified together with Dengue Haemorrhagic Fever, and the abbreviation DHF is used for the combined category.

Cases should be classified as suspected DF or DHF on the basis of clinical criteria. When available, added serological evidence will categorize them as confirmed cases.
Case definition for Suspected Dengue Fever

An acute febrile illness of 2-7 days duration with two or more of the following manifestations:

- headache,
- retro-orbital pain,
- myalgia/arthralgia,
- rash,
- haemorrhagic manifestations (positive tourniquet test, petecchiae)
- leukopenia.

Case definition for Suspected Dengue Haemorrhagic Fever

A patient fulfilling:

1. The criteria of a suspected dengue fever, AND
2. Haemorrhagic tendency evidenced by one or more of the following:
   - Positive tourniquet test,
   - Petechiae,
   - Ecchymosis or purpura,
   - Bleeding from mucosa, gastrointestinal tract, injection sites or other sites,
   - Haematemesis,
   - Melaena AND
3. Thrombocytopenia (\(\leq 100,000/ \text{mm}^3\)) AND
4. Plasma leakage due to increased capillary permeability manifested by one or more of the following:
   - A rise in haematocrit equal to or greater than 20 % above average for age and sex;
   - A drop in haematocrit following treatment with fluids equal to or greater than 20 % of baseline;
   - Signs of plasma leakage (pleural effusion, ascites or hypoproteinaemia).

Case definition for Suspected Dengue Shock Syndrome (for international surveillance classified together with DHF)

A patient fulfilling the criteria of a suspected dengue haemorrhagic fever and signs of circulatory failure manifested by:

- rapid and weak pulse,
- narrow pulse pressure (\(\leq 20 \text{ mm Hg}\)), or by
- hypotension for age, cold and clammy skin, and restlessness.

The distinction between DF and DHF makes it possible to use the incidence of DF as an early warning sign for outbreaks, and the number of cases of DHF as a denominator in the calculation of case fatality rates.
**Reportable cases of DHF or DSS**

Reportable cases of DHF OR DSS will have the above **PLUS**

One of the following:

- Virological or serological evidence of acute dengue infection; or
- A history of exposure in dengue endemic or epidemic areas (recognizing that during epidemic or significant levels of endemic transmission it is unlikely that many cases will have laboratory confirmation.

All levels of the health care system should be able to report DF cases. It is not very important that the case definition is highly specific, as an increase in the number of suspected DF cases will require investigation by the public health services before specific action is initiated. In contrast, a rigorous case definition of DHF is necessary for case fatality rates to be comparable over time and from place to place.
LARVAL SURVEY SUMMARY FORM SHOWING HOUSE, CONTAINER AND BRETEAU INDICES FOR *AE. AEGYPTI*

City, province of district ________________________________
Health unit reporting ________________________________

<table>
<thead>
<tr>
<th>Place or locality</th>
<th>Date of survey</th>
<th>Houses</th>
<th>Containers</th>
<th>Breteau Index: No. of containers with larvae per 100 houses</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. examined</td>
<td>No. of houses with larvae</td>
<td>% positive (House Index)</td>
<td>No. with water</td>
</tr>
</tbody>
</table>

|                   |               |                   |               |                                                        |         |
EXAMPLE OF NOTIFICATION OF DENGUE FEVER/
DENGUE HAEMORRHAGIC FEVER
BY PHONE AND WRITTEN COMMUNICATION

GOVERNMENT HOSPITALS/ CLINICS/GOVERNMENT HEALTH CENTRES

PRIVATE DOCTORS PRIVATE HOSPITALS

daily notification by phone, fax or e-mail, followed by letter of notification

daily notification by phone, fax or e-mail followed by weekly submission of form

HEALTH OFFICER-IN-CHARGE

DISTRICT/ MUNICIPAL HEALTH OFFICER

daily notification by phone, fax or e-mail followed by weekly submission of form

EPIDEMIOLOGY DEPARTMENT AT HEADQUARTERS

daily notification by phone, fax or e-mail followed by monthly submission of form

SENIOR STATE HEALTH OFFICER
ANNEX 4

QUANTITIES OF 1% TEMEPHOS (ABATE) SAND GRANULES
REQUIRED TO TREAT DIFFERENT SIZE OF WATER CONTAINERS
TO KILL MOSQUITO LARVAE

<table>
<thead>
<tr>
<th>Size of water jar, drum or other container in litres</th>
<th>Grams of 1% granules required</th>
<th>Number of teaspoons required assuming one teaspoon holds 5 grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 25</td>
<td>Less than 5</td>
<td>Pinch: small amount held between thumb and finger</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>200</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>250</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>500</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>1000</td>
<td>100</td>
<td>20</td>
</tr>
</tbody>
</table>

Methoprene (altosid) briquettes can also be used in large water drums or overhead storage tanks. One briquette is suitable to treat 284 litres of water. Briquettes of *Bacillus thuringiensis* H-14 can also be used in large cistern tanks.
PROCEDURE FOR TREATING MOSQUITO NETS AND CURTAINS

The steps described below mainly refer to treatment of mosquito nets with permethrin. The net treatment technique can be easily used for curtains.

a. Calculate the rate to be treated.

Measure the height, length and width of net. Assuming a rectangular mosquito net is 150 cm high, 200 cm long and 107 cm wide, the calculations are as follows:

\[
\begin{align*}
\text{Area of one end} & = 107 \times 150 = 16050 \text{ cm}^2 \\
\text{Area of one side} & = 200 \times 150 = 30000 \text{ cm}^2 \\
\text{Area of top} & = 107 \times 200 = 21400 \text{ cm}^2 \\
\end{align*}
\]

The sides and ends need to be multiplied by 2.

\[
\begin{align*}
2(16050+30000) & = 92100+21400 = 113500 \text{ cm}^2 \\
\text{(end)} & \text{(side)} & \text{(top)} \\
\text{If } 10000 \text{ cm}^2 & = 1 \text{ m}^2 \text{ then} \\
113500/10000 & = 11.35 \text{ m}^2 \text{ area of net}
\end{align*}
\]

b. Determine how much insecticide is needed.

Assume a permethrin emulsifiable concentrate will be used, and the dosage desired is 0.5 grams per square metre.

To determine the total grams required, multiply net size by dosage:

\[
11.35 \times 0.5 = 5.67 \text{ grams of insecticide needed}
\]

c. Determine the amount of liquid to saturate a net.

To determine the percentage solution to be used by dipping, it is first necessary to determine the approximate amount of water retained by a net. Another term for dipping is soaking.

Pour five litres of water, but preferably a dilute solution of the insecticide to be used, into a plastic pan or other suitable container. For cotton, a 0.3% solution can be tried, for polyethylene or other synthetic fiber, a 1.5% solution can be tried. Add one net to thoroughly wet and remove. Allow drips to fall into a bucket for 15 to 30 seconds. Set net aside. Repeat process with two other nets. Cotton nets can be lightly squeezed but not the synthetic ones. Measure water or
solution remaining in dripping/soaking container and in the bucket to calculate the amount of liquid used per net.

Assuming that one polyethylene net retained 280 ml of solution, the % concentration required for dripping is calculated as follows:

\[
\frac{\text{Grams required per net}}{\text{ml solution retained per set}} = \frac{5.67}{280} = 2\%
\]

d. Preparation of dripping solutions to treat bulk quantities of mosquito nets or curtains.

The general formula is:

\[
X = \left(\frac{A}{B}\right) - 1
\]

in which X=parts of water to be added to 1 part of emulsifiable concentrate

\[
A = \text{concentration of the emulsifiable concentrate (\%)}
\]

\[
B = \text{required concentration of the final solution (\%)}
\]

Example: A 2.0% solution of permethrin for dripping nylon mosquito nets or curtains is to be prepared from 1 25% concentrate.

\[
X = \left(\frac{25}{2.0}\right) - 1 = \frac{12.5 - 1}{1} = 11.5
\]

Therefore 11.5 parts of water to 1 part of concentrate are required, or more litre of concentrate to 11.5 litres of water.

Example: A 2.0% solution of permethrin for dripping nylon mosquito nets or curtains is to be prepared from a 50% concentrate.

\[
X = \left(\frac{50}{2}\right) - 1 = 24
\]

Therefore 24 parts of water to 1 part of concentrate are required, or one litre of concentrate to 24 litres of water.

Examples: A 0.3% solution of permethrin for dripping cotton mosquito nets or curtains is to be prepared from 25% concentrate.

\[
X = \left(\frac{25}{0.3}\right) - 1 = \frac{83.3 - 1}{1} = 82.3 \text{ rounded to } 82
\]

Therefore, 82 parts of water to 1 part concentrate are required, or one litre of concentrate to 82 litres of water, or one-half litre of concentrate to 41 litres of water to accommodate a smaller container.
Example: A 0.3% solution of permethrin for dripping cotton mosquito nets or curtains is to be prepared from a 50% concentrate.

$$X = \frac{0.3}{50} \times 1 = 166.6 - 1 = 165.6$$ or rounded to 166

Therefore 166 arts of water to 1 part of concentrate are required, or more litre of concentrate to 166 litres of water, or one half litre of concentrate to 83 litre of water to accumulate a smaller container.

e. Preparing a 2% dipping solution using a one litre bottle of 25% or 50% permethrin emulsifiable concentrate for soaking polyethylene or other synthetic fiber nets or curtains. This operational approach minimizes detailed measurements in the field.

For 25% concentrate

Add 11.5 litres water to container (with pre-measured marks to indicate volume)
Add 1 litre (1 bottle) concentrate to container
Total volume: 12.5 litres
Grams permethrin: 250
% concentration: 2%

For 50% concentrate

Add 24 litres water to container
Add 1 litre (1 bottle) concentrate to container
Total volume: 25 litres
Grams permethrin: 500
% concentration: 2%

f. Preparing a 0.3% dipping solution using a one litre bottle of 25% or 50% permethrin emulsifiable for soaking cotton nets or curtains.

For 25% concentrate

Add 82 litres of water to container
Add 1 litre (1 bottle) concentrate to container
Total volume: 83 litres
Grams permethrin: 250 litres
% concentration: 0.3%

g. Drying of nets

Polyethylene and synthetic nets are dried in horizontal position. Do not hang to dry. Drying the nets on mats removed from houses has proved to be convenient and acceptable. The nets are turned over about once every hour up to 3 to 4 hours. If the weather is good, the nets can be dried outside in the sun but not more than several hours. Under rainy conditions, they can be placed under sheltered areas or inside and left overnight to dry. When dripping no longer occurs, they can be hung up to dry. Treated cotton nets which are not over saturated and do not drip can be hung up to dry soon after the soaking procedure.
h. Treatment of one net in a plastic bag

As shown in (a) above, it is assumed that net size is 11.35 m$^2$, 5.67 grams or permethrin are needed to achieve a target dosage of 0.5 grams per square metre, and this size net absorbs 280 ml of solution.

The amount of 25% permethrin emulsifiable concentrate to use is determined as follows:

$$\text{grams required} \times 100 = 5.67 \times 100 = 22.68 \text{ ml, rounded to 23 ml}$$

% concentrated used

Therefore, 23 ml of 25% permethrin is mixed with 280 ml of water. The net is placed inside the bag and the solution added. The net and solution are mixed together, shaken and kneaded in the bag. The net is removed and dried on top of the bag or a mat as described in (g) above. The amount of water can be reduced by 23 ml if there is excess run off after the net is removed from the bag.

i. Summary of treatment procedures

Important points in the treatment are summarized as follows:

1. Dipping is the preferred method of net treatment. A 2% solution is usually sufficient to achieve a target dosage of 0.5 grams per square meter of permethrin on polyethylene, polyester, nylon or other type of synthetic fiber net or curtain. The residual effect lasts for 6 months or more. A 2% solution can be simply prepared by pouring the contents of a one-litre bottle of 25% permethrin emulsion concentrate into a container will 11.5 litres of water. With a 50% concentrate, one litre is poured into 24 litres of water. The container used can be marked to show one or both of these volume levels. A 0.3% solution is normally required for cotton material, which absorbs more liquid. Responsible staff need to check on the dosage applied and refine the operation accordingly.

With bamboo curtains or mats used over doors or windows, a higher dosage (1.0 grams per square meter) can be used.

2. Dipping the nets in a permethrin solution is a fast and simple method for treating nets and curtains under urban or rural housing conditions. Community members rapidly learn the technique which is required for follow-up treatment. A dish-pan type of plastic or aluminium container which holds 15 to 25 litres of solution has been found to be quite suitable. Normally, about one liter of solution can treat about 4 to 5 double (10m$^2$) size polyethylene or polyester nets. When the nets are removed from the solution, they should be held to drip in a bucket for no more than one minute before being laid out to dry in a horizontal position. Straw mats removed from houses are quite suitable for drying nets outside in open air. With one dipping station, about 150 nets or curtains can be treated in two hours or less.
3. One can assume that 100 treated double size nets or an equivalent area of curtain material can protect 250 persons. It is not reasonable to expect every person in a crowded household to sleep under a net. It is important that every house in a community or village has one or two treated nets to kill mosquitoes to reduce the vector density. When used in the manner, protection is provided to those who do not even sleep under the nets. Infants and small children can sleep under the nets during day time.

**Figure 17. Dipping mosquito nets in pyrethroid solution**

Nets (non-cotton) are dried in a horizontal position
Selected insecticide suitable for use as cold aerosols and thermal fogs for mosquito control

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dosage of active ingredient (g/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organophosphates</strong></td>
<td></td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>250-300</td>
</tr>
<tr>
<td>Malathion</td>
<td>112-600</td>
</tr>
<tr>
<td>Pirimiphos-methyl</td>
<td>250</td>
</tr>
<tr>
<td><strong>Pyrethroids</strong></td>
<td></td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>1-6</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>1-2</td>
</tr>
<tr>
<td>Permethrin</td>
<td>5-10</td>
</tr>
<tr>
<td>Resmethrin</td>
<td>2-4</td>
</tr>
</tbody>
</table>

Source: WHO/CDS/WHOPES/2002.5
PROCEDURE, TIMING AND FREQUENCY OF THERMAL FOGGING AND ULV SPACE SPRAY OPERATIONS

Basic steps

The following steps are followed in carrying out the space spraying of a designated area.

a. The street maps of the area to be sprayed must be studied carefully before the spraying operation begins.

b. The area covered should be at least 300 metres within the radius of the house where the dengue case was located.

c. Residents should be warned in advance before the operations so that food is covered, fires extinguished, and pets are moved out together with the occupants.

d. Ensure proper traffic control when conducting outdoor thermal fogging since it can pose a traffic hazard to motorists and pedestrians.

e. The most essential information about the operation area is the wind direction. Spraying should always be done from downwind to upwind, i.e. going against the direction of the wind.

Vehicle-mounted spraying

a. Doors and windows of houses and buildings in the area to be sprayed should be opened.

b. The vehicle is driven at a steady speed of 6-8 km/hr (3.5-4.5 mile/hr) along the streets. Spray production should be turned off when the vehicle is stationary.

c. Always spray downwind to upwind. If possible, drive the vehicle at right angles to the wind direction.

In areas where streets run parallel as well as perpendicular to the wind direction, spraying is only done when the vehicle travels upwind in road parallel to the wind direction.

d. In areas with wide streets with houses and buildings far from the roadside, the spray head should point at an angle to the left side of the vehicle (in countries where driving is on the left side of the road). The vehicle should also be driven close to the edge of the road.

e. In areas where the roads are narrow, with houses close to the roadside, the spray head should be pointed directly towards the back of the vehicle.

f. In dead-end roads, the spraying is done only when the vehicle is coming out of the dead-end, not while going in.

g. The spray head should be pointed at a 45° angle to the horizontal to achieve maximum throw of the droplets.
h. Vector mortality downwind increases as more streets are sprayed upwind in relation to the target area.

**Portable thermal fogging**

a. Thermal fogging with portable thermal foggers is done from house to house, always fogging from downwind to upwind.

b. All windows and doors should be shut for half an hour after the fogging to ensure good penetration of the fog and maximum destruction of the target mosquitoes.

c. In single storey houses, fogging can be done from the front door or through an open window without having to enter every room of the house. All bedroom doors should be left open to allow dispersal of the fog throughout the house.

d. In multi-storey buildings, fogging is carried out from upper floors to the ground floor, and from the back of the building to the front. This ensures that the operator has good visibility along his spraying path.

e. When fogging outdoors, it is important to direct the fog at all possible mosquito resting sites, including hedges, covered drains, bushes, and tree-shaded areas.

f. The most effective type of thermal fog for mosquito control is a medium/dry fog, i.e., it should just moisten the hand when the hand is passed quickly through the fog at a distance of about 2.5-3.0 metres in front of the fog tube. Adjust the fog setting so that oily deposits on the floor and furniture are reduced.

**Back-pack aerosol spraying with ULV attachments**

1. Basic points

   a. Each spray squad consists of 4 spraymen and one supervisor.

   b. Each sprayman sprays for 15-30 minutes and then is relieved by the next sprayman. He must not spray when tired.

   c. The supervisor must keep each sprayman in his sight during actual spraying in case he falls or needs help for any reason.

   d. Do not directly spray humans, birds or animals that are in front of spray nozzles less than 5 metres away.

   e. Spray at full throttle. For example, a ULV Fontan nozzle tip 0.4 can deliver 25 ml of malathion per minute, and a 0.5 tip, 65 ml. The smaller tip is usually preferred unless spraymen move quickly from house to house. Some machines can run for about one hour on a full tank of petrol.

2. House spraying technique

   a. Do not enter the house. House spraying means spraying in the vicinity of the house.
b. Stand three to five metres in front of house and spray for 10 to 15 seconds directing nozzle towards all open doors, windows and eaves. If appropriate, turn away from house and standing in the same place spray the surrounding vegetation for 10 to 15 seconds.

c. If it is not possible to stand 3 metres from the house due to closeness of houses and lack of space, spray nozzle should be directed towards house openings, narrow spaces and upwards.

d. While walking from house to house hold nozzle upwards so that particles can drift through area. Do not hold nozzle towards ground.

e. Spray particles drift through the area and into houses to kill mosquitos which become irritated and fly into the particles. The settled deposits can be residual for several day to kill mosquitos resting inside houses and on vegetation not exposed to the rain.

f. This technique permits treatment of a house with insecticide ranging from 1 to 25 grams in one minute. The dosage depends on discharge rate, concentration of insecticide applied, and time it takes to spray the house. For comparison, an indoor residual house spray may require 30 minutes of spraying to deposit 300' grams of insecticide. This assumes a dosage of 2 grams per square meter to 150 square metres of sprayable surface.

3. Information for inhabitants:
   a. time of spraying (for example 0630 to 1000 hours);
   b. all doors and windows should be open;
   c. dishes, food, fish tanks, and bird cages should be covered;
   d. stay away from open doors and windows during spraying, or temporarily leave the house and sprayed area until spraying is completed; and
   e. children or adults should not follow spray squad from house to house.

Timing of application

Spraying is carried out only when the right weather conditions are present and usually only at the prescribed time. These conditions are summarized below:

<table>
<thead>
<tr>
<th>Time</th>
<th>Most favourable conditions</th>
<th>Average conditions</th>
<th>Unfavourable conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early morning or late evening (0630-0830 hrs)</td>
<td>Early to mid-morning or late afternoon, early evening</td>
<td>Mid-morning to mid-afternoon</td>
<td></td>
</tr>
<tr>
<td>Wind</td>
<td>Steady, between 3-13 km/hr</td>
<td>0-3 km/hr</td>
<td>Medium to strong over 13 km/hr</td>
</tr>
<tr>
<td>Rain</td>
<td>No rain</td>
<td>Light showers</td>
<td>Heavy rain</td>
</tr>
<tr>
<td>Temperature</td>
<td>Cool</td>
<td>Mild</td>
<td>Hot</td>
</tr>
</tbody>
</table>
For optimum spraying conditions please note:

a. In the early morning and late evening hours, the temperature is usually cool. Cool weather is more comfortable for workers wearing protective clothing. Also, adult *Aedes* mosquitos are most active at these hours.

In the middle of the day, when the temperature is high, convection currents from the ground will prevent concentration of the spray close to the ground where adult mosquitos are flying or resting, thus rendering the spray ineffective.

b. An optimum wind speed of between 3-13 km/hr enables the spray to move slowly and steadily over the ground, allowing for maximum exposure of mosquitos to the spray. Air movements of less than 3 km/hr may result in vertical mixing, while winds greater than 13 km/hr disperse the spray too quickly.

c. In heavy rain, the spray generated loses its consistency and effectiveness. When rain is heavy, spraying should stop and the spray head of the ULV machine should be turned down to prevent water from entering the blower.

d. Spraying is permissible during light showers. Also, mosquito activity increases when the relative humidity reaches 90, especially during light showers.

How to calculate the daily spraying operation using Vehicle Mounted ULV Ground spraying equipment

A. Calculate the sprayed areas based on acre

- Vehicle speed, 5-10 miles per hour, swath width = 300 feet or 100 m
- If 1 mile = 5280 feet then 1 acre = 43 560 ft²

- For a 5 mph = \[
\frac{5 \times 5280 \times 300}{43 560} = 181.8 \text{ acres per hour}
\]

- For 4 hours operation a day = 181.8 acres x 4 hours = 727.2 acres/day of operation

- If vehicle speed maintained at 10 mph, this figure would be doubled (363.6 acres per hour).

B. Calculate the sprayed areas based on Hectare

- If vehicle speed 5 mph= 8 kph

- then 8 km x 1000 m = 8 000 m per hour

- 8000 m x 100 m swath width = 800 000 m² per hour

- let 10 000 m² = 1 ha

- 800 000 m² = 80 ha x 4 hours of operation/day = 320 ha/day
C. Insecticide Required

- If flow rate = 3 fluid ounce/minute (29.57 m1 x 3 fluid ounce) = 88.7 m1/minute
- Then one hour of operation = 60 minutes x 3 fluid ounces = 180 fluid ounces per hour or 5322 m1 per hour
- For one day of operation, 4 hours x 5322 m1 (or 5.3 litres) = 21.2 litres of technical grade of insecticide.

Calibration of back packed mist blower

Space spraying operations with portable backpacked UL V machines can be more accurately adjusted for droplet size and discharge rate. It is recommended that calibration of machines should be routinely carried out to ensure better performance of the machine.

The calibration procedures are:

1. Performance the calibration

   - Perform procedure under ideal operating conditions.
   - Pour about one litre of oil/petrol mixed into fuel tank (the mixture depending the type of machine used, read the instructions).
   - Pour one litre of test solution (recommended insecticide use for the vector control programme).
   - Start the unit (according to manufacturers instructions) and allow to run for three minutes.
   - Direct discharge horizontal.
   - Open solution tap and allow to discharge for 10 minutes (record the discharge time).
   - Close solution tap and note elapsed time.
   - Shut off engine and close petrol tap.
   - Disconnect discharge tube and allow insecticide tank contents to drain into a graduated cylinder.

2. Example of calculations

   - Original contents: 1000 ml
   - Balance: 720 ml
   - Amount discharged (10 minutes): 280 ml
   - Discharge per minute: 28 ml

Repeat discharge operation and average the results.

Frequency of application

The commencement and frequency of spraying generally recommended is as follows:

a. Spraying is started in an area (residential houses, offices, factories, schools) as soon as possible after a DF/DHF case from that area is suspected.
b. At least one treatment should be carried out within each breeding cycle of the mosquito (seven to ten days for *Aedes*). Therefore, a repeat spraying is carried out within seven to ten days after the first spraying. The time the dengue virus incubates (eight to ten days) in the mosquito is also important.

**Evaluation of epidemic spraying**

Within two days after spraying during outbreaks, a parous rate of 10% or less, in comparison to a much higher rate before spraying, indicates that most of the mosquito population is newly emerged and incapable of transmitting the disease. This also indicates the spray was effective and greatly reduced transmission by killing the older infected mosquito population. A low parous rate after spraying can occur in the absence of a marked reduction in vector density. This can be attributed to the emergence of a new population of mosquitoes which escaped the spray, a relatively low adult density before spraying and adult sampling methods which show considerable variations in density in the absence of control. An effective spray programme also should be accompanied by a reduction in hospitalized cases after the incubation of the disease in humans (about 5 to 7 days) has elapsed. The spraying should be repeated at 7 days intervals to eliminate the possibility of infected mosquitoes.
ANNEX 8

PREPARATION OF SPRAY SOLUTIONS TO KILL ADULT MOSQUITOS DURING DHF OUTBREAKS

The general formula is the same as for preparing solutions to treat mosquito nets as described in section (d), Annex 5.

\[ X = \frac{A}{B} - 1 \]

a. Example: A 1% solution of permethrin or other suitable pyrethroid is to be prepared from a 25% concentrate and used in a back-pack sprayer with ULV attachments.

\[ X = \frac{25}{1} - 1 = 24 \]

Therefore 24 parts of water to one part of concentrate are required or 250 ml of concentrate to six litres of water or kerosene. The total liquid volume should be minimized to reduce weight carried by the sprayman.

b. Example: A 1% solution of permethrin or other suitable pyrethroid is to be prepared from a 10% concentrate and used in a back-pack sprayer with ULV attachments.

\[ X = \frac{10}{1} - 1 = 9 \]

Therefore nine parts of water or kerosene to one part of concentrate are required, or 667 ml of concentrate to 6 litres of water or kerosene, or 600 ml to 5.4 litres of water or kerosene.

c. A 4% solution of malathion or pirimiphos-methyl is to be prepared from a 50% concentrate and used in a hand-carried thermal fogger.

\[ X = \frac{50}{4} - 1 \]
\[ 12.5 - 1 = 11.5 \]

Therefore 11.5 parts of diesel oil to one part of concentrate are required, or 100 ml of concentrate to 1150 ml of diesel oil.
d. A 4% solution of malathion is to be prepared from a ULV concentrate (98% technical) and used in a vehicle-mounted thermal fogger.

\[ X = \frac{98}{4} - 1 \]
\[ 24.5 - 1 = 23.5 \]

Therefore, 23.5 parts of diesel oil to 1 part of concentrate are required, or 425 ml of concentrate to 10 litres of diesel oil.

e. A ULV concentrate (95% technical) of malathion is to be used in a vehicle mounted ULV sprayer. No dilution is necessary.
Example of calculation of insecticide formulations for space spraying application

1. If the flow rate of sprayer per minute = 50 ml
2. and dosage of active ingredient (a.i.) use = 60 ml/ha or 60ml in 10000 m²
3. If the spray area = 1000 m²
4. then total a.i. required = 6 ml a.i.
5. If the spraying time is 20 minutes to cover 1,000 m²
6. then = 50 ml x 20 minutes
   = 1000 ml
   = 994 ml (water or diesel) + 6 ml a.i.

If the machine needs 6000 ml (6 Litres) for a 2 hours operation. So there is a need to increase the solution to 6000 ml by diluting the insecticide formulation accordingly:

6 ml active ingredient + 994 ml water (or diesel) = 1000 ml solution

\[
\begin{align*}
36\text{ml} & \quad \times 6 \\
5964\text{ml} & \quad \times 6 \\
6000\text{ml} & \quad \text{solution}
\end{align*}
\]

Overall, mixed 36 ml of active ingredient with 5964 ml of water (or diesel oil)
BEHAVIOURAL OBJECTIVES

Below is a list of behavioural objectives that could have an impact on prevention of dengue through control of *Aedes* mosquitoes. It is important to again stress that you should keep behavioural objectives to a minimum.

For larvae and adults control:

Prompt communities to:

- Clean and cover water storage containers.
- Keep surroundings clean and improve basic sanitation measures.
- Burn mosquito coils to kill or repel mosquitos.
- Burn coconut shells and husks to repel mosquitos which also eliminates these potential outdoor breeding sites.
- Screen houses, particularly bedrooms.
- Make available household type aerosols for killing mosquitoes.
- Use mosquito nets to protect infants and small children from bites during the daytime, and also insecticide-treated mosquito nets and curtains to kill mosquitoes attempting to bite through the nets or resting on the nets or curtains.

Larvae control:

Prompt communities to:

- Collect, remove, dispose, bury all unusable tin cans, jars, bottles, tyres, coconut shells and husks, coca pods and other items that can collect and hold water.
- Keep tyres, metal boxes, discarded appliances, sinks, basins, vehicle frames and parts of other items on industrial and commercial premises in sheltered areas protected from rainfall.
- Arrange clean-up campaigns once or twice a year by the local health authorities or community leaders in order to collect and remove all unusable containers and eliminate potential breeding sites in and around houses.
- Turn 200 litre water drums and small earthen jars upside down once a week. This emptying and cleaning procedures is easier when the water level is low.
- Periodically scrub the insides of water containers to destroy *Aedes* eggs.
- Properly cover 200 litres water drums with burlap bags or other material which allows rainwater to enter but not mosquito.
- Cover large volume (500 litres+) water storage tank inlets and overflow outlets with mosquito wire mesh.
- Empty water in flower vases at least once a week.
- Shred or cut old tyres into flat pieces and dispose of them in managed landfills away from populated areas.
- Turn canoes and small fishing boats upside down.
- Clean roof gutters and place salt in ant traps.
- Puncture holes in tyres used for recreational purposes by children in schools, parks and beaches.
- Filling tops of bamboo fences to prevent accumulation of water and breeding sites.
- Remove small copepod crustaceans of the genus *Mesocyclops* from a pond or lake and place several of them in a water storage container to kill mosquito larvae.
- Remove small larvivorous fish from a pond, stream or canal and place one or two of them in a water storage container to kill larvae.
SAMPLE ORDER FORM, BACKED BY LAW ENFORCEMENT, FOR DISTRIBUTION TO HOUSE OCCUPANTS TO ELIMINATE \textit{AEDES} LARVAE

To: 

The owner/occupier (address of premise)

Your premise at the above address has been found to breed dengue vector mosquitoes. You are hereby required under (section, law, act, order, etc.) to take the following action within 10 days from the date of the service of this order. Boxes checked below show the action required.

1.  

- [ ] Collection, removal, disposal, burying or burning of all unusable tin cans, jars, bottles, tyres, coconut shells and husks, and other container items that collect and hold water.
- [ ] Keep all tyres, metal boxes, sinks, abandoned appliances, basins, vehicle frames and parts, and related items in sheltered areas protected from rainfall.
- [ ] Turn 200 litre water drums and small water storage containers upside down once a week. This emptying and cleaning is simplified when the water level is low.
- [ ] Scrub the insides of water storage containers to destroy mosquito eggs at the time of cleaning.
- [ ] Cover large volume (500 litres +) water storage tank inlets and overflow outlets with wire mesh to prevent mosquitos from flying into the tank to lay eggs.
- [ ] Empty water in flower vases once a week.
- [ ] Puncture water logged tree holes with a knife, and level or fill in tops of bamboo fences to prevent accumulation of water.
- [ ] Place fish or suitable chemicals in large water storage tanks not easy to move or clean.
- [ ] Cut and clear weeds and tall grasses to keep premises clean and tidy.
Mosquito larvae were found breeding in ________________________

2. Measures checked above should continue until your premise is no longer favourable for the breeding of dengue vector mosquitos.

3. Failure to comply with this order shall render you liable to prosecution under (section, act of the law). This can involve a fine of (amount) and/or imprisonment of (number of days, weeks).

Dated this ______ day of _________________, 19____.

________________________________    Address:

Signature of Officer     ____________________________

textbox:
PROCEDURES FOR COLLECTION OF *AEDES* LARVAE WITH ENFORCED LEGISLATION

**Standard storage bottle for larvae**

Use a standard bottle for pathological specimen used in hospitals or any other specimen bottle available. The measurement of the bottle is approximately 80 x 30 mm.

**Larval search**

a. Explain to the occupant/house-owner the objective of the larval search.

b. Make sure that an adult from the house follows the Health Inspector during the larval search.

c. Enter all the findings whether positive or negative into the Daily Larval Survey Form, which can be easily designed locally.

d. If no larvae are found, thank the occupant/house-owner for their cooperation.

e. If live larvae are found, show the larvae to the occupant/house-owner and make a note in the official diary. Eliminate or treat the site with larvicide.

f. Collect at least 3-5 larvae from every breeding place.

g. If there is more than one positive container, the larvae from each container must be put in a separate bottle.

h. Get an acknowledgment (signature) from the occupant/house-owner on the premise form pertaining to collection of larvae. This form is attached hereto.

i. Sketch a map of the premise, and its breeding sites, if necessary.

**Labeling of specimen**

a. Every specimen collected from the field must be properly and clearly labelled.

b. Self-adhesive labels are recommended; however, other types of labels can also be used.

c. Except for the identification of the larvae which is to be done in the office, all other particulars on the label must be entered in the field.

d. The label must then be immediately affixed to the bottle to avoid possibility of mix-up.
e. The number on the label must correspond to that on the premise form.

f. The following particulars should be printed on the label:

- *mosquito larvae collection*
- *sample no:*
- *date/time of collection:*
- *type of breeding container:*
- *collection indoors/outdoors:*
- *address of premise:*
- *collection officer:*
- *findings:

**Identification of species**

a. The specimen must be identified immediately upon the return of the Health Inspector to the office. (Figures 1, 2 and 3)

b. The identification result must: be re-checked or confirmed by the Senior Officers in the Health Office.

c. Any problem in-identification' or re-checking should be referred to the Entomologist

d. Inspector must fill in the result on the label and on the premise form.

e. After identification, the specimen should be preserved in 70% ethyl alcohol. An specimens collected from a single positive container are to be preserved in the same bottle.

**Sealing of specimen bottle'**

The specimen bottle should be sealed with sealing wax and then stamped with the department seal.

**Handling and storage of specimen**

a. The specimen bottle is then handed over to the Senior Officer in the Department for safe-keeping.

b. The Senior Officer should initial the labels and record the items received and the date/time of receipt in a record book specifically designated for that purpose.

c. The items received should be kept in a locked cabinet accessible only to the officer himself and ready to be produced in court as exhibit if and when necessary in the implementation of legislative action.

d. The items should only be removed if:

- the offender has paid his fine, in which case the specimen will be destroyed;
- the items are required to be produced in court as evidence, in which case, they are handed to the Entomologist for verification, confirmation and issue of certificate of confirmation.
Confirmation by entomologist

a. Wherever possible, the Senior Officer should personally hand over the sealed specimens and a copy of the completed form to the Entomologist.
b. The Entomologist is to acknowledge receipt of the items by signing the despatch book.
c. The Entomologist should then break the seal on the specimen bottle and remove the larvae from the specimen bottle for identification and verification of species.
d. After verification, the larvae/pupae are returned to the same bottle and resealed with sealing wax and then stamped with the department seal.
e. A certificate of specimen confirmation is to be prepared by the Entomologist addressed to the Health Officer-In-Charge. This certificate will subsequently be produced in court as an exhibit.
f. All exhibits together with the certificate of confirmation by the Entomologist are subsequently returned to the Health Officer in charge, for safekeeping pending court action.
COLLECTION OF MOSQUITO LARVAE FROM PREMISES
WITH ENFORCED LEGISLATION

To: Health Office

Name: ___________________________ Our Reference: __________________
Address: _________________________ Date: __________________

Dear Sir,

Collection of Mosquito Larvae from Premises (Offense under the Anti-Insect Act).

I, together with ______________________________________ (IC No. _______________________) and __________________________________________________ (IC No.: ______________________ ), have examined and found the breeding of mosquito larvae in your premises at the following:
Date ______________________ and time ________________________.

I have taken ____________________larvae for examination, confirmation and further action.

<table>
<thead>
<tr>
<th>Type of breeding place</th>
<th>Confirmation on type of larvae found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottle No. 1</td>
<td></td>
</tr>
<tr>
<td>Bottle No. 2</td>
<td></td>
</tr>
<tr>
<td>Bottle No. 3</td>
<td></td>
</tr>
<tr>
<td>Bottle No. 4</td>
<td></td>
</tr>
</tbody>
</table>

I, resident of _________________________________________________ have

(i) Permitted the above-mentioned Health Inspector to enter and examine the premises.
(ii) Witnessed and confirmed the breeding of mosquito larvae in the above-mentioned premise

________________________
Signature of Resident
(IC No. ______________________)

Proof of submission

Time and date of delivery: ___________________________ Received from: ___________________________
Means of delivery: ___________________________ Name of recipient: ___________________________

Witnessed by:
Name: ___________________________ (Signature of Inspecting Officer)
IC No: ___________________________
Signature: ___________________________ (IC No: ___________________________)

94
ANNEX 13

HEALTH EDUCATION POSTERS ON DHF VECTOR CONTROL

Courtesy of the Ministry of Environment, Singapore

Courtesy of the Department of Health, Philippines
SAFETY MEASURES FOR INSECTICIDE USE

Safety measures in insecticide use are adopted to protect the health and lives of those applying insecticides. These measures seek to minimize the degree of poisoning by insecticides and the exposure to insecticides, prevent accidental poisoning, monitor sub-acute poisoning and provide adequate treatment for acute poisoning. These measures can be broken down into four broad categories:

a. the choice of insecticides to be used;
b. the safe use of the insecticides;
c. the monitoring of sub-acute insecticide poisoning; and
d. the treatment of insecticide poisoning.

The human population exposed to insecticide treatment is also given prime importance to ensure that health hazards are not a problem.

Choice of insecticides to be used

The choice of an insecticide for vector control is determined by the following factors:

a. toxicity and its safety to humans and to the environment;
b. effectiveness against the vector; and
c. cost of the insecticide,

In weighing the relative importance of the three factors above, the following are important from the safety standpoint:

a. An effective and/or cheap insecticide should not be used if the chemical is highly toxic to humans and other non-target organisms.
b. Pyrethroids generally have very low mammalian toxicity when compared to other groups of insecticides such as carbamates.
c. The liquid formulation of an insecticide is usually more dangerous than a solid formulation of the same strength. Certain solvents in the liquid formulation facilitate skin penetration.
d. With regard to occupational exposure, dermal exposure is more important than gastrointestinal or respiratory exposure. Thus, an insecticide with low dermal toxicity is preferred.
e. The latest information on the safety aspect of insecticides being considered must be available before a wise choice can be made.
The safe use of insecticides

The key to the use of insecticides is to control and minimize the level of routine or accidental exposure of an individual to a given insecticide. The level of exposure is in turn dependent on:

- insecticide storage conditions;
- personal hygiene and attitude of workers;
- knowledge and understanding of workers concerning insecticides;
- equipment used;
- method and rate of application;
- environmental conditions, such as prevailing winds, temperature, and humidity; duration of work; and
- protective clothing and mask used.

Thus, to minimize routine and accidental exposure of staff to insecticides, safety precautions must be observed at all stages of insecticide use.

Safety precautions during storage:

- Store insecticides in containers with the original label. Labels should identify the contents, nature of the material, preparation methods, and precautions to be employed.
- Do not transfer insecticides to other containers, or to containers used for food or beverages.
- All insecticide containers must be sealed.
- Keep insecticides in a properly-designated place, away from direct sunlight, food, medicine; clothing, children and animals, and protected from rain and flooding preferably in a locked room with posted warning signs such as "Dangerous - Insecticides - Keep Away"
- To avoid unnecessary and prolonged storage of insecticides, order only sufficient amounts needed for a given operation, or order on a regular basis (e.g. every three months depending on routine needs), or order only when the stockpile is getting low.
- Stocks received first must be used first. This avoids prolonged storage of any batch of insecticide.

Before insecticide use

- Read the label carefully and understand the directions for preparing and applying the insecticides as well as the precautions listed, then follow the directions and precautions exactly.
- Know the first aid measures and antidotes for the insecticides being used.
During mixing and spraying/fogging with insecticides

a. Do not drink, eat or smoke while working. This prevents accidental inhalation or ingestion of insecticides.
b. Mix insecticides in a well-ventilated area, preferably outdoors.
c. Mix only as much insecticide as is needed for each application. This will reduce the problem of storing and disposing of excess insecticide.
d. Do not smell or inhale insecticides.
e. Never mix insecticides directly with bare hands.
f. Stand with the wind blowing from behind when mixing insecticides.
g. Do not blow with the mouth to clear blocked spray nozzles.
h. Make sure that the spray equipment does not leak; check all joints regularly.
i. Keep all unconcerned people away from where insecticides are being mixed.
j. Exposure to spraying normally should not exceed five hours a day.
k. When spraying is undertaken, the hottest, most humid period of the day should be avoided if possible. It is best to apply insecticides early in the morning or late in the evening. This minimizes excessive sweating and encourages the use of protective clothing. Also, high temperatures increase skin absorption of insecticides.
l. Those applying insecticides should always wear long sleeved shirts and trousers.
m. Wear protective clothing and headgear where necessary to protect the main part of the body, as well as the head and neck, lower legs, hands, mouth, nose and eyes. Depending on the insecticide and type of application, boots, gloves, goggles and respirator may be required.
n. Mixers and baggers should wear rubber boots, gloves, aprons and masks, since they come in contact with technical material and concentrated formulations.
o. Those engaged in thermal fogging and UL V spraying should be provided with overalls, goggles, hats and masks.
p. Those engaged in larviciding (e.g. with temephos) need no special protective clothing because the risk of toxicity is low.
q. To protect yourself and your family, never work with insecticides in your street clothes.
r. Do not wear unwashed protective clothing. Make sure your gloves and boots have been washed inside and outside before you put them on.
s. Take heed of wind direction to avoid drift.

After spraying/fogging of insecticides

a. Wash all spray equipment thoroughly and return to the storeroom. It is important to maintain equipment in good working order after usage.
b. Empty insecticide containers should not be used in the household to store food or drinking water. They should be buried or burned. Larger metal containers can be punctured so that they cannot be reused.

c. Used containers can be rinsed two or three times with water, scrubbing the sides thoroughly. If a drum has contained an organophosphorus compound, an additional rinse should be carried out with washing soda, 50 g/l (5%), and the solution allowed to remain in the container overnight. A soakage pit should be provided for rinsings.

d. All workers must wash thoroughly with soap and water. This removes deposits of insecticides on the skin.

e. All protective clothing should be washed after each use.

f. All usage of insecticides must be recorded.

g. Eat only after thorough washing with soap and water.

Monitoring sub-acute insecticide poisoning

Regular medical surveillance of all spraymen may be required if space spray operations are done on a routine, long-term basis.

a. Mixers, baggers, and spraymen should be instructed to detect and report any early signs and symptoms of mild intoxication.

b. Any undue prevalence of illness not associated with well recognized signs and symptoms of poisoning by a particular insecticide should be noted and reported.

c. A regular medical examination, including the determination of blood cholinesterase for those applying organophosphorus compounds, should be conducted. If the level of cholinesterase activity decreases significantly (50% of a well-established pre-exposure value), the affected operator must be withdrawn from exposure until he recovers. Test kits for monitoring cholinesterase activity are available.

Symptoms of insecticide poisoning

Field workers should be taught to recognize the following symptoms:

a. DDT and other organochlorines:
   Apprehension, excitement, dizziness, hyperexcitability, disorientation, headache, muscular weakness and convulsions. These compounds are normally not used for DHF vector control.

b. Malathion, fenitrothion and other organophosphates:
   Early symptoms include nausea, headache, excessive sweating, blurred vision, lacrimation (tears from eyes), giddiness, hypersalivation, muscular weakness, excessive bronchial secretion, vomiting, stomach pains, slurred speech and muscular twitching. Later advanced symptoms may include diarrhea, convulsions, coma, loss of reflexes, and loss of sphincter control.
   (Note: Temephos has a very low toxicity rating and can safely be used in drinking water to kill mosquito larvae).
c. Carbamates:
Headache, nausea, vomiting, bradycardia, diarrhea, tremors, convulsive seizures of muscles, increased secretion of bronchial, lacrimal, salivary and sweat glands.

d. Pyrethroids (e.g., permethrin and S-bioallethrin):
These insecticides have very low mammalian toxicity, and it is deduced that only single doses above 15 gm may produce a serious hazard to an adult. In general, the effective dosages of pyrethroids for vector control are much, lower when compared with other major groups of synthetic insecticides. Although pyrethroids may be absorbed by ingestion, significant skin penetration is unlikely. Symptoms, if they develop, reflect stimulation of the central nervous system. No cases of accidental poisoning from pyrethroids have been reported in humans. Some pyrethroids, such as deltamethrin, cypermethrin and lambda-cyhalothrin can cause eye and skin irritation if adequate precautions are not taken.

e. Microbial insecticide (Bacillus thuringiensis H-14) and insect growth regulators (Methoprene):
These control agents have exceedingly low mammalian toxicity and cause no side effects. They can be safely used in drinking water.

Treatment of acute insecticide poisoning

a. Know the symptoms of poisoning due to different insecticides.
b. Call a physician.
c. Begin emergency treatment in the field. This treatment is continued during transport and ends in a medical centre.
d. Provide supportive treatment for the patient. This may include:
   i. Artificial respiration if spontaneous respiration is inadequate.
   ii. A free airway must be maintained. Excess vomitus and secretion should be removed.
   iii. Oxygen therapy for cyanosis (a blue or purplish coloration of the skin due to insufficient oxygen).
e. Decontaminate the patient as soon as possible. This may involve:
   i. Removal of contaminated clothing.
   ii. Thorough washing of the skin and hair with soap and water.
   iii. Flushing the contaminated eyes with water or saline solution for 10 minutes
   iv. Evacuation to fresh air.
f. Eliminate the poison. Determine whether the insecticide is in water emulsion or petroleum solution, if possible.
   i. If the insecticide is dissolved in a water emulsion, induce vomiting by finger or spoon down the throat. If this fails, give one tablespoon of salt in a glass of warm water until vomitus is clear.
   ii. If insecticide is dissolved in a petroleum product, have doctor or nurse perform gastric lavage, sucking the insecticide out of the stomach with a tube to prevent possibility of the
petroleum product entering lungs and causing pneumonia.

iii. Administer a laxative such as epsom salts or milk of magnesia in water to eliminate insecticide from alimentary tract. Avoid oily laxatives, such as castor oil, which might increase absorption of insecticide.

g. Administer an antidote where possible. This involves the following steps:

i. The insecticide container must be made available to the physician wherever possible. This will help in determining the group of insecticides involved in the poisoning. The label will indicate if it is a chlorinated hydrocarbon, an organophosphate, a carbamate, a pyrethroid or a bacterial insecticide.

ii. If the insecticide is an organophosphate, either atropine sulphate or 2-PAM chloride (Pralidoxime chloride) can be used as an antidote.

An injection of 2 to 4 mg atropine sulfate is given intravenously. More atropine may be required depending on severity of the poisoning. The dose of 2-PAM chloride is 1 gm for an adult and 0.25 gm for an infant.

iii. If the insecticide is a carbamate, atropine sulphate is used as an antidote. 2-PAM and other oximes are not to be used.
SELECTED REFERENCES


Destruction of Disease-bearing Insect Act (1975) [Law of Malaysia, Act 154].

Destruction of Disease-bearing Insects Act (1985) [The Statutes of Republic of Singapore, Chapter 79].


Yap, HH [et al.] (1988). Comparative adulticidal and larvicidal effects of thermal fogging formulations of Resigen (R) and malathion against *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* (Say) in urban areas, Malaysia. *Tropical Biomedicine*, 5(2): 125-130.


Insecticide-treated Mosquito Net Interventions, WHO/CDS/RBM/2002.45
