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Prevention and Control of Dengue and Dengue Haemorrhagic Fever

Comprehensive Guidelines

World Health Organization
Regional Office for South-East Asia
New Delhi

Contents

Preface

THROUGH the ages, dengue fever (DF) has been a cause of public health concern in the South-East Asia Region. After World War II, there was a dramatic increase in the frequency and number of epidemics in South-East Asia, with the emergence of the severe forms - dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Globally, 2.5 to 3 billion people are estimated to be at risk of infection with dengue viruses. Affecting mostly children, the case fatality rates range from less than 1% to 10% (average 5%).

Dengue haemorrhagic fever appeared for the first time in 1953 in the Philippines and later spread to most countries in the WHO South-East Asia (SEA) and Western Pacific (WP) Regions. In 1964, these two Regions organized the first Interregional Seminar on Mosquito-borne Haemorrhagic Fevers in Bangkok, Thailand. Since then, the World Health Organization has been actively involved in the planning, development, establishment and evaluation of dengue prevention and control programmes in endemic Member States.

In 1974, the two WHO Regions established a Technical Advisory Committee on DHF. In view of the increasing occurrence of epidemics, it was felt that guidelines for the diagnosis, treatment and control of dengue infection would be very useful to the physicians and health authorities. The first version of the Technical Guide for Diagnosis, Surveillance, Prevention and Control of Dengue Haemorrhagic Fever was published in 1975. The Regions also supported research on the pathophysiology and clinical and laboratory diagnosis of dengue. On the basis of these studies, revised guidelines on DHF were issued in 1980, 1986 and 1998. Simultaneously, this effort was strengthened at the regional level by the publication of technical guidelines by some WHO Regional offices.

Researchers and programme managers studying dengue in the South-East Asia Region have demonstrated that different geographic areas show a variable response to the infection and accordingly, present different epidemiological patterns. The complex epidemiology of DF/DHF may be further modified at the local level by different socioeconomic and sociocultural practices in the diverse

communities of the Region. These epidemiological complexities call for specific solutions for the prevention and control of DF/DHF.

The Comprehensive Guidelines for Prevention and Control of Dengue/DHF focus on the South-East Asia Region. While the key roles of Ministries of Health as well as the non-health sectors have been highlighted, emphasis has also been placed on community involvement particularly of students, welfare and civic organizations and NGOs. This is essential to achieve acceptable levels of vector control through cost-effective and sustainable activities.

Epidemic preparedness is another important area which requires attention. Efforts to make communities self-reliant to meet the problems posed by dengue in the domestic environment are essential.

It is hoped that these guidelines, drawn upon earlier guidelines and numerous WHO and other publications will prove useful in effectively meeting the challenge posed by DF/DHF in the Region.

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Introduction

DENGUE is caused by a virus spread by *Aedes* (*Stegomyia*) mosquitoes. Over the past two decades there has been a dramatic global increase in the frequency of dengue fever (DF) dengue haemorrhagic fever (DHF), and dengue shock syndrome (DSS) and their epidemics, with a concomitant increase in disease incidence (Box 1). *The World Health Report 1996*[1] stated, that the "re-emergence of infectious diseases is a warning that progress achieved so far towards global security in health and prosperity may be wasted." The report further indicated that "infectious diseases range from those occurring in tropical areas (such as malaria and DHF which are most common in developing countries) to diseases found worldwide (such as hepatitis and sexually transmitted diseases, including HIV/AIDS) and food-borne illnesses that affect large numbers of people in both the richer and poorer nations."

In May 1993, the 46th World Health Assembly (WHA) adopted a resolution on dengue prevention and control which urged that the strengthening of national and local programmes for the prevention and control of DF, DHF and DSS should be among the priorities of WHO Member States where the disease is endemic. The resolution also requested that: (1) strategies be developed to contain the spread and increasing incidence of dengue in a manner sustainable by countries, (2) community health education be improved, (3) health promotion be encouraged, (4) research be strengthened, (5) dengue surveillance be expanded, (6) guidance be given in vector control, and

Box I
Dengue and Dengue Haemorrhagic Fever: Key Global Issues

- 2.5-3 billion people are at risk.
- *Aedes aegypti* is the primary epidemic vector.
- Imported cases are common.
- Urban disease, but becoming rural.
- Estimated 50-100 million cases of dengue fever annually.
- 500,000 DHF cases require hospitalization, each year of which 90% are children less than 15 years of age.
- Mortality averages 5% of DHF cases.
- Epidemics are cyclical.

(7) the mobilization of external resources for disease prevention be given a priority.

In response to the WHA resolution, on dengue prevention and control, a global strategy for operationalization of vector control was developed based on five major components (Box 2). One of the major pillars of the global strategy is to increase active and accurate laboratory-based surveillance for DF/DHF and its vectors. Effective surveillance requires that DHF be made a reportable (notifiable) disease by all DF/DHF endemic countries. These guidelines are based on the regional strategy developed in 1995, which emphasizes disease surveillance, case management, integrated vector control and epidemic preparedness.

Box 2
Global Strategy for Control of DF/DHF Vectors

- Selective integrated mosquito control with community and intersectoral participation
- Active disease surveillance based on a strong health information system
- Emergency preparedness
- Capacity building and training
- Research on vector control

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Dengue and Dengue Haemorrhagic Fever

2.1 Historical Overview

Dengue epidemics are known to have occurred over the last three centuries in tropical, subtropical and temperate areas of the world. The first epidemic of dengue was recorded in 1635[2] in the French West Indies, although a disease compatible with dengue had been reported in China as early as 992 AD[3]. During the 18th, 19th and early 20th centuries, epidemics of dengue-like diseases were described globally in the tropics as well as in some temperate regions. Rush[4] was probably describing dengue when he wrote of "break-bone fever" occurring in Philadelphia in 1780. Most of these epidemics were clinical dengue fever, although some were associated with the severe haemorrhagic form of the disease. Efforts to control *Aedes aegypti* and economic development have markedly reduced the threat of epidemic dengue in temperate countries during the past 50 years.

The first recorded outbreak of a dengue disease compatible with DHF occurred in Australia in 1897. A similar haemorrhagic disease was recorded in 1928 during an epidemic in Greece and again in Taiwan in 1931. The first confirmed epidemic of DHF was recorded in the Philippines in 1953-1954. Since then, major outbreaks of DHF with significant mortality have occurred in most countries of the South-East Asia Region, including India, Indonesia, Maldives, Myanmar, Sri Lanka, and Thailand, as well as in Singapore, Cambodia, China, Laos, Malaysia, New Caledonia, Palau, Philippines, Tahiti and Vietnam in the Western Pacific Region. Over the past 20 years, there has been a dramatic increase in the incidence and geographical distribution of DHF, and epidemics now occur each year in some South-East Asian countries.

2.2 The Virus

The dengue viruses are members of the genus *Flavivirus* and family *Flaviviridae.* These small (50 nm.) viruses contain single-strand RNA. The virion consists of a nucleocapsid with cubic symmetry enclosed in a lipoprotein envelope. The dengue virus genome is

approximately 11,000 base pairs in length, and is composed of three structural protein genes encoding the nucleocaprid or core protein (C), a membrane-associated protein (M), an envelope protein (E), and seven nonstructural protein (NS) genes. The envelope glycoprotein is associated with viral haemagglutination and neutralization activity.

The dengue viruses form a distinct complex within the genus *Flavivirus* based on antigenic and biological characteristics. There are four virus serotypes which are designated as DEN-1, DEN-2, DEN-3 and DEN-4. Infection with any one serotype confers lifelong immunity to that virus serotype. Although all four serotypes are antigenically similar, they are different enough to elicit cross-protection for only a few months after infection by any one of them.

Dengue viruses of all four serotypes have been associated with epidemics of dengue fever in which there was little or no evidence of DHF. All four virus serotypes have also caused DHF epidemics associated with severe and fatal disease.

2.3 The Vector

Dengue viruses are transmitted from person to person by *Aedes (Ae.)* mosquitoes of the subgenus *Stegomyia. Ae. aegypti* is the most important epidemic vector, but other species such as *Ae. albopictus, Ae. polynesiensis,* members of *Ae. scutellaris* complex, and *Ae. (Finlaya) niveus* have also been incriminated as secondary vectors. All except *Ae. aegypti* have their own restricted geographical distribution and, although they may be excellent hosts for dengue viruses, they are generally less efficient epidemic vectors than *Ae. aegypti.*

2.4 The Host

Dengue viruses infect humans and several species of lower primates. Humans are the main urban reservoir of the viruses. Studies in Malaysia and Africa have shown that monkeys are infected and are the likely reservoir hosts, although the epidemiological significance of this observation remains to be established[4,5,6]. Dengue virus strains grow well in insect tissue cultures and on mammalian cell cultures after adaptation.

2.5 Global Situation

Significant recent dengue outbreaks have occurred in five of the six WHO Regions, with the European Region being the only exception. However, imported dengue has been reported in significant numbers in several countries of that Region. The global population at risk is estimated to range from 2.5 to 3 billion individuals living mainly in urban areas in tropical and subtropical regions. However, while dengue was formerly thought to be strictly an urban problem, it is now recognized as also being of significance in rural areas of South-East Asia. It is estimated that there are at least 100 million cases of dengue fever annually and 500,000 cases of DHF which require hospitalization. Of the latter, 90% are children under the age of 15 years. DHF mortality rates average 5%, with approximately 25,000 deaths each year[7].

Box 3
The Global Problem of Dengue

Africa – 20 endemic countries

- Epidemics have been caused by all four virus serotypes in the past 18 years
- Recent major epidemic in the Comores and Eritrea
- DHF not reported

Eastern Mediterranean – 4 endemic countries

- Recent major epidemics in Dibouti, Saudi Arabia and Pakistan
- Multiple virus serotypes circulating
- Sporadic cases of DHF documented

Western Pacific – 29 endemic countries

- Recent major epidemics in Singapore, Cambodia, Vietnam, Philippines, Tahiti, Fiji and Palau
- All four virus serotypes circulating
- DHF is endemic and is a major public health problem in many countries

Americas – 42 endemic countries

- Recent major epidemics in Central America, Colombia, Peru, Venezuela, Brazil, Mexico, Cuba, Puerto Rico, Barbados and Trinidad
- All four serotypes circulating
- DHF is a newly emergent disease and now occurs in 24 countries

South-East Asia – 7 endemic countries

- Recent major epidemics in India, Sri Lanka, Thailand, Myanmar and Indonesia
- All four virus serotypes circulating
- DHF is a leading cause of hospitalization and death among children

The world distribution of DF/DHF has recently been reviewed[8]. Between 1975 and 1995, DF/DHF was present in 102 countries of five WHO Regions: 20 countries in Africa, 42 in the Americas, 7 in South-East Asia, 4 in the Eastern Mediterranean, and 29 in the Western Pacific (Box 3).

All tropical regions of the world have now become hyperendemic, with all four virus serotypes circulating simultaneously in the Americas, Asia, the Pacific and Africa[8]. Northern Queensland, Australia has reported three serotypes (DEN-1, DEN-2 and DEN-3) and the Middle East has reported two serotypes (DEN-I and DEN-2). The current situation of DF/DHF in different WHO Regions has been described by Gratz and Knudsen (1996)[9] and Gubler (1998)[10]. Factors responsible for the resurgence of dengue infection are summarized in Box 4[10].

2.6 Dengue/Dengue Haemorrhagic Fever in South-East Asia

The reported DHF cases and deaths between 1985-1996 in the ten countries of the WHO South-East Asian Region are presented in Table 1. Boxes 5 and 6 and Figure 1 underscore the public health importance of this disease in the Region, which continues to be hyperendemic. The number of cases have increased over the last three to five years, with recurring epidemics. Moreover, there has been an increase in the proportion of dengue cases with severe disease, particularly in India, Sri Lanka and Myanmar.

Box 4
Factors Responsible for the Resurgence of the Dengue Epidemic

- Unprecedented human population growth
- Unplanned and uncontrolled urbanization
- Inadequate waste management and water supply
- Increased distribution and densities of vector mosquitoes
- Lack of effective mosquito control
- Increased movement and spread of dengue viruses
- Development of hyperendemicity
- Deterioration in public health infrastructure

Box 5
Dengue Haemorrhagic Fever as a Major Public Health Problem in South-East Asia

- Seven of the ten countries have a serious DHF problem.
- DHF is a leading cause of hospitalization and death among children in these countries.
- The incidence of DHF in the Region has increased dramatically in the past 17 years; and approximately five times more cases have been reported since 1980 than in the previous 30 years.
- The geographic distribution has expanded within countries as well as to new countries in the Region.

Box 6
Stratification of Dengue / Dengue Haemorrhagic Fever in the South-East Asia Region

Category A (Indonesia, Myanmar, Thailand)

- Major public health problem
- Leading cause of hospitalization and death among children
- Cyclical epidemics in urban centres with 3-5 year periodicity
- Spreading to rural areas
- Multiple virus serotypes circulating
- *Aedes aegypti* is the principal epidemic vector
- Role of *Aedes albopictus* is uncertain

Category B (Bangladesh, India, Maldives, Sri Lanka)

- DHF is an emergent disease
- Cyclical epidemics are becoming more frequent
- Multiple virus serotypes circulating
- Expanding geographically within countries
- *Aedes aegypti* is the principal epidemic vector
- Role of *Aedes albopictus* is uncertain

Category C (Bhutan, Nepal)

- No reported cases
- Endemicity uncertain

Category D (DPR Korea)

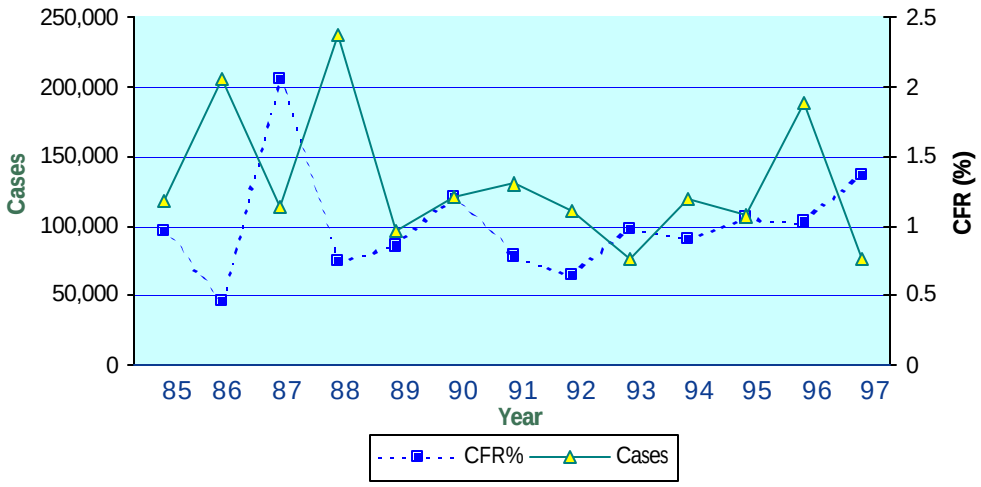
- Non-endemic

Table 1. **Number of Reported Cases and Deaths of DF and DHF in the South-East Asia Region**
By Country, Years 1985-97

	Country	India	Indonesia	Maldives	Myanmar	Sri Lanka	Thailand	Total
1985	Case		13 588		2 666		80 076	96 330
	Death	NA	460	NA	134	NA	542	1 136
	CFR (%)		3.39		5.03		0.68	1.18
1986	Case		16 529		2 092		27 837	46 458
	Death	NA	608	NA	111	NA	236	955
	CFR (%)		3.68		5.31		0.85	2.06
1987	Case		23 864		7 231		174 285	205 380
	Death	NA	1 105	NA	227	NA	1 007	2 339
	CFR(%)		4.63		3.14		0.58	1.14
1988	Case		44 573	2 054	1 178	10	26 925	74 741
	Death	NA	1 527	9	64	0	179	1 779
	CFR(%)		3.43	0.43	5.43	0.00	0.636	2.38
1989	Case		10 362		1 196	203	74 391	86 152
	Death	NA	464	NA	62	20	290	836
	CFR(%)		4.48		5.18	9.85	0.39	0.97
1990	Case		22 807		5 242	1 350	92 002	121 401
	Death	NA	821	NA	179	54	414	1468
	CFR(%)		3.60		3.41	4.00	0.44	1.21
1991	Case	6 291	21 120		6 772	1048	43 511	78 742
	Death	3	578	NA	282	31	137	1 031
	CFR(%)	0.05	2.74		4.16	2.96	0.31	1.31
1992	Case	2 683	17 620		1 685	656	41 125	63 769
	Death	12	509	NA	37	15	136	709
	CFR(%)	0.45	2.89		2.20	2.29	0.33	1.11
1993	Case	11 125	17 418		2 279	750	67 017	98 589
	Death	36	418	NA	67	7	222	750
	CFR(%)	0.32	2.40		2.94	0.93	0.33	0.76
1994	Case	7 494	18 783		11 647	582	51 688	90 194
	Death	4	471	NA	461	7	140	1 083
	CFR(%)	0.05	2.51		3.96	1.20	0.27	1.20
1995	Case	7 847	35 102		2 477	440	59 911	105 777
	Death	10	885	NA	53	11	183	1 142
	CFR(%)	0.13	2.52		2.14	2.50	0.31	1.08
1996	Case	16 517	44 650		1 655	1 298	38 109	102 229
	Death	545	1 192	NA	18	54	114	1 923
	CFR(%)	3.30	2.67		1.09	4.16	0.30	1.88
1997	Case	1 177	30 730		3 993	980	99 150	136 030
	Death	36	681	NA	76	17	227	1 037
	CFR(%)	3.05	2.22		1.90	1.73	0.27	0.76

NA: Not available

Figure 1 Number of Reported Cases and Case Fatality Rate of DF/DHF in the South-East Asia Region, 1985-1997



2.7 Transmission Cycle

The female *Aedes (Stegomyia)* mosquito usually becomes infected with dengue virus when she takes blood from a person during the acute febrile (viraemic) phase of illness (Box 7). After an extrinsic incubation period of 8 to 10 days, the salivary glands of the mosquito become infected and the virus is transmitted when the infective mosquito bites and injects the salivary fluid into the wound of another person. Following an incubation period in humans of 3-14 days (4-6 days average), there is often a sudden onset of the disease, with fever, headache, myalgias, loss of appetite, and a variety of nonspecific signs and symptoms, including nausea, vomiting and rash.

Viraemia is usually present at the time of or just before the onset of symptoms and lasts an average of five days after the onset of illness. This is the crucial period when the patient is most infective for the vector mosquito and contributes to maintaining the transmission cycle if the patient is not protected against vector mosquito bites.

There is evidence that the vertical transmission of dengue virus from infected female mosquitoes to the next generation occurs in several species including *Ae. aegypti* and *Ae. albopictus*[11]. This may be an important mechanism for virus maintenance, but does not appear to be important in epidemics[10,11].

2.8 Epidemiological Pattern

Virus-host interactions

In order to understand the various epidemiological situations, it is important to

Box 7
Transmission Cycle

- Vectors: *Aedes aegypti*, other *Aedes (Stegomyia)* spp.
- Extrinsic incubation period 8-10 days
- Dengue virus infection in person from mosquito bite
- Intrinsic incubation 3-14 days (Average 4-7 days)
- Viraemia appears before the onset of symptoms and lasts an average of five days after the onset
- Possible vertical transmission, which may be important in virus maintenance, but not in epidemic cycles

recognize the fundamental aspects of virus-host interaction. These are:

- Dengue infection frequently causes mild illness in children.
- Dengue infection in adults frequently produces symptoms, with the infection: apparent illness ratio approaching 1 in some epidemics. Some virus strains, however, produce very mild illness in both adults and children which is often not recognized as dengue and circulates silently in the community.

Box 8
Risk Factors For Dengue Haemorrhagic Fever

- Immune status of individuals
- Infecting virus strain/serotype
- Age of patient
- Genetic background of patient

- Primary as well as secondary dengue infections in adults may result in severe gastrointestinal haemorrhage, as well as cases with increased vascular permeability. For example, many adults with severe haemorrhage associated with DEN-1 in Taiwan in 1988 had underlying peptic ulcer disease.

Risk factors for DHF

Secondary dengue infection is a risk factor for DHF, including passively-acquired antibodies in infants. The strain of virus is also a risk factor for DHF; not all wild type viruses have epidemic potential or cause severe disease (Box 8). Finally, the age of the patient and host genetics are risk factors of DHF. Although DHF can and does occur in adults, most cases are in children less than 15 years of age, and circumstantial evidence suggests that some population groups may be more susceptible to vascular leak syndrome than others.

3

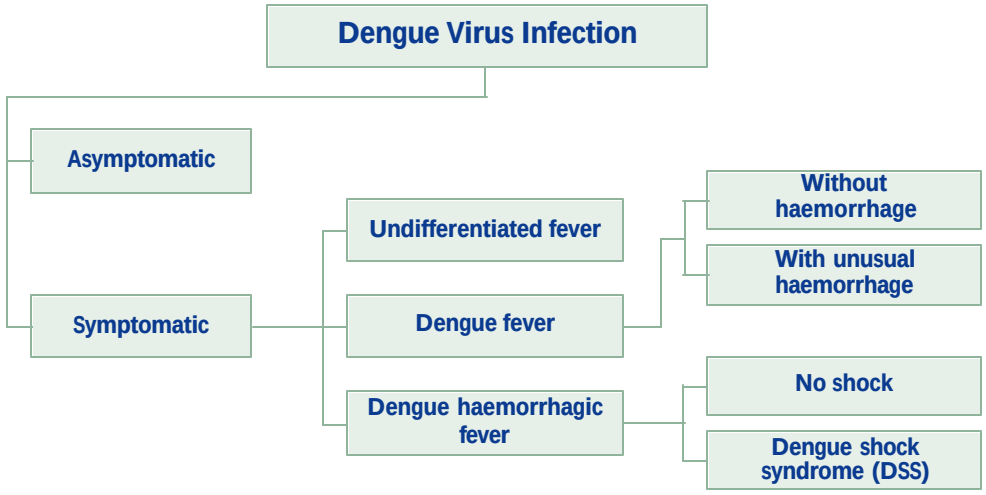
Clinical Manifestations and Diagnosis

3.1 Clinical Presentation

Dengue virus infection may be asymptomatic or may cause undifferentiated febrile illness (viral syndrome), dengue fever (DF), or dengue haemorrhagic fever (DHF) including dengue shock syndrome (DSS). Infection with one dengue serotype gives lifelong immunity to that particular serotype, but there is no cross-protection for the other serotypes. The clinical presentation depends on age, immune status of the host, and the virus strain (Box 9).

(i) Undifferentiated fever: Infants, children and some adults who have been infected with dengue virus for the first time (i.e. primary dengue infection) will develop a simple fever

Box 9
Manifestation of Dengue Infection



indistinguishable from other viral infections. Maculopapular rashes may accompany the fever or may appear during defervescence.

(ii) Dengue fever: Dengue fever is most common in older children and adults. It is generally an acute biphasic fever with headache, myalgias, arthralgias, rashes and leucopenia. Although DF is commonly benign, it may be an incapacitating disease with severe muscle and joint pain (break-bone fever), particularly in adults, and occasionally with unusual haemorrhage. In dengue endemic areas, DF seldom occurs among indigenous people.

(iii) Dengue haemorrhagic fever: Dengue haemorrhagic fever is most common in children less than 15 years of age, but it also occurs in adults. DHF is characterized by the acute onset of fever and associated non-specific constitutional signs and symptoms. There is a haemorrhagic diathesis and a tendency to develop fatal shock (dengue shock syndrome). Abnormal haemostasis and plasma leakage are the main patho-physiological changes, with thrombocytopenia and haemoconcentration presenting as constant findings. Although DHF occurs most commonly in children who have experienced secondary dengue infection, it has also been documented in primary infections.

Dengue fever

Clinical Symptoms

After an average incubation period of 4-6 days (range 3-14 days), various non-specific, undifferentiated prodomes, such as headache, backache and general malaise may develop. Typically, the onset of DF in adults is sudden, with a sharp rise in temperature occasionally accompanied by chillis, and is invariably associated with severe headache and flushed face[12]. Within 24 hours there may be retro-orbital pain, particularly on eye movement or eye pressure, photophobia, backache and pain in the muscles and joints/bones of the extremities. The other common symptoms include anorexia and altered taste sensation, constipation, colicky pain and abdominal tenderness, dragging pains in the inguinal region, sore throat, and general depression. These symptoms vary in severity and usually persist for several days.

Fever: The body temperature is usually between 39°C and 40°C, and the fever may be biphasic, lasting 5-7 days.

Rash: Diffuse flushing or fleeting pinpoint eruptions may be observed on the face, neck and chest during the first half of the febrile period, and a conspicuous rash that may be maculopapular or scarlatiniform appears on approximately the third or fourth day. Towards the end of the febrile period or immediately after defervescence, the generalized rash fades and localized clusters of petechiae may appear over the dorsum of the feet, on the legs, and on the hands and arms. This confluent petechial rash is characterized by scattered, pale, round areas of normal skin. Occasionally the rash is accompanied by itching.

Skin Haemorrhage: A positive tourinquet test and/or petechiae.

Course: The relative duration and severity of DF varies between individuals in a given

epidemic, as well as from one epidemic to another. Convalescence may be short and uneventful, but may also often be prolonged. In adults it sometimes lasts for several weeks and may be accompanied by pronounced asthenia and depression. Bradycardia is common during convalescene. Haemorrhagic complications, such as epistaxis, gingival bleeding, gastrointestinal bleeding, haematuria and hypermenorrhoea, may accompany epidemics of DF. Severe bleeding has occasionally caused deaths in some epidemics. *Dengue fever* with haemorrhagic manifestations must be differentiated from *dengue haemorrhagic fever.*

Clinical Laboratory Findings

The laboratory findings during an acute DF episode of illness are as follows:

- Total WBC is usually normal at the onset of fever; then leucopenia develops and lasts throughout the febrile period.
- Platelet counts are usually normal, as are other components of the blood clotting mechanism. However, thrombocytopenia is common in some epidemics.
- Serum biochemistry and enzymes are usually normal, but liver enzyme levels may be elevated.

Differential Diagnosis: The differential diagnoses associated with DF include a wide variety of viral (including chikungunya), bacterial, rickettsial and parasitic infections that produce a similar syndrome. It is impossible to diagnose mild dengue infection clinically, particularly when there are only sporadic cases. A definitive diagnosis is confirmed by virus isolation and/or serology.

Dengue haemorrhagic fever and dengue shock syndrome

Typical cases of DHF are characterized by high fever, haemorrhagic phenomena, hepatomegaly, and often circulatory failure[12,13]. Moderate to marked thrombocytopenia with concurrent haemoconcentration are distinctive clinical laboratory findings. The major pathophysiologic changes that determine the severity of the disease in DHF and differentiate it from DF are abnormal haemostasis and leakage of plasma as manifested by thrombocytopenia and rising haematocrit.

DHF commonly begins with a sudden rise in temperature which is accompanied by facial flush and other non-specific constitutional symptoms resembling dengue fever, such as anorexia, vomiting, headache, and muscle or joint pains (Table 2)[14].

Some DHF patients complain of sore throat, and an injected pharynx may be found on examination. Epigastric discomfort, tenderness at the right costal margin, and generalized abdominal pain are common. The temperature is typically high and in most cases continues for two to seven days, then falls to a normal or subnormal level. Occasionally the temperature may be as high as 40ºC, and febrile convulsions may occur.

The most common haemorrhagic phenomenon is a positive tourniquet test. Easy bruising and bleeding at venipuncture sites are present in most cases. Fine petechiae scattered on the extremities, axillae, face and soft palate may be seen during the early febrile phase. A confluent petechial rash with

Table 2. **Non-specific constitutional symptoms observed in haemorrhagic fever patients with dengue and chikungunya virus infection[a]**

Symptom	DHF (%)	Chikun-gunya fever (%)
Injected pharynx	98.9	90.3
Vomiting	57.9	59.4
Constipation	53.3	40.0
Abdominal pain	50.0	31.6
Headache	44.6	68.4
Generalized lymphadenopathy	40.5	30.8
Conjunctival injection	32.8[b]	55.6[b]
Cough	21.5	23.3
Restlessness	21.5	33.3
Rhinitis	12.8	6.5
Maculopapular rash	12.1[b]	59.6[b]
Myalgia/arthralgia	12.0[b]	40.0[b]
Enanthema	8.3	11.1
Abnormal reflex	6.7	0.0
Diarrhoea	6.4	15.6
Palpable spleen (in infants < 6 months)	6.3	3.1
Coma	3.0	0.0

[a] Based on: Nimmannitya S, et al, American Journal of Tropical Medicine and Hygiene, 1969, 18:954-971

[b] Statistically significant difference

characteristic small, round areas of normal skin is sometimes seen in convalescence after the temperature has returned to normal. A maculopapular or rubella-type rash may be observed early or late in the disease. Epistaxis and gum bleeding are less common. Mild gastrointestinal haemorrhage is occasionally observed. Haematuria is rarely observed.

The liver is usually palpable early in the febrile phase, varying from just palpable to 2-4 cm below the right costal margin. Liver size is not correlated with disease severity, but hepatomegaly is more frequent in shock cases. The liver is tender, but jaundice is not usually observed, even in patients with an enlarged, tender liver. In some epidemics, hepatomegaly is not a consistent finding. Splenomegaly is rarely observed in infants under six months, however, the spleen is sometimes prominent on X-ray examination. Chest X-rays show/reveal pleural effusion, mostly on the right side, as a constant finding. The extent of pleural effusion is positively correlated with disease serverity.

In mild or moderate cases, all signs and symptoms abate after the fever subsides. Fever lysis may be accompanied by profuse sweating and mild changes in pulse rate and blood pressure, together with coolness of the extremities and skin congestion. These changes reflect mild and transient circulatory disturbances as a result of some degree of plasma leakage. Patients usually recover either spontaneously or after fluid and electrolyte therapy.

In severe cases, the patient's condition suddenly deteriorates a few days after onset of fever. At the time of or shortly after the temperature drop, between three and seven days after the onset, there are signs of circulatory failure: the skin becomes cool, blotchy and congested, circumoral cyanosis is frequently observed, and the pulse becomes weak and rapid. Although some patients may appear lethargic, they become restless and then rapidly go into a critical stage of shock. Acute abdominal pain is a frequent complaint shortly before the onset of shock.

The early stage of shock is characterized by a rapid and weak pulse with narrowing of

the pulse pressure ≤ 20 mmHg, with a minimal difference between systolic and diastolic blood pressure levels, e.g (100/90) or hypotension, with cold clammy skin and restlessness. Patients in shock are in danger of dieing if they do not promptly get appropriate treatment. Patients may pass into a stage of profound shock with blood pressure and/or pulse becoming imperceptible. Most patients remain conscious almost to the terminal stage. Shock lasts for a short time; the patient may die within 12 to 24 hours, or recover rapidly following appropriate volume-replacement therapy. Alternatively, uncorrected shock may give rise to a more complicated course with metabolic acidosis, severe bleeding from the gastrointestinal tract as well as from various other organs, and a poor prognosis. Patients with intracranial haemorrhage may have convulsions and go into coma. Encephalopathy may occur in association with metabolic and electrolyte disturbances.

Convalescence in DHF with or without shock is short and uneventful. Even in cases with profound shock, once the shock is overcome, the surviving patients recover within two to three days. The return of appetite is a good prognostic sign. Common findings in convalescence include sinus bradycardia or arrythmia and the characteristic dengue confluent petechial rash as described for DF.

3.2 Pathogenesis and Pathophysiology

The pathogenesis of DHF is not fully understood, but two main pathophysiologic changes occur:

- Increased vascular permeability resulting in plasma leakage, hypovolaemia and shock. DHF appears unique in that there is selective leakage of plasma into the pleural and peritoneal cavities and the period of leakage is short (24-48 hours).
- Abnormal haemostasis due to vasculopathy, thrombocytopenia and coagulopathy, leading to various haemorrhagic manifestations.

Activation of the complement system is a constant finding in patients with DHF. Levels of C3 and C5 are depressed, and C3a and C5a are elevated. The mechanisms of complement activation are not known. The presence of immune complexes has been reported in DHF cases, however, the contribution of antigen-antibody complexes to complement activation in patients with DHF has not been demonstrated.

It has been hypothesized that the severity of DHF compared with DF is explained by the enhancement of virus multiplication in macrophages by heterotypic antibodies resulting from a previous dengue infection. There is evidence, however, that viral factors and a cell-mediated immune response are also involved in the pathogenesis of DHF.

3.3 Clinical Laboratory Findings of DHF

The laboratory findings in DHF are as follows:

- The WBC may be normal, but leucopenia is common initially, with neutrophils predominating. Towards the end of the febrile phase there is a drop in the total number of white cells as well as in the

number of polymorphonuclear cells. A relative lymphocytosis with more than 15% atypical lymphocytes is commonly observed towards the end of the febrile phase (critical stage) and at the early stage of shock.

- Thrombocytopenia and haemo-concentration are constant findings in DHF. A drop in platelet count to below 100,000/mm^3 is usually found between the third and eighth days of illness. A rise in haematocrit occurs in all DHF cases, particularly in shock cases. Haemo-concentration with haematocrit increased by 20% or more is considered objective evidence of increased vascular permeability and leakage of plasma. It should be noted that the level of haematocrit may be affected by early volume replacement and by bleeding.
- A transient mild albuminuria is sometimes observed.
- Occult blood is often found in the stool.
- In most cases, assays of coagulation and fibrinolytic factors show reductions in fibrinogen, prothrombin, factor VIII, factor XII, and antithrombin III. A reduction in antiplasmin (plasmin inhibitor) has been noted in some cases. In severe cases with marked liver dysfunction, reduction is observed in the vitamin K-dependent prothrombin family, such as factors V, VII, IX and X.
- Partial thromboplastin time and prothrombin time are prolonged in about one-half and one-third of DHF cases respectively. Thrombin time is also prolonged in severe cases.
- Serum complement levels are reduced.
- Other common findings are hypoproteinemia, hyponatremia, and mildly elevated serum aspartate aminotransferase levels. Metabolic acidosis is frequently found in cases with prolonged shock. Blood urea nitrogen is elevated in the terminal stage of cases with prolonged shock.

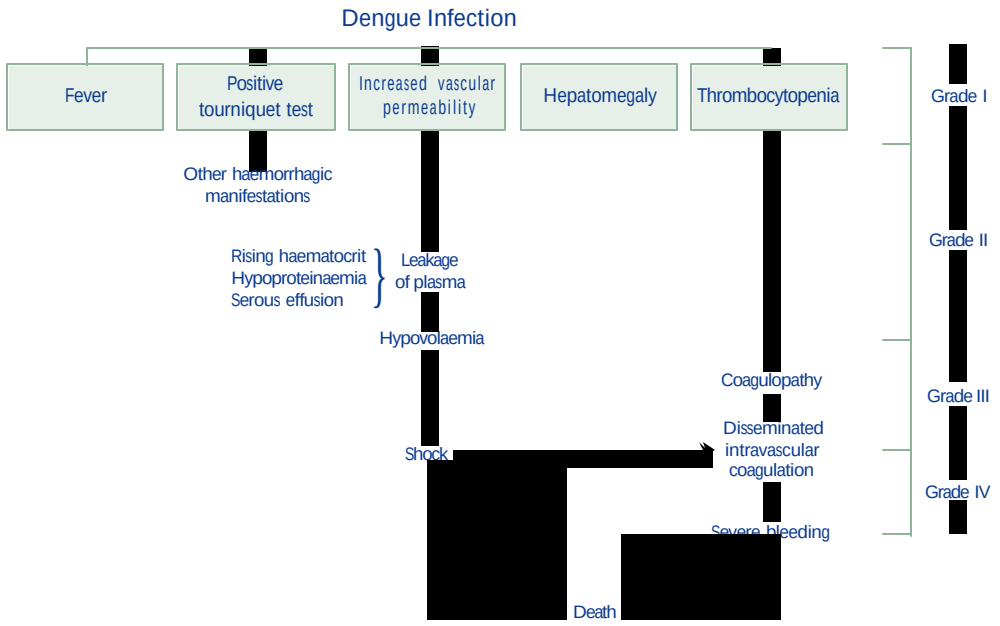
3.4 Criteria for Clinical Diagnosis of DHF/DSS

Clinical Manifestations:

- Fever: acute onset, high and continuous, lasting 2 to 7 days.
- Any of the following haemorrhagic manifestations (including at least a positive tourniquet test*): petechiae, purpura, ecchymosis, epistaxis, gum bleeding, and haematemesis and/or melena.
  - Enlargement of the liver (hepatomegaly) is observed at some stage of the illness in 90-98% of Thai children, but its frequency may be variable in other countries.
  - Shock, manifested by rapid and weak pulse with narrowing of the pulse pressure (20mm Hg or less), or hypotension, with the presence of cold, clammy skin and restlessness.

* The tourniquet test is performed by inflating a blood pressure cuff to a point midway between the systolic and diastolic pressures for five minutes. The test is considered positive when 10 or more petechiae per 2.5 cm^2 (1 square inch) are observed. In DHF the test usually gives a definite positive result with 20 petechiae or more. The test may be negative or only mildly positive during the phase of profound shock. It usually becomes positive, sometimes strongly positive, if it is conducted after recovery from shock.

Box 10
The Spectrum of Dengue Haemorrhagic Fever



Source: Dengue haemorrhagic fever - Diagnosis, treatment, prevention and control, 2nd edition. World Health Organization, Geneva[14]

Laboratory Findings:

- Thrombocytopenia (100,000/mm^3 or less).*
- Haemoconcentration; haematocrit increased by 20% or more.

The first two clinical criteria, plus thrombocytopenia and haemoconcentration or a rising haematocrit, are sufficient to establish a clinical diagnosis of DHF. Pleural effusion (seen on chest X-ray) and/or hypoalbuminaemia provide supporting evidence of plasma leakage. This is particularly useful in those patients who are anaemic and/or having severe haemorrhage. In cases with shock, a high haematocrit and marked thrombocytopenia support the diagnosis of DHF/DSS.

The physical and laboratory findings associated with the various grades of severity of DHF are shown in Box 10 (see section 3.5 for a description of the DHF severity grades).

* Direct count using a phase-contrast microscope (normal 200,000-500,000/mm^3). In practice, for outpatients, an approximate count from a peripheral blood smear is acceptable. In normal persons, 4-10 platelets per oil-immersion field (the average observed from 10 fields is recommended) indicate an adequate platelet count. An average of 2-3 platelets per oil-immersion field or less is considered low (less than 100,000/mm^3).

3.5 Grading the Severity of Dengue Haemorrhagic Fever

The severity of DHF is classified into four grades[12,13] (Box 11).

The presence of thrombocytopenia with concurrent haemoconcentration differentiates Grade I and Grade II DHF from dengue fever.

Grading the severity of the disease has been found clinically and epidemiologically useful in DHF epidemics in children in the South-East Asia, Western Pacific, and American Regions of WHO. Experiences in Cuba, Puerto Rico and Venezuela suggest that this classification is also useful for adults.

Box 11
Grading the Severity of DHF

Grade I	Fever accompanied by non-specific constitutional symptoms; the only haemorrhagic manifestation is a positive tourniquet test.
Grade II	Spontaneous bleeding in addition to the manifestations of Grade I patients, usually in the form of skin and/or other haemorrhages.
Grade III	Circulatory failure manifested by rapid and weak pulse, narrowing of pulse pressure (20 mmHg or less) or hypotension, with the presence of cold clammy skin and restlessness.
Grade IV	Profound shock with undetectable blood pressure and pulse.

3.6 Differential Diagnosis of DHF

Early in the febrile phase, the differential diagnoses associated with DHF include a wide spectrum of viral, bacterial, and protozoal infections. Diseases such as leptospirosis, malaria, infectious hepatitis, chikungunya, meningococcaemia, rubella and influenza should be considered. The presence of marked thrombocytopenia with concurrent haemoconcentration differentiates DHF/DSS from other diseases. In patients with severe bleeding, evidence of pleural effusion and/or hypoproteinemia indicates plasma leakage. A normal erythrosedimentation rate in DHF/DSS helps to differentiate this disease from bacterial infection and septic shock.

3.7 Complications and Unusual Manifestations of DF/DHF in Childhood

Encephalitic signs such as convulsion and coma are rare in DHF. They may, however, occur as a complication in cases of prolonged shock with severe bleeding in various organs including the brain. Water intoxication, as a result of inappropriate use of hypotonic solution to treat DHF patients with hyponatraemia, is a relatively common iatrogenic complication that leads to encephalopathy. A subtle form of seizure is occasionally observed in infants under one year of age during the febrile phase and, in some cases, is considered to be febrile convulsions since the cerebrospinal fluid is normal. Subdural effusions have been observed in some cases.

In recent years there has been an increasing number of reports of DF or DHF

with unusual manifestations. Unusual central nervous system manifestations, including convulsions, spasticity, change in consciousness and transient paresis, have been observed. Some of these cases may have encephalopathy as a complication of DHF with severe disseminated intravascular coagulation that may lead to focal occlusion or haemorrhage.

Fatal cases with encephalitic manifestations have been reported in Indonesia, Malaysia, Myanmar, India and Puerto Rico. However, in most cases there have been no autopsies to rule out bleeding or occlusion of the blood vessels. Although limited, there is some evidence that, on rare occasions, dengue viruses may cross the blood-brain barrier and infect the CNS. Further studies are needed to identify the factors contributing to these unusual manifestations. Attention should be given to the study of underlying host factors such as convulsive disorders and concurrent diseases.

Encephalopathy associated with acute liver failure is commonly observed and renal failure usually occurs at the terminal stage. Liver enzymes are markedly elevated in these cases, with serum aspartate aminotransferase about 2-3 times higher than serum alanine aminotransferase.

Other rarely observed, unusual manifestations of DF/DHF include acute renal failure and haemolytic uraemic syndrome. Some of these cases have been observed in patients with underlying host factors (e.g. G6P deficiency and haemoglobinopathy) that lead to intravascular haemolysis. Dual infections with other endemic diseases, such as leptospirosis, viral hepatitis B, and melioidosis, have been reported in cases with unusual manifestations.

3.8 Clinical Manifestations of DF/DHF in Adults

Cuba's experience in 1981, with 130 adult cases (26 with fatal outcome), showed that the infection was usually manifested by the clinical symptoms of dengue fever (high fever, nausea/vomiting, retro-orbital headache, myalgias and asthenia), regardless of whether the patient had a fatal outcome or not. Less frequently, patients demonstrated thrombocytopenia and haemorrhagic manifestations, the most common of which were skin haemorrhages, menorrhagia, and haematemesis. Overt shock in adults was less frequently observed than in children, but was severe when it did occur. It was found mostly in white adults with a history of bronchial asthma and other chronic diseases. In one series of 1,000 adult cases studied in Cuba, the persons who were severely ill usually showed thrombocytopenia and haemoconcentration. In five cases with hypovolemic shock not associated with haemorrhage, the disease responded, as in children, to vigorous fluid replacement[15]. In the 1986 Puerto Rico outbreak, DHF with overt shock in adults was not rare, but did occur less frequently than in children[16]. Similar observations were reported in the recent outbreak in New Delhi, India in 1996[17].

4

Clinical Management of DF/DHF

EFFECTIVE case management of DF/DHF requires well-trained physicians and nurses, modern state-of-the-art and reliable laboratory facilities, functioning pharmacies and adequate blood supply systems. Early diagnosis of the disease and admission of patients to hospital are therefore important in order to reduce case fatality rates. Depending upon the severity of infection, three disease entities – DF, DHF and DSS – are recognized. The treatment of each of these is discussed below.

4.1 Dengue Fever

The management of DF is symptomatic and supportive.

- Bed rest is advisable during the acute febrile phase.
- Antipyretics or sponging are required to keep the body temperature below 40°C. Aspirin should be avoided since it may cause gastritis, bleeding and acidosis; paracetamol is preferable.
- Analgesics or mild sedatives may be required for patients with severe pain.
- Oral fluids and electrolyte therapy are recommended for patients with excessive sweating or vomiting.

In DHF-endemic areas, patients should be monitored until after they become afebrile and after platelet counts and haematocrit determinations are normal.

4.2 Dengue Haemorrhagic Fever/ Dengue Shock Syndrome

General considerations

The major pathophysiologic hallmarks that distinguish DHF/DSS from DF and other diseases are abnormal haemostasis and increased vascular permeability that lead to leakage of plasma. The clinical features of DHF/DSS are rather stereotyped, with acute onset of high (continuous) fever, haemorrhagic diathesis (most frequently on the skin), hepatomegaly, and circulatory disturbance (in the most severe form as shock). It is thus possible to make an early and yet accurate clinical diagnosis of DHF/DSS before the critical stage or before shock occurs, by using

the pattern of clinical presentations together with thrombocytopenia and concurrent haemoconcentration, which represent abnormal haemostasis and plasma leakage respectively.

The prognosis of DHF depends on early recognition of plasma leakage. This can be achieved by frequent monitoring for a drop in the platelet count and a rise in the haematocrit level. The critical period is at the time of defervescence which occurs approximately on or after the third day of illness. A drop in the platelet count to $\leq$100,000/mm^3 or less than 1-2 platelets per oil-immersion field (average of 10 oil-immersion field counts), usually precedes a rise in haematocrit and may occur before defervescence. A rise in haematocrit of 20% or more (e.g. increase from 35% to 42%) reflects a significant plasma loss and indicates the need for intravenous fluid therapy. Early volume replacement of lost plasma with isotonic salt solution can modify the severity of disease and prevent shock.

In mild to moderate cases of DHF (Grades I and II), intravenous fluid therapy may be given for a period of 12-24 hours at an outpatient clinic. Patients who continue to have elevated haematocrit, platelet counts below 50,000/mm^3, or present with any type of spontaneous haemorrhage other than petechiae should be hospitalized. In general, there is no need to hospitalize all patients with suspected DHF, since only about one-third will develop shock.

Febrile phase

The management of DHF during the febrile phase is similar to that of DF. Antipyretics may be indicated but salicylates should be avoided. It should be noted that antipyretics do not shorten the duration of fever in DHF. Paracetamol is recommended and should be used only to keep the temperature below 39^{o}C. The following dosages are recommended: under-one year old: 60 mg/dose; 1-2 years old: 60-120 mg/dose; 3-6 years old: 120 mg/dose; and 7-12 years old: 240 mg/dose. Patients with hyperpyrexia are at risk of convulsions.

High fever, anorexia and vomiting lead to thirst and dehydration. Therefore, copious amounts of fluids should be given orally, to the extent tolerated. Oral rehydration solutions, such as those used for the treatment of diarrhoeal diseases* and/or fruit juices are preferable to plain water.

Patients should be closely monitored for the initial signs of shock. The critical period is during the transition from the febrile to the afebrile phase, and usually occurs after the third day. Serial haematocrit determinations are an essential guide for treatment, since they reflect the degree of plasma leakage and the need for intravenous administration of fluids. Haemoconcentration usually precedes the blood pressure and pulse changes. Haematocrit should be determined daily from the third day, until the temperature has remained normal for one or two days. If haematocrit

* If the WHO oral rehydration solution (ORS) (90 mmol of Na per litre) is to be used in children under two years of age, additional fruit juice or water should be given in the proportion of one volume of fruit juice (or water) for each two volumes of ORS. The WHO oral rehydration solution consists of: 3.5 g sodium chloride, 2.9 g trisodium citrate dihydrate, 1.5 g potassium chloride, and 20.0 g glucose, dissolved in 1 litre of potable water.

determination is not possible, haemoglobin determination may be carried out as an alternative, but this is less sensitive.

Volume replacement in DHF

Although there is massive plasma leakage, particularly in shock cases, judicious volume replacement is mandatory. The required volume should be charted on a two or three hourly basis or even more frequently in shock cases. The rate of intravenous fluid replacement should be adjusted throughout the 24-48 hour period of leakage by serial haematocrit determinations, with frequent assessments of vital signs and urine output, in order to ensure adequate volume replacement and to avoid over-volume infusion. The volume of fluid replacement should be the minimum that is sufficient to maintain effective circulation during the period of leakage. Excessive volume replacement and continuation after leakage stops will cause massive pleural effusion, ascites, and pulmonary congestion/oedema with respiratory distress when reabsorption of the extravasated plasma occurs in the convalescent stage. In general, the volume required is maintenance plus 5-8% deficit.

Parenteral fluid therapy can be administered in outpatient rehydration units in mild or moderate cases when vomiting produces or threatens to produce dehydration or acidosis or when haemoconcentration is present. The fluid administered to correct dehydration from high fever, anorexia and vomiting is calculated according to the degree of dehydration and electrolyte loss and should have the following composition: 5% glucose in one-half or one-third physiological saline solution (PSS). In the case of acidosis, one-fourth of the total fluids should consist of 0.167 mol/litre of sodium bicarbonate (i.e. three-quarters PSS plus glucose plus one-quarter sodium bicarbonate).

When there is significant haemo-concentration, i.e. haematocrit elevated 20% or more of the baseline value (alternatively, the normal haematocrit value of children in the same age group in the general population may be used to estimate the degree of haemoconcentration), the fluids used for replacement therapy should have a composition similar to plasma. The volume and composition are similar to those used in the treatment of diarrhoea with mild to moderate isotonic dehydration (5-8% deficit).

The necessary volume of replacement fluid is equivalent to the amount of fluids and elecrolytes lost: thus, 10ml/kg should be administered for each 1% of normal body weight lost. Maintenance fluid requirements, calculated according to the Halliday and Segar[18] formula (Table 3) should be added to the replacement fluid. Since the rate of plasma leakage is not constant (it is more rapid when body temperature drops), the volume and rate of intravenous fluid therapy should be adjusted according to the volume and rate of plasma loss. Plasma loss can be monitored by changes in the haematocrit, vital signs or volume of urine output. However, even where there is massive plasma loss, judicious fluid replace-ment is necessary to avoid overhydration.

The schedule shown in Table 3 is recommended as a guideline, and has been

calculated for moderate dehydration of about 6% deficit (plus maintenance). In older children and adults who weigh more than 40 kgs, the volume needed for 24 hours should be calculated as twice that required for maintenance.

Patients should be hospitalized and treated immediately if there are any of the following signs and symptoms of shock: restlessness/lethargy; cold extremities and circumoral cyanosis; oliguria; rapid and weak pulse; narrowing pulse pressure (20 mm Hg or less) or hypotension, and a sudden rise of haematocrit to a high level or continuously elevated haematocrit levels despite administration of intravenous fluids.

Table 3. **Calculations for Maintenance of Intravenous Fluid Infusion***

Body weight (kg)	Maintenance volume (ml) administered over 24 hours
<10	100/kg
10-20	1000 + 50 for each kg in excess of 10
>20	1500 + 20 for each kg in excess of 20

* Halliday MA, Segar WE. Maintenance need for water in parenteral fluid therapy. *Pediatrics*. 1957, 19:823.

Type of fluid:

• Crystalloid:

5% dextrose in lactated Ringer's solution (5% D/RL)

5% dextrose in acetated Ringer's solution (5% D/RA)

5% dextrose in half strength normal saline solution (5% D/1/2/NSS)

5% dextrose in normal saline solution (5% D/NSS)

• Colloidal:

Dextran 40
Plasma

An example of treatment:

The patient: A two year old child has DHF grade II, with the following presentation:

- High fever for 3 days
- Symptoms worsen on day 4 when temperature drops
- Physical examination findings: temperature 37°C, pulse rate 120 per minute, blood pressure 100/70 mmHg, petechiae and a positive tourniquet test; the liver was tender and enlarged by 2 cm
- Laboratory findings: platelets 0 to 1 per oil-immersion field, haematocrit 45% (baseline 35%)
  Administration of intravenous fluid is indicated because the patient has a more than 20% increase in haematocrit level, and early signs of circulatory disturbance are indicated by a rapid pulse and a generally worsening condition.

The following steps should be taken:

- Calculate the volume of intravenous fluid needed for mild isotonic dehydration (5% deficit) based on a 10-kg body-weight.
  Maintenance fluid: 10 x 100 = 1000ml
  5% deficit, 50ml/kg
  10x50 = 500ml
  Total volume needed: = 1500ml
- Order 500ml of glucose in Ringer's lactate or Ringer's acetate (50 g/litre), or glucose in a half-strength physiological saline (50 g/litre) (if the serum sodium level is normal):

Fluid volume per order should not exceed 500 ml, and fluid therapy should not take longer than 6 h

Written orders should state the type of solution and the rate of administration. In this example, the rate is 63 ml per hour, or 21 drops per minute (one ml is equal to 21 drops)

- Follow up vital signs every 1 to 2 h and haematocrit every 3 to 4 h. Periodically record urine output and assessment of the patient's condition
- Adjust the volume and rate of intravenous fluid according to vital signs, haematocrit and urine output as shown in Box 12[20].

The fluid replacement should be the minimum volume that is sufficient to maintain effective circulation during the period of leakage (24-48 hours). Excessive replacement will cause respiratory distress (from massive pleural effusion and ascites), pulmonary congestion and oedema.

4.3 Dengue Shock Syndrome

Shock is a medical emergency. Volume replacement is the most important treatment measure, and ***immediate administration of intravenous fluid*** to expand plasma volume is essential. Children may go into and out of shock during a 48-hour period. Close observation with good nursing care 24 hours a day is imperative (see Box 12).

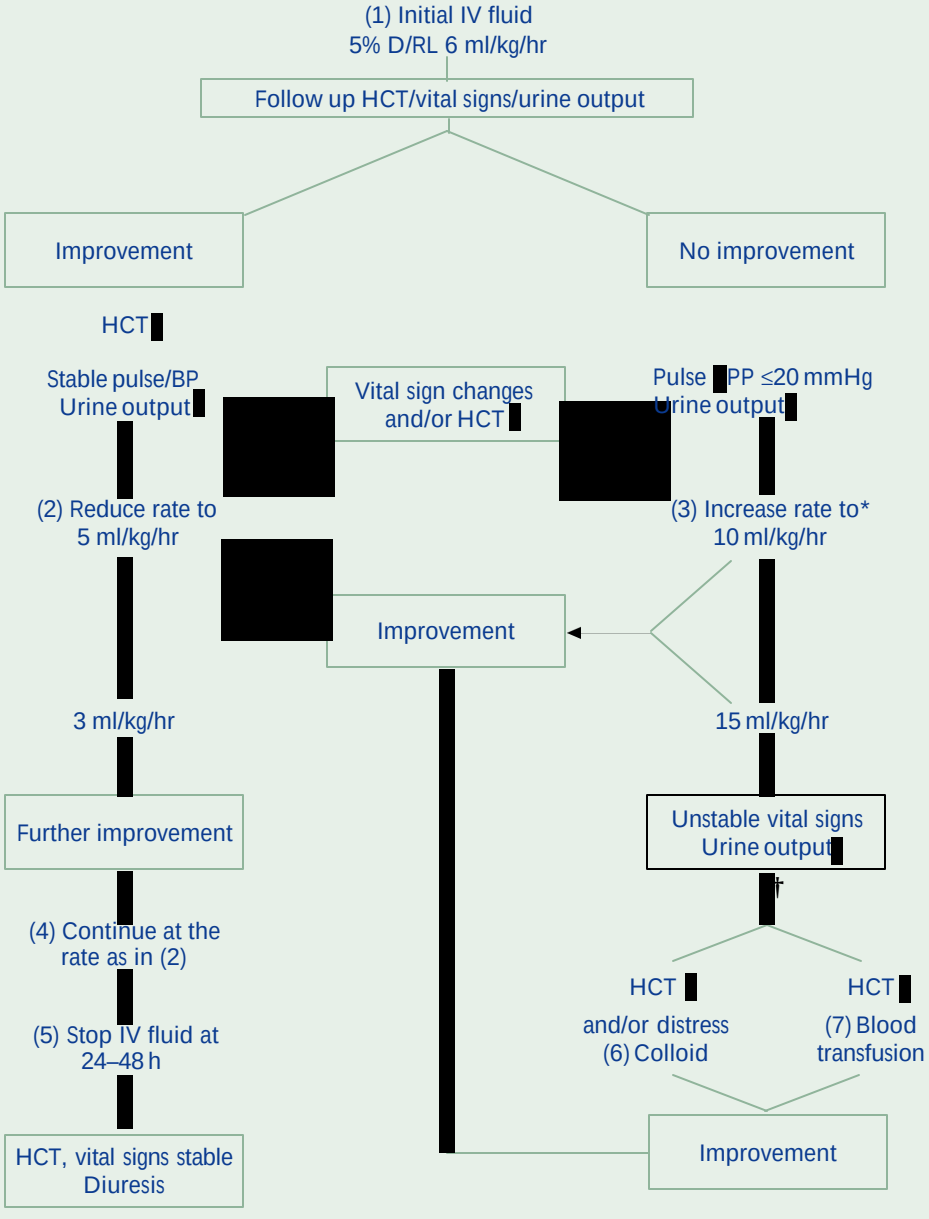
Immediate replacement of plasma

Start initial intravenous fluid therapy with Ringer's acetate or 5% glucose in normal saline solution at the rate of 10-20 ml/kg body weight per hour. Run fluids as rapidly as possible. Positive pressure may be necessary in cases of profound shock. If shock persists after initial fluid resuscitation with 10-20 ml/kg body weight per hour, colloidal solution plasma or plasma expander (10% Dextran of medium related molecular mass in normal saline solution) should be administered at the rate of 10-20 ml/kg per hour. In most cases, no more than 30 ml per kg of body weight of plasma or Dextran 40 is needed. In cases of persistent shock after adequate initial resuscitation with crystalloid and colloidal solutions, despite a decline in the haematocrit level, significant internal bleeding should be suspected, and fresh whole-blood transfusion is indicated. If the haematocrit level is still above 40%, a small volume of blood (10 ml per kg body weight per hour) is recommended. When improvement in vital signs is apparent, the intravenous infusion rate should be reduced. Thereafter, it should be adjusted according to the haematocrit levels and vital signs.

Continued replacement of plasma, based on frequent micro-haematocrit determinations

Intravenous administration of fluids should be continued even when there is a definite improvement in the vital signs and the haematocrit has decreased. The rate of fluid replacement should be decreased to 10 ml per kilogram per hour, and readjusted thereafter to the rate of plasma loss, which may continue for 24 to 48 hours. The determination of central venous pressure may also be necessary in the treatment of severe cases of shock that are not easily reversible.

Box 12. Volume replacement flow chart in dengue hemorrhagic fever



RL = Ringer's lactated; HCT = haematocrit; BP = blood pressure; PP = pulse pressure; *with signs of shock; †establish CVP catheter and urinary catheter

Intravenous administration of fluids should be discontinued when the haematocrit decreases to a stable level, around 40%, and the patient's appetite returns. Good urinary output indicates that there is sufficient fluid circulating. In general, there is no need to administer fluid therapy for more than 48 hours after the termination of shock. Reabsorption of extravasated plasma occurs 2 to 3 days thereafter (manifested by a further drop in haematocrit after the intravenous administration of fluid has been terminated) and may cause hypervolaemia, pulmonary oedema or heart failure if more fluid is given. It is of the utmost importance that a decrease in the haematocrit in this phase is not interpreted as a sign of internal haemorrhage. Strong pulse and blood pressure (with wide pulse pressure) and diuresis are good vital signs during this reabsorption phase. They rule out the likelihood of gastrointestinal haemorrhage, which is found primarily in the shock phase.

Other electrolyte and metabolic disturbances that may require specific correction

Hyponatraemia occurs commonly and metabolic acidosis occurs occasionally in DHF/DSS patients. Electrolyte levels and blood gases should be determined periodically in severely ill patients and in those who do not respond as quickly as expected. This will provide an estimate of the magnitude of the electrolyte (sodium) deficit and help determine the presence and degree of acidosis. Acidosis in particular, if unresolved, may lead to disseminated intravascular clotting and to a more complicated course of recovery. The use of heparin may be indicated in some of these cases, but extreme caution should be exercised when it is administered. In general, early volume replacement and early correction of acidosis with sodium bicarbonate result in a favourable outcome and preclude the need for heparin.

Sedatives

In some cases, treatment with sedatives is necessary to calm an agitated child. Hepatotoxic drugs should be avoided. Chloral hydrate, administered orally or rectally, is highly recommended at a dosage of 12.5-50 mg per kilogram of body weight (but no more than 1 g) as a single hypnotic dose. Agitation/ restlessness that results from poor tissue perfusion often subsides when adequate fluid volume replacement is given.

Oxygen therapy

Oxygen therapy should be provided for all patients in shock, but it must be remembered that an oxygen mask or tent may lead to increased patient anxiety.

Blood transfusion

Blood grouping and cross-matching should be carried out as a systematic precaution on every patient in shock, particularly in cases with prolonged shock. Blood transfusion is indicated in cases with significant haemorrhagic manifestations.

It may be difficult to recognize internal haemorrhage if there is haemoconcentration. A decrease in the haematocrit - e.g. from 0.5

(50%) to 0.4 (40%) - without clinical improvement, despite the administration of sufficient fluids, indicates significant internal haemorrhage. Fresh whole blood is preferable and the volume of blood administered should be only enough to raise the red blood cell concentration to normal. Fresh frozen plasma and/or concentrated platelets may be indicated in some cases when disseminated intravascular coagulation causes massive bleeding.

Disseminated intravascular coagulation is common in severe shock, and may play an important role in the development of massive bleeding and lethal shock. The results of haematological tests (e.g. prothrombin time, partial thromboplastin time, and fibrinogen degradation products) should be studied in all patients with shock to monitor the onset and severity of disseminated intravascular coagulation. Results of these tests will determine the prognosis.

Essential laboratory tests

In addition to serial haematocrit and platelet determinations, the following tests are recommended to evaluate the patient's status: studies of the serum electrolytes and blood gases; platelet count, prothrombin time, partial thromboplastin time and thrombin time; and liver function tests - serum aspartate aminotransferase [(previously known as serum glutamic oxaloacetic transaminase, (SGOT)], serum alanine aminotransferase [(previously known as serum glutamic pyruvic transaminase (SGPT)], and serum proteins.

Monitoring and anti-shock therapy

Frequent recording of vital signs and haematocrit determinations are important in evaluating treatment results. If the patient presents some indication of secondary shock, vigorous anti-shock therapy should be instituted promptly. These patients should be under constant and careful observation until there is reasonable assurance that the danger has passed. In practice:

- The pulse, blood pressure, respirations and temperature should be recorded every 15 to 30 minutes or more frequently, until the shock has been overcome.
- Haematocrit levels should be determined every two hours during the first six hours, and later every four hours until stable.
- A fluid balance sheet should be kept, recording the type, rate and quantity of fluid administered, in order to determine whether there has been sufficient replacement and correction of fluids and electrolytes. The frequency and volume of urine excreted should also be recorded.

4.4 Criteria for Discharging Patients Hospitalized with DHF/DSS

All of the following six criteria must be met before a patient is discharged:

- Absence of fever for 24 hours without the use of antipyretics and a return of appetite.
- Visible improvement in clinical picture.
- Stable haematocrit.
- Three days after recovery from shock.
- Platelet count greater than 50,000/mm^3.

- No respiratory distress from pleural effusion/ascites.

4.5 Management of Unusual Manifestations/Complications

The most frequently encountered unusual manifestations are acute hepatic failure and renal failure (which usually follow prolonged shock) that require specific and appropriate treatment. Early blood transfusion in cases of hepatic encephalopathy or Reye's-like syndrome has proved to be life saving in a number of cases, as has haemodialysis in renal failure cases.

Some DHF patients present unusual manifestations with signs and symptoms of CNS involvement, such as convulsion and/or coma. This has generally been shown to be encephalopathy, not encephalitis, which may be a result of intracranial haemorrhage or occlusion associated with DIC. In recent years, however, several cases with CNS infections have been documented by virus isolations from the CSF or brain[21].

4.6 DHF Special Unit

For the purpose of more effective management, DHF patients should be hospitalized in a semi-intensive care unit that is a mosquito-free area. Paramedical workers or parents can assist in oral fluid therapy and monitor the IV fluid and the general status of the patient. Experience at the Children's Hospital, Bangkok,[19] where a great number of DHF cases are seen each year, has shown that management without using corticosteroids or any vasopressure drugs, results in a steady decline in mortality in the case of shock cases. The case fatality rate dropped from about 5% in 1971 to 2% in 1984 and 0.2% in 1990. Studies on the use of corticosteroids in treating DSS have shown no benefit. The prognosis of DHF/DSS thus depends on: early diagnosis, early recognition of shock, careful clinical observations, and volume replacement guided by simple laboratory tests[20].

4.7 Role of WHO Collaborating Centres

Additional information, practice advice and consultation regarding case management of DF/DHF/DSS can be obtained from the WHO Collaborating Centres (CC) for Case Management of Dengue/DHF/DSS (see Annex 1). The WHO Regional Office for South-East Asia (SEARO) has supported the training of 30 physicians from dengue endemic countries of the Region on clinical management of dengue/DHF/DSS at this CC. SEARO and the WHO CC will provide technical support to dengue-training wards proposed to be established during 1998-99 for clinical management of DF/DHF/DSS in dengue endemic countries of the Region. Also, it is expected that, through networking, it will be possible not ony to standardize the case management of DF/DHF/DSS patients, but also to obtain rapid information on the occurrence of cases which is essential for establishing early warning systems for dengue outbreaks and their management (see Box 13).

Box 13
Important Considerations in the Clinical Diagnosis and Management of DHF/DSS

- A child with acute onset of high fever, flushed face without coryza, with petechiae and/or a positive tourniquet test should suggest a possibility of dengue infection.
- The appearance of hepatomegaly (+ tenderness) increases the possibility of DHF.
- The critical stage of the disease is at the time of defervescence. The presence of thrombocytopenia with concurrent haemoconcentration (rising HCT), which occur before the temperature drop and/or onset of shock, are essential to the clinical diagnosis of DHF/DSS.
- Moderate marked leukopenia near the end of the febrile period helps in the differential diagnosis.
- Antipyretics cannot shorten the duration of fever. Inappropriate use may lead to severe complications, e.g. severe bleeding, acidosis, hepatic failure.
- Rising haematocrit (by 20% or more) reflects significant plasma loss and a need for IV fluid therapy. Although early IV replacement can prevent shock and modify severity, IV fluid therapy before leakage is not recommended.
- DSS is hypovolemic shock due to plasma loss: volume replacement with isotonic salt solution, plasma or plasma substitute for the period of plasma leakage (24-48 hrs) is life-saving. Dextran 40 is as effective as plasma (maximum dose 30 ml/kg/day), and has some advantages.
- Volume replacement should be carefully monitored according to the rate of plasma leakage (as reflected by HCT, vital signs, urine output) to avoid fluid overload (the rate of leakage is more rapid in the first 6-12 hours)
- Over replacement with more volume and/or for a longer period of time than necessary will cause pulmonary congestion/oedema, particularly when reabsorption of extravasated plasma occurs.
- Stagnant acidaemia blood promotes the occurrence/enhances the severity of DIC; acidosis must be corrected. Coagulogram should be evaluated.
- Platelet-rich plasma transfusion as prophylaxis for bleeding in all shock cases is not recommended.
- There are abnormal haemostatic changes that potentiate bleeding in DHF/DSS. Severe bleeding (may be concealed) often occurs in cases with prolonged shock and further perpetuates shock.
- Refractory shock despite adequate volume replacement and a drop in HCT (at any rate, e.g. from 50% to 40%) indicate significant bleeding and a need for fresh whole blood transfusion (10ml/kg/dose).

5

Laboratory Diagnosis

LABORATORY tests essential for confirmatory diagnosis of dengue infection include: (a) isolation of the virus, (b) demonstration of a rising titre of specific serum dengue antibodies, and (c) demonstration of a specific viral antigen or RNA in the tissue or serum[21, 22]. Isolation of the virus is the most definitive approach, but the techniques presently available require a relatively high level of technical skill and equipment. Serological tests are simpler and more rapid, but cross-reactions between antibodies to dengue and other flaviviruses may give false positive results. In addition, accurate identification of the infecting dengue virus serotype is not possible with most serological methods. New technologies available for the laboratory diagnosis of dengue infection include immunohistochemistry on autopsy tissues and polymerase chain reaction (PCR) to detect viral RNA in the tissue or serum[22].

5.1 Collection of Specimens

An essential aspect of the laboratory diagnosis of dengue is proper collection, processing, storage and shipment of specimens. The types of specimens and their storage and shipment requirements are presented in Table 4.

Table 4. **Collecting and processing specimens for laboratory diagnosis of dengue**

Specimen Type	Time of collection	Clot retraction	Storage	Shipment
Acute phase blood (S1)	0-5 days after onset	2-6 hours, 4°C	Serum - 70°C	Dry ice
Convalescent phase blood (S2 + S3)	14-21 days after onset	2-24 hours, ambient	Serum – 20°C	Frozen or ambient
Tissue	As soon as possible after death		70°C or in formalin	Dry ice or ambient

Source: Gubler DJ, and Sather GE. 1988[21]

- Collect a specimen as soon as possible after the onset of illness, hospital admission or attendance at a clinic (this is called the acute serum, S1).
- Collect a specimen shortly before discharge from the hospital or, in the event of a fatality, at the time of death (convalescent serum, S2).
- Collect a third specimen, in the event hospital discharge occurs within 1-2 days of the subsidence of fever, 7-21 days after the acute serum was drawn (late convalescent serum, S3).

The optimal interval between the acute (S1) and the convalescent (S2 or S3) serum is 10 days. The above recommendations allow for the collection of at least two serum samples for comparison, and ideally will provide for an adequate interval between sera. Serological diagnoses are predicated on the identification of changes in antibody levels over time. Serial (paired) specimens are required to confirm or refute a diagnosis of acute flavivirus or dengue infection.

- The type of specimens to be collected, the way they should be processed for a laboratory diagnosis of dengue, and the information required are presented in this chapter. Effective laboratory support for proactive DF/DHF surveillance requires close and frequent communication between staff in the laboratory and those in the epidemiology unit of the ministry of health. It also requires, at a minimum, weekly evaluation of laboratory results, including monitoring the geographic location of positive cases, the sero-positivity rate, the virus serotypes isolated, and the occurrence of severe and fatal disease. This information must be communicated on a weekly basis to the epidemiology unit for dissemination to other offices in the ministry of health and for further action. Weekly laboratory results are clearly the driving force which determine the response to be taken.
- The above data obtained from a proactive surveillance system can be used effectively if they are disseminated to the proper government and community agencies. Thus, an effective communi-cation or reporting system is also a critical component of the surveillance system. The availability of inexpensive yet powerful desktop computers that are networked can revolutionize surveillance reporting since, with the touch of a button, all responsible persons/agencies can be informed of the latest data needed for decision making.
- Samples of suitable request and reporting forms for arbovirus laboratory examination are provided in Annex II. Blood is preferably collected in tubes or vials, but filter paper may be used if this is the only option. Filter-paper samples cannot be used for virus isolation.

Blood collection in tubes or vials

- Aseptically collect 2-10 ml of venous blood.
- Use adhesive tape marked with pencil, indelible ink, or a typewritten self-adhesive label to identify the container. The name of the patient, identification number and date of collection must be indicated on the label.

- Use vacuum tubes or vials with screw caps, if possible. Fix the cap with adhesive tape, wax or other sealing material to prevent leakage during transport.
- Ship specimens to the laboratory on wet ice (blood) or dry ice (serum) as soon as possible. Do not freeze whole blood, as haemolysis may interfere with serology test results.
- If there will be more than a 24-hour delay before specimens can be submitted to the laboratory, the serum should be separated from the red blood cells and stored frozen.

Blood collection on filter paper

- With a pencil, write the patient's initials or number on two or three filter-paper discs or strips of standardized absorbent paper.*
- Collect sufficient finger-tip blood (or venous blood in a syringe) on the filter paper to fully saturate it through to the reverse side. Most standard filter-paper discs or strips will absorb 0.1 ml of serum.
- Allow the discs or strips to dry in a place that is protected from direct sunlight and insects. Preferably, the blood-soaked papers should be placed in a stand which allows aeration of both sides. For unusually thick papers, a drying chamber may be useful, e.g. dessicator jar, air-conditioned room, or warm-air incubator.
- Place the dried strips in plastic bags and staple them to the laboratory examination request form. Store without refrigeration.

Dried filter-paper discs may be sent through the mail.

One of the recommended methods for eluting the blood from filter-paper discs and preparing it for the HI or IgM and IgG tests is as follows :

- Elute the disc at room temperature for 60 minutes or at 4°C overnight, in 1 ml of kaolin in borate saline (125 g/litre), pH 9.0, in a test-tube.
- After elution, keep the tube at room temperature for 20 minutes, shaking periodically.
- Centrifuge for 30 minutes at 600g.
- For HI tests using goose erythrocytes, without removing the kaolin, add 0.05 ml of 50% suspension of goose cells to the tube, shake without disturbing the pellet, and incubate at 37°C for 30 minutes.
- Add 1 ml of borate saline, pH 9.0, to the tube.
- Centrifuge at 600g for 10 minutes and decant the supernatant.
- This is equivalent to a 1:30 serum dilution.
- Each laboratory must standardize the filter-paper technique against results with venous blood from a panel of individuals.

5.2 Isolation of Dengue Virus

Isolation of most strains of dengue virus from clinical specimens can be accomplished in a majority of cases provided the sample is taken in the first few days of illness and processed without delay. Specimens that may be suitable

* Whatman No.3 filter-paper discs 12.7 mm (1/2 inch) in diameter are suitable for this purpose, or Nobuto Type 1 blood-sampling paper made by Toyo Roshi Kaisha Ltd., Tokyo, Japan.

for virus isolation include acute phase serum, plasma or washed buffy coat from the patient, autopsy tissues from fatal cases, especially liver, spleen, lymph nodes and thymus, and mosquitoes collected in nature.

For short periods of storage (up to 48 hours), specimens to be used for virus isolation can be kept at +4 to +8°C. For longer storage, the serum should be separated and frozen at -70°C, and maintained at such so that thawing does not occur. If isolation from leucocytes is to be attempted, heparinized blood samples should be delivered to the laboratory within a few hours. Whenever possible, original material (viraemic serum or infected mosquito pools) as well as laboratory-passaged materials should be preserved for future study.

Tissues and pooled mosquitoes are triturated or sonicated prior to inoculation. The different methods of inoculation and the methods of confirming the presence of dengue virus are shown in Table 5.[22]

The choice of methods for isolation and identification of dengue virus will depend on local availability of mosquitoes, cell culture, and laboratory capability. Inoculation of serum or plasma into mosquitoes is the most sensitive method of virus isolation, but mosquito cell culture is the most cost-effective method for routine virologic surveillance. It is essential for health workers interested in making a diagnosis by means of virus isolation to make contact with the appropriate virology laboratory prior to the collection of specimens. The acquisition, storage and shipment of the samples can then be organized to have the best chance of successful isolation.

In order to identify the different dengue virus serotypes, mosquito head squashes and slides of infected cell cultures are examined by indirect immunoflourescence using serotype-specific monoclonal antibodies.

5.3 Serological Tests for the Diagnosis of DF/DHF

Five basic serologic tests are routinely used for the diagnosis of dengue infection[21,23] haemagglutination-inhibition (HI), complement

Table 5. **Dengue virus isolation methods**

Recommended methods	Confirmation of dengue virus infection
Inoculation of mosquitoes	Presence of antigen in head squashes demonstrated by immunofluorescence
Inoculation of insect cells or mammalian cultures	(a) Presence of antigen in cells demonstrated by immunofluorescence (b) Cytopathic effect and plaque formation in mammalian cells

fixation (CF), neutralization test (NT), IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA), and indirect IgG ELISA. Regardless of the test used, unequivocal serologic confirmation depends upon a significant (4-fold or greater) rise in specific antibodies between acute-phase and convalescent-phase serum samples. The antigen battery for most of these serologic tests should include all four dengue serotypes, another flavivirus, such as Japanese encephalitis, a non-flavivirus such as chikungunya, and an uninfected tissue control antigen, when possible.

Haemagglutination inhibition (HI) test

Of the above tests, HI has been the most frequently used for routine serologic diagnosis of dengue infections. It is sensitive, easy to perform, requires only minimal equipment, and is very reliable if properly done. Because HI antibodies persist for long periods (up to 50 years or longer), the test is ideal for seroepidemiologic studies. The HI test is based on the fact that the dengue viruses, under controlled conditions of pH and temperature, can agglutinate goose red blood cells, and this effect can be inhibited by specific antibodies. The antigens employed are prepared from infected suckling mice brains by extraction with sucrose and acetone to remove the lipids, or from infected mosquito cell cultures that have been concentrated or purified. Serum specimens must be treated to remove non-specific inhibitors and agglutinins.

The HI antibody usually begins to appear at detectable levels (titer of 10) by day five or six of illness, and antibody titers in convalescent-phase serum specimens are generally at or below 1:640 in primary infections, although there are exceptions. By contrast, there is an immediate anamnestic response in secondary and tertiary dengue infections, and antibody titers increase rapidly during the first few days of illness, often reaching 1:5,120 to 1:10,240 or more. Thus, a titer of 1:1,280 or greater in an acute-phase serum is considered a presumptive diagnosis of current dengue infection. High levels of HI antibody may persist for 2-3 months in some patients, but in most antibody titers will generally begin to wane by 30-40 days and fall below the 1:1,280 level.

The major disadvantage of the HI test is lack of specificity, which makes the test unreliable for identifying the infecting virus serotype. However, some primary infections may show a relatively monotypic HI response that generally correlates with the virus isolated[21].

Complement fixation (CF) test

The CF test is not widely used for routine dengue diagnostic serology. It is more difficult to perform and requires highly-trained personnel. The CF test is based on the principle that the complement is consumed during antigen-antibody reactions. Two reactions are involved, a test system and an indicator system. Antigens for the CF test are prepared in the same manner as those for the HI test.

CF antibodies generally appear later than HI antibodies, are more specific in primary

infections, and usually persist for shorter periods, although low-level antibodies may persist in some persons. Because of the late appearance of CF antibodies, some patients may show a diagnostic rise by CF, but have only stable antibody titers by HI. The greater specificity of CF test in primary infections is demonstrated by the monotypic CF responses, whereas HI responses are broadly heterotypic. The CF test is not specific in secondary infections. The CF test is useful for patients with current infections, but is of limited value for seroepidemiologic studies where detection of persistent antibodies is important.

Neutralization test (NT)

The NT is the most specific and sensitive serologic test for dengue viruses. The most common protocol used in most dengue laboratories is the serum dilution plaque reduction neutralization test (PRNT). It is based on the fact that dengue viruses produce cytopathic effects (CPE) which can be observed as plaques in susceptible cell cultures. This CPE is neutralized by the presence of specific antibodies. In general, neutralizing antibodies rise at about the same time or at a slightly slower rate than HI antibodies, but more quickly than CF, and persist for at least 50 years or longer. Because NT is more sensitive, neutralizing antibodies may be detectable in the absence of detectable HI antibodies in some persons with past dengue infection.

The NT can be used to identify the infecting virus in primary dengue infections, provided the serum samples are properly timed. Relatively monotypic responses are observed in properly timed convalescent-phase serum. As noted above, the HI and CF tests may also give monotypic responses to dengue infection that generally agree with NT results. In those cases where the responses are monotypic, the interpretation is generally reliable. In secondary and tertiary infections, it is not possible to reliably determine the infecting virus serotype by NT. Because of the long persistence of neutralizing antibodies, the test may also be used for seroepidemiologic studies. The major disadvantages are the expense, time required to perform the test, and technical difficulty. It is therefore not routinely used in most laboratories.

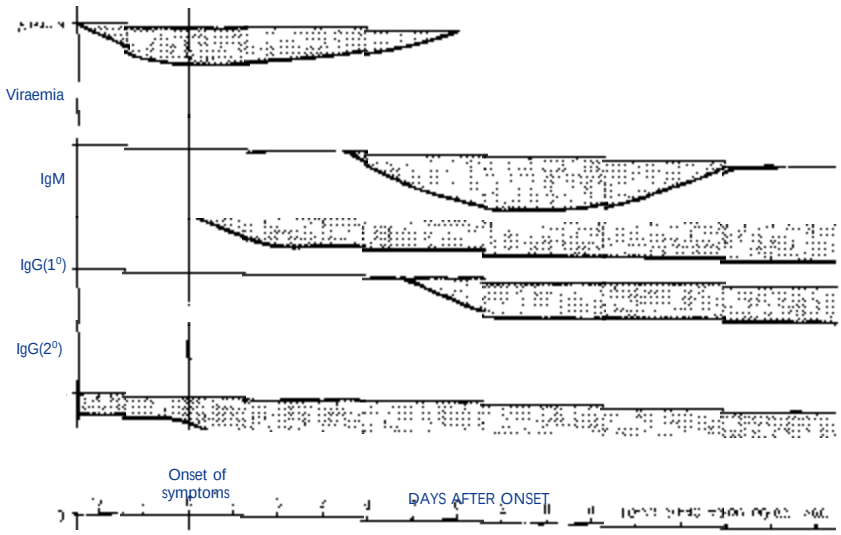
IgM-capture enzyme-linked immuno-sorbent assay (MAC-ELISA)

MAC-ELISA has become widely used in the past few years. It is a simple, rapid test that requires very little sophisticated equipment. MAC-ELISA is based on detecting the dengue-specific IgM antibodies in the test serum by capturing them out of solution using anti-human IgM that was previously bound to the solid phase[24]. If the IgM antibody from the patient's serum is anti-dengue antibody, it will bind the dengue antigen that is added in the next step and can be detected by subsequent addition of an enzyme labelled anti-dengue antibody, which may be human or monoclonal antibody. An enzyme-substrate is added to give a colour reaction.

The anti-dengue IgM antibody develops a little faster than IgG, and is usually detectable by day five of the illness. However, the rapidity with which IgM develops varies considerably among patients. Some patients have detectable IgM on days two to four after the onset of illness, while others may not develop IgM for seven to eight days after the onset[22]. IgM antibody titers in primary infections are significantly higher than in secondary infections, although it is not uncommon to obtain IgM titers of 320 in the latter cases. In some primary infections, detectable IgM may persist for more than 90 days, but in most patients it wanes to an undetectable level by 60 days[21] (Fig.2).

MAC-ELISA is slightly less sensitive than the HI test for diagnosing dengue infection. It has the advantage, however, of frequently requiring only a single, properly timed blood sample. Considering the difficulty in obtaining second blood samples and the long delay in obtaining conclusive results from the HI test, this low error rate would be acceptable in most surveillance systems. It must be emphasized, however, that because of the persistence of IgM antibody, MAC-ELISA positive results on single serum samples are only provisional and do not necessarily mean that the dengue infection is current. It is reasonably certain, however, that the person had a dengue infection sometime in the previous two to three months.

Figure 2. Representation of the temporal appearance of virus, IgM, and IgG antibodies in persons infected with dengue virus.



Shaded areas represent approximate time periods when virus or antibody can be detected using current methods; 1° = primary infection; 2° = secondary infection. Gubler DJ 1993, unpublished, prepared for Scientific Publication No.548, PAHO 1994.[25]

MAC-ELISA has become an invaluable tool for surveillance of DF/DHF/DSS. In areas where dengue is not endemic, it can be used in clinical surveillance for viral illness or for random, population-based serosurveys, with the certainty that any positives detected are recent infections[21]. It is especially useful for hospitalized patients, who are generally admitted late in the illness after detectable IgM is already present in the blood.

IgG-ELISA

An indirect IgG-ELISA has been developed that compares well to the HI test[23]. This test can also be used to differentiate primary and secondary dengue infections. The test is simple and easy to perform, and is thus useful for high-volume testing. The IgG-ELISA is very non-specific and exhibits the same broad cross-reactivity among flaviviruses as the HI test; it cannot be used to identify the infecting dengue serotype. However, it has a slightly higher sensitivity than the HI test. It is expected that as more data are accumulated on the IgG ELISA, it will replace the HI test.

Rapid serologic test kits

A number of commercial serologic test kits for anti-dengue IgM and IgG antibodies have become available in the past few years, some producing results within 15 minutes[23]. Unfortunately, the accuracy of most of these tests is unknown since they have not yet been properly validated. Some of the kits that have been independently evaluated at CDC have had a high rate of false positive results compared to standard tests, while others have agreed closely with standard tests. It is anticipated that these test kits can be reformulated to make them more accurate, thus making global laboratory-based surveillance for DF/DHF an obtainable goal in the near future. It is important to note that these kits should not be used in the clinical setting to guide management of DF/DHF cases because many serum samples taken in the first five days after the onset of illness will not have detectable IgM antibodies. The tests would thus give a false negative result. Reliance on such tests to guide clinical management could, therefore, result in an increase in case fatality rates. The relative sensitivity and interpretation of serological tests are given in Annex III.

6

Epidemiological Surveillance

EPIDEMIOLOGICAL surveillance of DF/DHF must cover both disease (case) and entomological (vector) surveillance.

6.1 Case Surveillance

Effective surveillance of DF/DHF infection is essential for monitoring endemic transmission and for early recognition of impending epidemics. It depends on close collaboration between the epidemiologic, clinical and laboratory components as well as on an efficient reporting system.

Passive surveillance

Every dengue endemic country should have a surveillance system and it should be mandated by law that DF/DHF is a reportable disease. The system should be based on standardized case definitions (see Box 14) and formalized mandated reporting. Although passive systems are not sensitive and have low specificity since cases are not laboratory confirmed, they are most useful in monitoring long-term trends in dengue transmission.

The clinical spectrum of illnesses associated with dengue infection ranges from non-specific viral syndrome to severe haemorrhagic disease or fatal shock. It may sometimes be difficult to differentiate the illnesses from those caused by other viruses, bacteria and parasites. Therefore, surveillance should be supported by laboratory diagnosis. However, the reporting of dengue disease generally has to rely on clinical diagnosis combined with simple clinical laboratory tests and available epidemiological information.

Passive surveillance should require case reports from every clinic, private physician and health centre or hospital that provides medical attention to the population at risk. However, even when mandated by law, passive surveillance is insensitive because not all clinical cases are correctly diagnosed during periods of low transmission, when the level of suspicion among medical professionals is low. Moreover, many patients with mild, non-specific viral syndrome self-medicate at home and do not seek medical treatment. By the time dengue cases are detected and reported by physicians under a passive surveillance system, substantial transmission has already occured and may even have peaked. In this case, it is often too late to control the epidemic.

Box 14
Recommended case definition

Dengue Fever

Clinical description

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, leucopenia

Laboratory criteria for diagnosis

One or more of the following:

- Isolation of the dengue virus from serum, plasma, leucocytes, or autopsy samples
- Demonstration of a fourfold or greater change in reciprocal IgG or IgM antibody titres to one or more dengue virus antigens in paired serum samples
- Demonstration of dengue virus antigen in autopsy tissue by immunohistochemistry or immunofluorescence or in serum samples by ELISA
- Detection of viral genomic sequences in autopsy tissue, serum or CSF samples by polymerase chain reaction (PCR)

Case classification

Suspected: A case compatible with the clinical description

Probable: A case compatible with the clinical description with **one or more** of the following:
- supportive serology (reciprocal haemagglutination-inhibition antibody titre $\geq$ 1280, comparable IgG ELISA titre or positive IgM antibody test in late acute or convalescent-phase serum specimen)
- occurrence at same location and time as other confirmed cases of dengue fever

Confirmed: A case compatible with the clinical description that is laboratory-confirmed

Criteria For Dengue Haemorrhagic Fever And Dengue Shock Syndrome

Dengue Haemorrhagic Fever

A probable or confirmed case of dengue **and** haemorrhagic tendencies evidenced by **one or more of the following:**
- positive tourniquet test
- petechiae, ecchymoses or purpura
- bleeding from mucosa, gastrointestinal tract, injection sites or other sites
- haematemesis or melaena

and thrombocytopenia (100,000 cells per mm^3 or less)
and evidence of plasma leakage due to increased vascular permeability, manifested by **one or more** of the following:
- a rise in average haematocrit for age and sex $\geq$ 20%
- a $\geq$ 20% drop in haematocrit following volume replacement treatment compared to baseline
- signs of plasma leakage (pleural effusion, ascites, hypoproteinaemia)

Dengue shock syndrome

All the above criteria for DHF **plus** evidence of circulatory failure manifested by rapid and weak pulse, and narrow pulse pressure ($\leq$ 20 mm Hg) **or** hypotension for age, and cold, clammy skin and restlessness

Source: WHO Recommended Surveillance Standards 1997[14]

Active surveillance

The goal of an active dengue surveillance system is to allow health authorities to monitor dengue transmission in a community and be able to tell, at any point in time, where transmission is occurring, which virus serotypes are circulating, and what kind of illness is associated with the dengue infection[10]. In order to accomplish this, the system must be active and have good diagnostic laboratory support. Effectively managed, such a surveillance system should be able to provide an early warning or predictive capability for epidemic transmission. The rationale is that if epidemics can be predicted, then they can be prevented.

This type of proactive surveillance system must have at least three components that place the emphasis on the inter or pre-epidemic period, and include a sentinel clinic/physician network, a fever alert system that uses community health workers, and a sentinel hospital system (Table 6). The sentinel clinic/physician and fever alert components are designed to monitor non-specific viral syndromes in the community. This is especially important for dengue viruses because they are frequently maintained in tropical urban centres in a silent transmission cycle, often presenting as non-specific viral syndromes. The sentinel clinic/physician and fever alert systems are also very useful for monitoring other common infectious diseases, such as influenza, measles, malaria, typhoid, leptospirosis, and others that present in the acute phase as non-specific febrile illnesses.

Table 6. **Components of Laboratory-Based, Proactive Surveillance for Dengue and Dengue Haemorrhagic Fever during Interepidemic Periods[a]**

Type of Surveillance	Samples[b]	Approach
Sentinel Clinic/ Physician	Blood from representative cases of viral syndrome, taken from 3 to 15 days after the onset of symptoms	Representative samples taken year round and processed weekly for virus isolation and for IgM antibodies
Fever Alert	Blood samples from representative cases of febrile illness	Increased febrile illness in the community is investigated immediately
Sentinel Hospital	Blood and tissue samples taken during hospitalization and/or at death	All haemorrhagic disease and all viral syndromes with fatal outcome are investigated immediately

[a] During an epidemic, after the virus serotype(s) is known, the case definition should be more specific and surveillance focused on severe disease.

[b] All samples are processed weekly for virus isolation and/or for dengue specific IgM antibodies.

In contrast to the sentinel clinic/physician component, which requires sentinel sites to monitor routine viral syndromes, the fever alert system relies on community health, sanitation and other workers to be alert to any increase in febrile activity in their community and to report this to the central epidemiology unit of the health department. Investigation by the latter should be immediate, but flexible. It may involve telephone follow up or active investigation by an epidemiologist who visits the area to take samples.

The sentinel hospital component should be designed to monitor severe disease. Hospitals used as sentinel sites should include all of those that admit patients for severe infectious diseases in the community. This network should also include the infectious disease physicians who usually consult on such cases. The system can target any type of severe disease, but for dengue it should include all patients with any haemorrhagic manifestation, an admission diagnosis of viral encephalitis, aseptic meningitis and meningococcal shock, and/or a fatal outcome following a viral prodrome[10].

All three surveillance components require a good public health laboratory to provide diagnostic support in virology, bacteriology and parasitology. The laboratory does not need to be able to test for all agents, but should know where to refer specimens for testing, i.e. to WHO collaborating centres for reference and research.

An active surveillance system is designed to monitor disease activity during the inter-epidemic period, prior to increased transmission. Individually, the three components are not sensitive enough to provide effective early warning, but used collectively, they can often accurately predict epidemic activity. Table 6 outlines the active surveillance system for DF/DHF, giving the types of specimens and approaches required. It must be emphasized that once epidemic transmission has begun, the active surveillance system is refocused on severe disease rather than on viral syndromes. Surveillance systems should be designed and adapted to the areas where they will be initiated.

6.2 Vector Surveillance

Surveillance for *Ae. aegypti* is important in determining the distribution, population density, major larval habitats, spatial and temporal risk factors related to dengue transmission, and levels of insecticide susceptibility or resistance[26], in order to prioritize areas and seasons for vector control. These data will enable the selection and use of the most appropriate vector control tools, and can be used to monitor their effectiveness. There are several methods available for the detection and monitoring of larval and adult populations. The selection of appropriate sampling methods depends on surveillance objectives, levels of infestation, and availability of resources.

Larval surveys

For practical reasons, the most common survey methodologies employ larval sampling procedures rather than egg or adult collections. The basic sampling unit is the house or premise, which is systematically searched for water-holding containers.

Box 15
Indices used to assess the levels of *Ae. aegypti* infestations

House index (HI): percentage of houses infested with larvae and/or pupae.

$$HI = \frac{\text{Number of houses infested}}{\text{Number of houses inspected}} \times 100$$

Container index(CI): percentage of water-holding containers infested with larvae or pupae.

$$CI = \frac{\text{Number of positive containers}}{\text{Number of containers inspected}} \times 100$$

Breteau index (BI): number of positive containers per 100 houses inspected.

$$BI = \frac{\text{Number of positive containers}}{\text{Number of houses inspected}} \times 100$$

Containers are examined for the presence of mosquito larvae and pupae. Depending on the objectives of the survey, the search may be terminated as soon as *Aedes* larvae are found, or it may be continued until all containers have been examined. The collection of specimens for laboratory examination is necessary to confirm the species present. Three indices that are commonly used to monitor *Ae. aegypti* infestation levels[25, 26] are presented in Box 15.

The house index has been most widely used for monitoring infestation levels, but it does not take into account the number of positive containers nor the productivity of those containers. Similarly, the container index only provides information on the proportion of water-holding containers that are positive. The Breteau index establishes a relationship between positive containers and houses, and is considered to be the most informative, but again there is no reflection of container productivity. Nevertheless, in the course of gathering basic information for calculating the Breteau index, it is possible and desirable to obtain a profile of the larval habitat characteristics by simultaneously recording the relative abundance of the various container types, either as potential or actual sites of mosquito production (e.g. number of positive drums per 100 houses, number of positive tyres per 100 houses, etc.). These data are particularly relevant for focusing control efforts on the management or elimination of the most common habitats and for the orientation of educational messages for community-based initiatives.

The rate of contribution of newly-emerged adults to the adult mosquito population from different container types can vary widely. The estimates of relative adult production may be based on pupal counts[26] (i.e. counting all pupae found in each container). The corresponding index is the Pupal index (Box 16).

Box 16
***Pupal index:* number of pupae per 100 houses**

$$PI = \frac{\text{Number of pupae}}{\text{Number of houses inspected}} X\ 100$$

In order to compare the relative importance of larval habitats, the pupal index can be broken down to "useful", "non-essential" and "natural" containers, or by specific habitat types, such as tyres, flower vases, drums, clay pots, etc. Given the practical difficulties and labour-intensive efforts entailed in obtaining pupal counts, especially from large containers, this method does not need to be used in every survey, but may be reserved for special studies or used once in each locality during the wet season and once during the dry season, to determine the most productive container types. The pupal index has been most frequently used for operational research purposes.

Adult surveys

Adult vector sampling procedures can provide valuable data for specific studies, such as seasonal population trends, transmission dynamics, transmission risk, and evaluation of adulticiding interventions. However, results may be less reproducible than those obtained from sampling of immature stages. The collection methods also tend to be labour-intensive and heavily dependent on the collector's proficiency and skill.

Landing/biting collections

Landing/biting collections on humans are a sensitive means of detecting low-level infestations, but are very labour-intensive. Both male and female *Ae. aegypti* are attracted to humans. Because adult males have low dispersal rates, their presence can be a reliable indicator of close proximity to hidden larval habitats. The rates of capture, typically using hand nets or aspirators as mosquitoes approach or land on the collector, are usually expressed in terms of *landing/biting counts per man hour.*

As there is no prophylaxis for dengue or other viruses transmitted by *Aedes* mosquitoes, it is highly desirable, for ethical reasons, that adult captures of *Aedes* vectors should be based on "landing collections" only. Instruction must be clearly given to all field staff involved in entomological work in DF/DHF control programmes that every effort should be made to avoid being bitten.

Resting collections

During periods of inactivity, adult mosquitoes typically rest indoors, especially in bedrooms, and mostly in dark places, such as clothes closets and other sheltered sites. Resting collections require systematic searching of these sites for adult mosquitoes with the aid of a flashlight. A labour-intensive method is to capture the adults using mouth or battery-powered aspirators and hand-held nets with the aid of flashlights. Recently, a much more productive, standardized and less labour-intensive method using battery-operated back-pack aspirators has been developed[27]. Following a standardized, timed collection routine in selected rooms of each house, densities are recorded as the number of adults per house (females, males or both) or the number of adults per human-hour of effort. When the mosquito population density is low, the percentage of houses positive for adults is sometimes used.

Oviposition traps

"Ovitraps" are devices used to detect the presence of *Ae. aegypti* and *Ae. albopictus*

where the population density is low and larval surveys are largely unproductive (e.g. when the Breteau index is less than 5), as well as under normal conditions. They are particularly useful for the early detection of new infestations in areas from which the mosquitoes have been previously eliminated. For this reason, they are used for surveillance at international ports of entry, particularly airports, which comply with international sanitary regulations and which should be maintained free of vector breeding. An ovitrap enhanced with hay infusion has been shown to be a very reproducible and efficient method for *Ae. aegypti* surveillance in urban areas and has also been shown to be useful to evaluate control programmes, such as the impact of adulticidal space spraying on adult female populations[28].

The standard ovitrap is a wide-mouthed, pint-sized glass jar, painted black on the outside. It is equipped with a hardboard or wooden paddle clipped vertically to the inside with its rough side facing inwards. The jar is partially filled with water and is placed appropriately in a suspected habitat, generally in or around homes in the environment. The "enhanced CDC ovitrap" has yielded eight times more *Ae. aegypti* eggs than the original version. In this method, double ovitraps are placed. One jar contains an olfactory attractant made from a "standardized" seven-day-old infusion, while the other contains a 10 percent dilution of the same infusion. Ovitraps are usually serviced on a weekly basis, but in the case of enhanced ovitraps, they are serviced every 24 hours. The paddles are examined under a dissecting microscope for the presence of *Ae. aegypti* eggs, which are then counted and stored. In areas where both *Ae. aegypti* and *Ae. albopictus* occur, eggs should be hatched and larvae or adults identified, since the eggs of those species cannot be reliably distinguished from each other. The percentage of positive ovitraps provides a simple index of infestation levels, or if the eggs are counted, it can provide an estimate of the adult female population.

Tyre section larvitraps

Tyre section larvitraps of various designs have also been used for monitoring oviposition activity, the simplest being a water-filled radial section of an automobile tyre. A prerequisite for any design is that it either facilitates visual inspection of the water *in situ* or allows the ready transfer of the contents to another container for examination. Tyre larvitraps differ from ovitraps in that water level fluctuations brought about by rainfall induce hatching of eggs, hence the presence of larvae is noted rather than the paddles on which eggs have been deposited. The placement and use of this method is discussed in more details in reference 26.

Epidemiological interpretations of vector surveillance

Adult surveillance

The epidemiology of dengue infection may be complicated because *Ae. aegypti* may probe repeatedly on one or more persons during a single blood meal. The correlation of different entomological indices in terms of actual disease transmission is difficult. The interpretation of

the epidemiology of dengue transmission must take into account inter-urban population movement, focality of *Aedes* populations within the urban area, and fluctuations in adult population densities, which influence transmission intensity. More attention should be given to understanding the relationships among adult vector densities, densities of the human population in different areas of the city, and the transmission of dengue viruses.

Larval surveillance

The commonly-used larval indices (house, container and Breteau) are useful for determining general distribution, seasonal changes and principal larval habitats, as well as for evaluating environmental sanitation programmes. However, they generally have no relevance to the dynamics of disease transmission. The precise levels of vector infestation that constitute a "risk" level for dengue transmission are influenced by many factors, including mosquito longevity and immunological status of the human population. There are examples (e.g. Singapore) where dengue transmission occurred even when the House Index was less than 2%. Therefore, the limitations of these indices must be recognized and studied more carefully to determine how they correlate with adult female population densities, and how all indices correlate with the disease-transmission risk. The development of alternative, practical and more sensitive entomological surveillance methodologies is an urgent need. The level and type of vector surveillance selected by each country or control programme should be determined by operational research activities conducted at the local level.

Sampling strategies

The sample size for routine larval surveys should be calculated using statistical methods based on the expected level of infestation and the desired level of confidence in the results. Annex IV gives tables and examples for determining the number of houses to be inspected. Several approaches can be used.

Systematic sampling

Every nth house is examined throughout a community or along linear transects through the community. For example, if a sample of 5% of the houses is to be inspected, every 20th house would be inspected. This is a practical option for rapid assessment of vector population levels, especially in areas where there is no house numbering system.

Simple random sampling

The houses to be examined are obtained from a table of random numbers (found in statistical text books or from a calculator or computer-generated list). This is a more laborious process, as detailed house maps or lists of street addresses are a prerequisite for identifying the selected houses.

Stratified random sampling

This approach minimizes the problem of under- and over-representation by subdividing the localities into sectors or "strata". Strata are usually based on identified risk factors, such as areas without piped water supply, areas not served by sanitation services, and densely-populated areas. A simple random sample is taken from each stratum, with the number of houses inspected being in proportion to the number of houses in that sector.

Frequency of sampling

Control programmes using integrated strategies do not require sampling at frequent intervals to assess the impact of the applied control measures. This is especially true where the effect of the alternative strategies outlasts residual insecticides (for example, larvivorous fish in large potable water storage containers, source reduction or mosquito-proofing of containers) or when larval indices are high (HI greater than 10%). On the other hand, feedback on at least a monthly basis may be desirable to monitor and guide community activities and to identify the issues that need more scrutiny, especially when the HI is 10% or lower. For specific research studies, it may be necessary to sample on a weekly, daily or even hourly basis (e.g. to determine the diurnal pattern of biting activity).

Insecticide susceptibility testing

Information on the susceptibility of *Ae. aegypti* to insecticides for the planning and evaluation of control is of fundamental importance. The status of resistance in a population must be carefully monitored to ensure that timely and appropriate decisions are made to use alternative insecticides or to change control strategies.

Standard WHO susceptibility test procedures and kits are available to determine the susceptibility or level of resistance of mosquito larvae and adults to insecticides (WHO, 1981)[29]. Test kits can be ordered and purchased through WHO Representatives (WRs) at country level and WHO regional offices. Biochemical and immunologic techniques for testing individual mosquitoes have also been developed but are not yet available for routine field use.

Additional information for entomological surveillance

In addition to the evaluation of aspects directly pertaining to vector density and distribution, community-oriented, integrated pest management strategies require that other parameters be measured or periodically monitored. These include the distribution and density of the human population, settlement characteristics, and conditions of land tenure, housing styles and education. The monitoring of these parameters is relevant and of importance to planning purposes and for assessing the dengue risk. The knowledge of changes over time in the distribution of water supply services, their quality and reliability, as well as in domestic water storage and solid waste disposal practices is also particularly relevant. Meteorological data are also important. Such information aids in planning targeted source reduction and management activities, as well as in organizing epidemic intervention measures.

Some of these data sets are generated by the health sector, but other sources of data may be necessary. In most cases, annual or even less frequent updates will suffice for programme management purposes. In the case of meteorologic data, especially rainfall patterns, humidity and temperature, a more frequent weekly analysis is warranted if it is to be of predictive value in determining the seasonal trends and short-term fluctuations of vector populations.

7

Vector Distribution and Bioecology

IN the South-East Asia Region, *Aedes aegypti* is the principal epidemic vector of dengue viruses. *Aedes albopictus* has been recognized as a secondary vector, which also is important in the maintenance of the viruses. The distribution and biology of these two species are described below.

7.1 *Aedes aegypti*

Taxonomic status

Aedes aegypti exhibits a continuous spectrum of scale patterns across its range of distribution from a very pale form to a dark form, with associated behavioural differences[30]. It is essential to understand the bionomics of the local mosquito population as a basis for its control.

Geographical distribution in South-East Asia

Distribution

Ae. aegypti is widespread in tropical and subtropical areas of South-East Asia, and is common in most urban areas. The rural spread of *Ae. aegypti* is a relatively recent occurrence associated with the development of rural water supply schemes and improved transport systems (see Figure 3).

In semi-arid areas, i.e. India, *Ae. aegypti* is an urban vector and populations typically fluctuate with rainfall and water storage habits[31]. In other countries of South-East Asia, where the annual rainfall is greater than 200 cm, *Ae. aegypti* populations are more stable and are established in urban, semi-urban and rural areas. Because of traditional water storage practices in Indonesia, Myanmar and Thailand, their densities are higher in semi-urban areas than in urban areas.

Urbanization tends to increase the number of habitats suitable for *Ae. aegypti*. In some cities where vegetation is abundant, both *Ae. aegypti* and *Ae. albopictus* occur together, but generally *Ae. aegypti* is the dominant species, depending on the availability and type of larval habitat and the extent of urbanization. In Singapore, for example, the premise index was highest for *Ae. aegypti* in slum houses, shop houses and multistoried flats. *Ae. albopictus,* on the other hand, did not seem to be related to the

prevailing housing types, but was more common in areas with open spaces and vegetation.

Altitude

Altitude is an important factor in limiting the distribution of *Ae. aegypti.* In India, *Ae. aegypti* ranges from sea level to 1000 metres above sea level. Lower elevations (less than 500 meters) have moderate to heavy mosquito populations[32] while mountainous areas (greater than 500 meters) have low populations. In countries of South-East Asia, 1000 to 1500 metres appears to be the limit for Ae. *aegypti* distribution. In other regions of the world, it is found at even higher altitudes, i.e. up to 2200 metres[33] in Columbia.

Ecology and bionomics

Eggs

Eggs are deposited singly on damp surfaces just above the water line. Most female *Ae. aegypti* will lay eggs in several oviposition sites during a single gonotrophic cycle. Embryonic development is usually completed in 48 hours in a warm and humid environment. Once embryonation development is complete, the eggs can withstand long periods of desiccation (more than a year). Eggs hatch once the containers are flooded, but not all eggs hatch at the same time. The capacity of eggs to withstand desiccation facilitates the survival of the species during adverse climatic conditions.

Figure 3. World distribution map of Dengue and *Aedes aegypti* in 1998

Areas infested with *Aedes aegypti*
Areas with *Aedes aegypti* and dengue epidemic activity

Source: Gubler DJ. 1998[10]

Larvae and pupae

The larvae pass through four developmental stages. The duration of larval development depends on temperature, availability of food, and larval density in the receptacle. Under optimal conditions, the time taken from hatching to adult emergence can be as short as seven days, including two days in the pupal stage. At low temperatures, however, it may take several weeks for adults to emerge.

Throughout most of South-East Asia, *Ae.aegypti* oviposits almost entirely in domestic, man-made water receptacles. These include a multitude of receptacles found in and around urban environments (households, construction sites and factories), such as water-storage jars, plates on which flower pots stand, flower vases, cement baths, foot baths, wooden and metal barrels, metal cisterns, tyres, bottles, tin cans, polystyrene containers, plastic cups, discarded wet-cell batteries, glass containers associated with "spirit houses" (shrines), drain pipes and ant-traps in which the legs of cupboards and tables often stand. Natural larval habitats are more rare, but include tree-holes, leaf axils and coconut shells. In hot and dry regions, overhead tanks, groundwater storage tanks and septic tanks may be primary habitats. In areas where water supplies are irregular, inhabitants store water for household use, thereby increasing the number of available larval habitats.

Adults

Soon after emergence, the adult mosquitoes mate and the inseminated female may take a blood meal within 24-36 hours. Blood is the source of protein essential for the maturation of eggs.

Feeding behaviour

Ae. aegypti is highly anthropophilic, although it may feed on other available warm blooded animals. Being a diurnal species, females have two periods of biting activity, one in the morning for several hours after daybreak and the other in the afternoon for several hours before dark[34,35,36]. The actual peaks of biting activity may vary with location and season. In the case of interrupted feeding, *Ae. aegypti* may feed on more than one person. This behaviour greatly increases the epidemic transmission efficiency. Thus, it is not uncommon to see several members of the same household with an onset of illness occurring within 24 hours, suggesting that they were infected by the same infective mosquito[10]. *Ae. aegypti* generally does not bite at night, but it will feed at night in lighted rooms[35].

Resting behaviour

Ae. aegypti prefers to rest in dark, humid, secluded places inside houses or buildings, including bedrooms, closets, bathrooms and kitchens. Less often it can be found outdoors in vegetation or other protected sites. The preferred indoor resting surfaces are the undersides of furniture, hanging objects such as clothes and curtains, and on walls.

Flight range

The dispersal of adult female *Aedes aegypti* is influenced by a number of factors including availability of oviposition sites and blood meals, but appears to be often limited to within 100 meters of the site of emergence. However,

recent studies in Puerto Rico indicate that they may disperse more than 400 meters primarily in search of oviposition sites.[37] Passive transportation can occur via eggs and larvae in containers.

Longevity

Aedes aegypti has an average adult survival of only eight days[36]. During the rainy season, when survival is longer, the risk of virus transmission is greater. More research is required on the natural survival of *Ae. aegypti* under various environmental conditions.

Virus transmission

A vector mosquito may become infected when it feeds on a viraemic human host. In the case of DF/DHF, viraemia in the human host may occur 1-2 days before the onset of fever and lasts for about five days after the onset of fever[38]. After an intrinsic incubation period of 10-12 days, the virus grows through the midgut to infect other tissues in the mosquito, including the salivary glands. If it bites other susceptible persons after the salivary glands become infected, it transmits dengue virus to those persons by injecting the salivary fluid.

7.2 *Aedes albopictus*

Aedes albopictus belongs to the same subgenus (*Stegomyia*) as *Ae. aegypti*. This species is widely distributed in Asia from tropical to temperate countries. During the past two decades, the species has extended its range to North and South America, the Caribbean, Africa, Southern Europe and some Pacific islands[39].

Ae. albopictus is primarily a forest species that has become adapted to rural, suburban and urban human environments. It oviposits and develops in tree holes, bamboo stumps and leaf axils in forest habitats; and in these plus artificial containers in urban settings. It is an indiscriminate blood feeder and more zoophagic than Ae. *aegypti*. Its flight range may be up to 500 metres. Unlike *Ae. aegypti*, some strains are cold adapted in Northern Asia and America, with eggs that spend the winter in diapause.

In some areas of Asia and in the Seychelles, *Ae. albopictus* has been occasionally incriminated as the vector of epidemic DF/DHF, though it is much less important than *Ae. aegypti*. In the laboratory, both species can transmit dengue virus vertically from a female through the eggs to her progeny, although *Ae. albopictus* does so more readily[9].

7.3 Vector Identification

Pictorial keys to *Aedes (Stegomyia)* mosquitoes breeding in domestic containers are given in Annex V[40]. The keys include *Culex quinquefasciatus* which may be found in the same habitats.

8

Prevention and Control Measures

NO VACCINE is available yet for the prevention of dengue infection and there are no specific drugs for its treatment. Hence DF/DHF control is primarily dependent on the control of *Ae. aegypti.*

Dengue control programmes in the Region have in general not been very successful, primarily because they have relied almost exclusively on space spraying of insecticides for adult mosquito control. However, space spraying requires specific operations which were often not adhered to, and most countries found it cost prohibitive.

In order to achieve sustainability of a successful DF/DHF vector control programme, it is essential to focus on larval source reduction and to have complete cooperation with non-health sectors, such as nongovernmental organizations, civic organizations and community groups, to ensure community understanding and involvement in implementation. There is, therefore, a need to adopt an integrated approach to mosquito control by including all appropriate methods (environmental, biological and chemical) which are safe, cost-effective and environmentally acceptable. A successful, sustainable *Ae. aegypti* control programme must involve a partnership between government control agencies and the community. The approaches described below are considered necessary to achieve long-term, sustainable control of *Ae. aegypti*.

8.1 Environmental Management

Environmental management involves any change that prevents or minimizes vector breeding and hence reduces human-vector contact. The control of *Ae. aegypti* in Cuba and Panama in the early part of this century was based mainly on environmental management. Such measures remain applicable wherever dengue is endemic. The World Health Organization[41] (1982) has defined three kinds of environmental management (Box 17).

Environmental methods to control *Ae. aegypti* and *Ae. albopictus* and to reduce man-vector contact are source reduction, solid waste management, modification of man-

made breeding sites, and improved house design. The major environmental management methods used for the control of the immature stages of dengue vectors are summarized in Box 18.

Environmental modification

Improved water supply

Whenever piped water supply is inadequate and available only at restricted hours or at low pressure, the storage of water in varied types of containers is encouraged, thus leading to increased *Aedes* breeding. The majority of such containers are large and heavy (e.g storage jars) and can neither be easily disposed of nor cleaned. In rural areas, unpolluted, disused wells become breeding grounds for *Ae. aegypti*. It is essential that potable water supplies be delivered in sufficient quantity, quality and consistency to reduce the necessity and use of water storage containers that serve as the most productive larval habitats.

Mosquito-proofing of overhead tanks/ cisterns or underground reservoirs

Where *Ae. aegypti* larval habitats include overhead tanks/cisterns and masonary chambers of piped waterlines, these structures should be mosquito-proofed[42]. A suggested design is illustrated in Annex VI. Similarly, mosquito-proofing of domestic wells and undergroundwater storage tanks should be undertaken. Masonary chambers of sluice valves and water meters are required to be provided with soak pits as part of preventive maintenance (Annex VI).

Environmental manipulation

Draining of water supply installations

Water collection/leakages in masonry chambers, distribution pipes, valves, sluice valves, surface boxes for fire hydrants, water meters, etc. collect water and serve as important *Ae.aegypti* larval habitats in the absence of preventive maintenance.

Domestic storage

The major sources of *Ae. aegypti* breeding in most urban areas of South-East Asia are containers storing water for household use including clay, ceramic and cement water jars of 200 litre size, 210 litre (50 gallon) metal drums, and smaller containers storing fresh water or rain water. Water storage containers should be covered with tight-fitting lids or

Box 17
Environmental Management Methods

- Environmental modification: long-lasting physical transformation of vector habitats.
- Environmental manipulation: temporary changes to vector habitats that involve the management of “essential” and “nonessential” containers; and management or removal of “natural” breeding sites.
- Changes to human habitation or behaviour: efforts to reduce man-vector-virus contact.

Box 18
Environmental measures for the control of *Aedes aegypti* production sites

Production site	Clean	Cover	Store under roof	Modify design	Fill (sand/ soil)	Collect recycle/ dispose	Puncture or drain
Essential							
Water storage tank/cistern	+	+		+			
Drum (40-55 gal)	+	+		+			
Flower vase with water	+				+		
Potted plants with saucers	+						
Ornamental pool/fountain	+						
Roof gutter/sun shades	+						
Animal water container	+						
Ant trap	+						
Non-essential							
Used tyres		+	+		+	+	
Discarded large appliances						+	
Discarded buckets						+	+
Tin cans						+	+
Natural							
Treeholes					+		
Rock holes					+		

screens, care being taken to replace them after water is used. An example of the efficacy of this approach has recently been demonstrated in Thailand[43].

Flower pots/vases and ant traps

Flower pots, flower vases and ant traps are common sources of *Ae. aegypti* breeding. They should be punctured to produce a drain hole. Alternatively, live flowers can be placed in a mixture of sand and water. Flowers should be removed and discarded weekly and vases scrubbed and cleaned before reuse. Brass flower pots, which make poor larval habitats, can be used in cemeteries in place of traditional glass containers. Ant traps to protect food storage cabinets can be treated with common salt or oil.

Aedes breeding in incidental water collections

Desert (evaporation) water coolers, condensation collection pans under refrigerators, and air conditioners should be regularly inspected, drained and cleaned. Desert water coolers generally employed in arid/semi-arid regions[44] of South-East Asia to cool houses during summer contain two manufacturing defects. These are as follows:
(1) The exit pipe at the bottom of the water-holding tray is generally fixed a few centimetres above the bottom. This exit pipe should be fitted at such a level that while emptying the tray, all the water should get drained off without any retention at the bottom.
(2) Desert coolers are normally fitted to windows with the exit pipe located on the exterior portion of the tray. These sites are usually difficult to access, and, therefore, there is a need to change the design so that both the filling and emptying of the water-holding trays can be manipulated from the room, thus eliminating the need of climbing to approach the exit pipe at the exterior of the building.

It is recommended that each country should develop regulatory mechanisms to ensure the design specifications as outlined above for manufacturing desert coolers.

Building exteriors

The design of buildings is important to prevent *Aedes* breeding. Drainage pipes of rooftops sunshades/porticos often get blocked and become breeding sites for *Aedes* mosquitoes. There is a need for periodic inspection of buildings during the rainy season to locate potential breeding sites.

Mandatory water storage for fire fighting

Fire prevention regulations may require mandatory water storage. Such storage tanks need to be kept mosquito-proofed. In some municipalities in India[45], timber merchants are required to maintain two metal drums (50 gallons) full of water for fire fighting. These drums should be kept covered with tight lids. Also, metal drums used for water storage at construction sites should be mosquito-proofed.

Solid waste disposal

Solid wastes, namely tins, bottles, buckets or any other waste material scattered around

houses, should be removed and buried in land fills. Scrap material in factories and warehouses should be stored appropriately until disposal. Household and garden utensils (buckets, bowls and watering devices) should be turned upside down to prevent the accumulation of rain water. Similarly, canoes and small boats should be emptied of water and turned upside down when not in use. Plant waste (coconut shells, cocoa husks) should be disposed of properly and without delay.

Tyre management

Used automobile tyres are of major importance as breeding sites for urban *Aedes,* and are therefore a significant public health problem. Imported used tyres are believed responsible for the introduction of *Ae. albopictus* into the United States, Europe and Africa[46]. Tyre depots should always be kept under cover to prevent the collection of rain water.

New technologies for tyre recycling and disposal are continually coming into use, but most of them have proved to be of limited application or cost-effectiveness. Used tyres can be filled with earth or concrete and used for planters or traffic/crash barriers. They may also be used as soil erosion barriers, or used to create artificial reefs and reduce beach erosion by wave action. Tyres can also be recycled for sandals, floormats, industrial washers, gaskets, buckets, garbage pails and carpet backing, while truck tyres have been made into durable, low-cost refuse containers.

Filling of cavities of fences

Fences and fence posts made from hollow trees such as bamboo should be cut down to the node, and concrete blocks should be filled with packed sand, crushed glass, or concrete to eliminate potential *Aedes* larval habitats.

Glass bottles and cans

Glass bottles, cans and other small containers should be buried in land fills or crushed and recycled for industrial use.

8.2 Personal Protection

Protective clothing

Clothing reduces the risk of mosquito biting if the cloth is sufficiently thick or loosely fitting. Long sleeves and trousers with stockings may protect the arms and legs, the preferred sites for mosquito bites. Schoolchildren should adhere to these practices whenever possible. Impregnating clothing with chemicals such as permethrin can be especially effective in preventing mosquito bites.

Mats, coils and aerosols

Household insecticidal products, namely mosquito coils, pyrethrum space spray and aerosols have been used extensively for personal protection against mosquitoes. Electric vaporizer mats and liquid vaporizers are more recent additions which are marketed in practically all urban areas.

Repellents

Repellents are a common means of personal protection against mosquitoes and other biting insects. These are broadly classified into two categories, natural repellents and chemical

repellents. Essential oils from plant extracts are the main natural repellent ingredients, i.e. citronella oil, lemongrass oil and neem oil. Chemical repellents such as DEET (N, N-Diethyl-m-Toluamide) can provide protection against *Ae. albopictus, Ae. aegypti* and anopheline species for several hours. Permethrin is an effective repellant when impregnated in cloth.

Insecticide-treated mosquito nets and curtains

Insecticide-treated mosquito nets (ITMN) have limited utility in dengue control programmes, since the vector species bites during the day. However, treated nets can be effectively utilized to protect infants and night workers who sleep by day. They can also be effective for people who generally have an afternoon sleep. For details of insecticide treatment of mosquito nets and curtains, see Annex VII.

"Olyset net", a wide mesh net woven from polyethylene thread containing 2% permethrin, is yet another improvement in ITMN technology. This net has two advantages over traditional nets in that the wide mesh permits better ventilation and light, and the treated thread enables a slow release of permethrin to the fibre surface, ensuring a long residual effect (over a year). In studies carried out in Malaysia, four washings with soap and water did not diminish the efficacy and the mortality of *Ae. aegypti* was 86.7%[47]. For control of DF/DHF in Vietnam, Olyset net curtains were hung on the inside against doors/windows; *Ae. aegypti* was adversely affected and dengue virus transmission was interrupted[48]. Further studies on impregnated fabrics appear warranted.

8.3 Biological Control

The application of biological control agents which are directed against the larval stages of dengue vectors in South-East Asia has been somewhat restricted to small-scale field operations.

Fish

Larvivorus fish (*Gambusia affinis* and *Poecilia reticulata*) have been extensively used for the control of *An. stephensi* and/or *Ae. aegypti* in large water bodies or large water containers in many countries in South-East Asia. The applicability and efficiency of this control measure depend on the type of containers.

Bacteria

Two species of endotoxin-producing bacteria, *Bacillus thuringiensis* serotype H-14 *(Bt.H-14)* and *Bacillus sphaericus (Bs)* are effective mosquito control agents. They do not affect non-target species. *Bt.H-14* has been found to be most effective against *An. stephensi* and *Ae. aegypti*, while *Bs* is the most effective against C*ulex quinquefasciatus* which breeds in polluted waters. There is a whole range of formulated *Bti* products produced by several major companies for control of vector mosquitoes. Such products include wettable powders and various slow-release formulations including briquettes, tablets and pellets. Further developments are expected in slow-release formulations. *Bt.H-14* has an extremely low-

level mammalian toxicity and has been accepted for the control of mosquitoes in containers storing water for household use.

Cyclopoids

The predatory role of copepod crustaceans* was documented between 1930-50, but scientific evaluation was taken up only in 1980 in Tahiti, French Polynesia, where it was found that *Mesocyclops aspericornis* could effect a 99.3% mortality rate among *Aedes (Stegomyia)* larvae and 9.7% and 1.9%, respectively among *Cx. quinquefasciatus* and *Toxorhynchities amboinensis* larvae.[49] Trials in crab burrows against *Ae. polynesiensis* and in water tanks, drums, and covered wells met with mixed results. In Queensland, Australia, out of seven species evaluated in the laboratory, all but *M. notius* were found to be effective predators of both *Ae. aegypti* and *An. farauti* but not against *Cx. quinquifasciatus*. Field releases in both northern and southern Queensland, however, showed mixed results. In Thailand, results were also mixed, but in Vietnam, results were more successful, contributing to the eradication of *Ae. aegypti* from one village[50].

Although the lack of nutrients and frequent cleaning of some containers can prevent the sustainability of copepods, they could be suitable for large containers which cannot be cleaned regularly (wells, concrete tanks and tyres)[50]. They can also be used in conjunction with *Bt.H-14.* Copepods have a role in dengue vector control, but more research is required on the feasibility of operational use.

Autocidal ovitraps

Autocidal ovitraps were successfully used in Singapore as a control device in the eradication of *Ae. aegypti* from the Changgi international airport. In Thailand, this autocidal trap was further modified as an auto-larval trap using plastic material available locally. Unfortunately, under the local conditions of water storage practices in Thailand, the technique was not very efficient in reducing natural populations of *Ae. aegypti*. Better results can be expected if the number of existing potential larval habitats is reduced, or more autocidal traps are placed in the area under control, or both activities are carried out simultaneously. It is believed that, under certain conditions, this technique could be an economical and rapid means of reducing the natural density of adult females as well as serve as a device for monitoring infestations in areas where some reduction in population densities of the vector have already taken place. However, the successful application of autocidal ovitraps/larval traps depends on the number placed, the location of placement, and their attractiveness as *Ae. aegypti* female oviposition sites[51].

8.4 Chemical Control

Chemicals have been used to control *Ae. aegypti* since the turn of the century. In the first campaigns against the yellow fever vector in Cuba and Panama, in conjunction with widespread clean-up campaigns, *Aedes* larval habitats were treated with oil and houses were fumigated with pyrethrins. When the

* Copepods should not be used in countries where guineaworm and gnathostomiasis are endemic, as they may act as intermediate hosts for these parasites.

insecticidal properties of DDT were discovered in the 1940s, this compound became a principal method of *Ae. aegypti* eradication programmes in the Americas. When resistance to DDT emerged in the early 1960s, organophosphate insecticides, including fenthion, malathion and fenitrothion were used for *Ae. aegypti* adult control and temephos as a larvicide. Current methods for applying insecticides include larvicide application and space spraying[51].

Chemical larviciding

Larviciding or "focal" control of *Ae. aegypti* is usually limited to domestic-use containers that cannot be destroyed, eliminated, or otherwise managed. It is difficult and expensive to apply chemical larvicides on a long-term basis. Therefore chemical larvicides are best used in situations where the disease and vector surveillance indicate the existence of certain periods of high risk and in localities where outbreaks might occur. Establishing the precise timing and location are essential for maximum effectiveness. Control personnel distributing the larvicide should always encourage house occupants to control larvae by environmental sanitation. There are three insecticides that can be used for treating containers that hold drinking water.

Temephos 1% sand granules

One per cent temephos sand granules are applied to containers using a calibrated plastic spoon to administer a dosage of 1 ppm. This dosage has been found to be effective for 8-12 weeks, especially in porous earthen jars, under normal water use patterns. The quantity of sand granules required to treat various size water containers is shown in Annex VIII. Although resistance to temephos in *Ae. aegypti* and *Ae. albopictus* populations has not been reported from the South-East Asia Region, the susceptibility level of *Aedes* mosquitoes should be monitored regularly in order to ensure the effective use of the insecticide.

Insect growth regulators

Insect growth regulators (IGRs) interfere with the development of the immature stages of the mosquito by interference of chitin synthesis during the molting process in larvae or disruption of pupal and adult transformation processes. Most IGRs have extremely low mammalian toxicity (LD50 value of acute oral toxicity for methoprene (Altosid) is 34 600 mg/kg). In general, IGRs may provide long-term residual effects (three to six months) at relatively low dosages when used in porous earthen jars. Because IGRs do not cause immediate mortality of the immature mosquitoes, countries with legislation stipulating that the breeding of *Aedes* larvae is an offense, will require some alteration of the law, so as not to penalize home owners who use these compounds.

Bacillus thuringiensis H-14 (Bt.H-14)

Bt.H-14, which is commercially available under a number of trade names, is a proven, environmentally-nonintrusive mosquito larvicide. It is entirely safe for humans when the larvicide is used in drinking water in normal dosages[52]. Slow-release formulations of *Bt.H-14* are being developed. Briquette formulations that appear to have greater

residual activity are commercially available and can be used with confidence in drinking water. The use of *Bt.H-14* is described in the section on biological control. The large parabasal body that forms in this agent contains a toxin that degranulates solely in the alkaline environment of the mosquito midgut. The advantage of *Bt.H-14* is that an application destroys larval mosquitoes but spares any entomophagus predators and other non-target species that may be present. *Bt.H-14* formulations tend to rapidly settle at the bottom of water containers, and frequent applications are therefore required. The toxin is also photolabile and is destroyed by sunlight.

Space sprays

Space spraying involves the application of small droplets of insecticide into the air in an attempt to kill adult mosquitoes. It has been the principal method of DF/DHF control used by most countries in the Region for 25 years. Unfortunately, it has not been effective, as illustrated by the dramatic increase in DHF incidence in these countries during the same period of time. Recent studies have demonstrated that the method has little effect on the mosquito population, and thus on dengue transmission [53,54,55]. Moreover, when space spraying is conducted in a community, it creates a false sense of security among residents, which has a detrimental effect on community-based source reduction programmes. From a political point of view, however, it is a desirable approach because it is highly visible and conveys the message that the government is doing something about the disease. This, however, is poor justification for using space sprays. The current recommendations are that space spraying of insecticides (fogging) should not be used except in the most extreme conditions during a major DHF epidemic. However, the operations should be carried out at the right time, at the right place, and according to the prescribed instructions with maximum coverage, so that the fog penetration effect is complete enough to achieve the desired results.

When space sprays are employed, it is important to follow the instructions on both the application equipment and the insecticide label and to make sure the application equipment is well maintained and properly calibrated. Droplets that are too small tend to drift beyond the target area, while large droplets fall out rapidly. Nozzles for ultra-low volume ground equipment should be capable of producing droplets in the 5 to 27 micron range and the mass median diameter should not exceed the droplet size recommended by the manufacturer. Desirable spray characteristics include a sufficient period of suspension in the air with suitable drift and penetration into target areas with the ultimate aim of impacting adult mosquitoes. Generally, there are two forms of space-spray that have been used for *Ae. aegypti* control, namely "thermal fogs" and "cold fogs". Both can be dispensed by vehicle-mounted or hand-operated machines.

Thermal fogs

Thermal fogs containing insecticides are normally produced when a suitable

formulation condenses after being vaporized 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 vaporized and condensed rapidly with only negligible formulation breakdown. Thermal fogging formulations can be oil-based or water-based. The oil(diesel)-based formulations produce dense clouds of white smoke, whereas water-based formulations produce a colorless 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.

Ultra-low volume (ULV), aerosols (cold fogs) and mists

ULV involves the application of a small quantity of concentrated liquid insecticides. The use of less than 4.6 litres/ha of an insecticide concentrate is usually considered as an ULV application. ULV is directly related to the application volume and not to the droplet size. Nevertheless, droplet size is important and the equipment used should be capable of producing droplets in the 10 to 15 micron range, although the effectiveness changes little when the droplet size range is extended to 5-25 microns. The droplet size should be monitored by exposure on teflon or silocone-coated slides and examined under a microscope. Aerosols, mists and fogs may be applied by portable machines, vehicle-mounted generators or aircraft equipment.

House-to-house application using portable equipment

Portable spray units can be used when the area to be treated is not very large or in areas where vehicle-mounted equipment cannot be used effectively. This equipment is meant for restricted outdoor use and for enclosed spaces (buildings) of not less than 14m^3. Portable application can be made in congested low-income housing areas, multistoried buildings, godowns and warehouses, covered drains, sewer tanks and residential or commercial premises. Operators can treat an average of 80 houses per day, but the weight of the machine and the vibrations caused by the engine make it necessary to allow the operators to rest, so that two or three operators are required per machine.

Vehicle-mounted fogging

Vehicle-mounted aerosol generators can be used in urban or suburban areas with a good road system. One machine can cover up to 1500-2000 houses (or approximately 80 ha) per day. It is necessary to calibrate the equipment, vehicle speed, and swath width (60-90m) to determine the coverage obtained by a single pass. A good map of the area showing all roads is of great help in undertaking the application. An educational effort may be required to persuade the residents to cooperate by opening doors and windows.

The speed of the vehicle and the time of day of application are important factors to consider when insecticides are applied by ground vehicles. The vehicle should not travel faster than 16 kph (10 mph). When the wind speed is greater than 16 kph or when the

ambient air temperature is greater than 28°C (82°F), the insecticide should not be applied[25]. The best time for application is in the early morning (approximately 0600-0830 hours) or late afternoon (approximately 1700-1930 hours). For details of procedures, timing, frequency of thermal fogging and ULV space operation please see Annex IX.

Performance of fogging machines

Estimates have been made of the average coverage per day with certain aerosol and thermal fog procedures (Box 19).

Box 19
Average coverage per day with space spraying procedures

	Equipment	Possible daily coverage
1.	Vehicle-mounted cold fogger	225 ha
2.	Vehicle-mounted thermal fogger	150 ha
3.	Back-pack ULV mist blower	30 ha
4.	Hand carried thermal fogger. Swing fog	5 ha
5.	Hand carried ULV aerosol generators	5 ha or 250 houses

Insecticide formulations for space sprays

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 spraying and thermal fogging respectively. For undiluted technical grade ULV malathion applications from vehicles, the dosage on an area basis is 0.5 liters per hectare.

Apart from the above-mentioned formulations, a number of companies produce pyrethroid formulations containing either permethrin, deltamethrin, lambda-cyhalothin or other compounds which can be used for space spray applications. It is important not to under-dose during operational conditions. Low dosages of pyrethroid insecticides are usually more effective indoors than outdoors. Also, low dosages 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 a 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 spraying operations. Regardless of the type of equipment and spray formulations and concentrations used, an evaluation should be made from time to time to ensure that effective vector control is being achieved. Insecticides suitable as cold aerosols and thermal fogging for mosquito control are included in Annex X.

Integrated control approach

The use of insecticides for the prevention and control of dengue vectors should be integrated into environmental methods wherever possible. During periods of little or no dengue virus activity, the routine source reduction measures described earlier can be integrated into larvicide application in containers that cannot be eliminated, covered, filled or otherwise managed. For emergency control to suppress a dengue virus epidemic or to prevent an imminent outbreak, a programme of rapid and massive destruction of the *Ae. aegypti* population should be undertaken with both insecticides and source reduction, using the techniques described in these guidelines in an integrated manner.

Insecticide susceptibility monitoring

During the past 40 years, chemicals have been widely used to control mosquitoes and other insects from spreading diseases of public health importance. As a result, *Ae. aegypti* and other dengue vectors in several countries have developed resistance to commonly-used insecticides, including temephos, malathion, fenthion, permethrin, propoxur and fenitrothion. It is therefore advisable to obtain baseline data on insecticide susceptibility before insecticidal control operations are started, and to continue monitoring susceptibility levels periodically. WHO kits are available for testing the susceptibility of adult and larval mosquitoes and other arthropod vectors to commonly-used insecticides. These can be obtained from the Communicable Diseases Cluster, World Health Organization, 1211 Geneva 27, Switzerland, or through WHO Regional Offices or WHO Representatives in the countries.

Safety precautions for chemical control

All pesticides are toxic to some degree. Safety precautions should therefore be followed, including care in the handling of pesticides, safe work practices for those who apply them, and their appropriate use in and around occupied housing. A safety plan for insecticide application is included in Annex XI.

9

Sustainable Prevention and Control Measures

9.1 Community Participation

Community participation (CP) has been defined "as a process whereby individuals, families and communities are involved in the planning and conduct of local vector control activities so as to ensure that the programme meets the local needs and priorities of the people who live in the community, and promotes community's self-reliance in respect to development." In short, CP entails the creation of opportunities that enable all members of the community and extended society to actively contribute to, influence the development of, and share equitably in the fruits of accrued benefits.

Objectives of community participation in dengue prevention and control

(1) To extend the coverage of the programme to the whole community by creating community awareness. This however often requires intensive inputs.
(2) To make the programme more efficient and cost-effective, with greater coordination of resources, activities and efforts pooled by the community.
(3) To make the programme more effective through joint community efforts to set goals, objectives and strategies for action.
(4) To promote equity through sharing of responsibility, and through solidarity in serving those in greatest need and at greatest risk .
(5) To promote self-reliance among community members and increase their sense of control over their own health and destiny.

How to invoke community participation

By showing concern

Community and government organizers should reflect the true concern for human suffering, i.e. morbidity and mortality due to dengue in the country, economic losses to the families and the country, and how the benefits of the programme fit into the people's needs and expectations.

Initiating dialogue

Community organizers and opinion leaders or other key personnel in the power structure of

the community, namely women's groups, youth groups and civic organizations, should be identified. Dialogue should be undertaken through personal contacts, group discussions and film shows. Interaction should generate mutual understanding, trust and confidence, enthusiasm and motivation. The interaction should not be a one-time affair, but should be a continuing dialogue to achieve sustainability.

Creating community ownership

Organizers should use community ideas and participation to initiate the programme, community leaders to assist the programme, and community resources to fund the programme. Mosquito control, abatement agency and community partnerships should be strong, but limited to providing technical guidance and expertise.

Health education (HE)

Health education should not be based on telling people the do's and don'ts through a vertical, top-down communication process. Instead, health education should be based on formative research to identify what is important to the community and should be implemented at three levels, i.e. the community level, systems level and political level.

Community level

People should not only be provided with knowledge and skills on vector control, but education materials should empower them with the knowledge that allows them to make positive health choices and gives them the ability to act individually and collectively.

Systems level

To enable people to mobilize local actions and societal forces beyond a single community, i.e. health, development and social services.

Political level

Mechanisms must be made available to allow people to articulate their health priorities to political authorities. This will facilitate placing vector control high on the priority agenda and effectively lobby for policies and actions.

Defining community actions

For sustaining DF/DHF prevention and control programmes, the following community actions are essential[56]:

(1) At the individual level, encourage each household to adopt routine health measures that will help in the control of DF and DHF, including source reduction and implementation of proper personal protection measures.

(2) At the community level, organize "clean-up" campaigns two or more times a year to control the larval habitats of the vectors in public and private areas of the community. Some key factors for the success of such campaigns include extensive publicity via mass media, posters and pamphlets, proper planning, pre-campaign evaluation of foci, execution in the community as promised, and follow-up evaluations. Participation by municipal sanitation services should be promoted.

(3) Where community-wide participation is difficult to arrange for geographical, occupational or demographic reasons, participation can be arranged through

voluntary associations and organizations. The people in these organizations may interact daily in work or institutional settings, or come together for special purposes, i.e. religious activities, civic clubs, women's groups and schools.

(4) Emphasize school-based programmes targeting children and parents to eliminate vector breeding at home and at school.

(5) Challenge and encourage the private sector to participate in the beautification and sanitary improvement of the community as sponsors, emphasizing source reduction of dengue vectors.

(6) Combine community participation in DHF prevention and control with other priorities of community development. Where municipal services (such as refuse collection, wastewater disposal, provision of potable water, etc.) are either lacking or inadequate, the community and their partners can be mobilized to improve such services, and at the same time reduce the larval habitats of *Aedes* vectors as part of an overall effort at community development.

(7) Combine dengue vector control with the control of all species of disease-bearing and nuisance mosquitoes as well as other vermin, to ensure greater benefits for the community and consequently greater participation in neighbourhood campaigns.

(8) Arrange novel incentives for those who participate in community programmes for dengue control. For example, a nationwide competition can be promoted to identify the cleanest communities or those with the lowest larval indices within an urban area.

Detailed requirements for sustainable participation in a vector-borne disease control programme are presented in Annex XII.

9.2 Intersectoral Coordination

Developing economies in countries of the South-East Asia Region have recognized many social, economic and environmental problems which promote mosquito breeding. The dengue problem thus exceeds the capabilities of ministries of health. The prevention and control of dengue requires close collaboration and partnerships between the health and non-health sectors (both government and private), nongovernmental organizations (NGOs) and local communities. During epidemics such cooperation becomes even more critical, since it requires pooling of resources from all groups to check the spread of the disease. Intersectoral cooperation involves at least two components: (i) resource-sharing, and (ii) policy adjustments among the various ministries and nongovernmental sectors.

Resource sharing

Resource sharing should be sought wherever the dengue control coordinator can make use of underutilized human resources, e.g. for local manufacture of needed tools, seasonal government labourers for water supply improvement activities, or community and youth groups to clean up discarded tyres and containers in neighbourhoods.

Policy adjustment

The dengue control programme should seek the accommodation or adjustment of existing policies and practices of other ministries, sectors, and municipal governments to include public health as a central focus for their goals. For instance, the public works sector could be encouraged to adjust its policies to give first priority to water supply improvements for communities at highest risk of dengue. In return, the Ministry of Health could authorize the use of some of its field staff to assist the ministry responsible for public works to repair water supply and sewerage systems in other urban areas.

Role of non-health sectors in dengue control

The following examples show how several government ministries may contribute to dengue vector control efforts.

Role of the ministry responsible for public works

The ministry responsible for public works and its municipal counterparts should play a key role in dengue control. They can contribute to source reduction by providing a safe, dependable water supply, adequate sanitation, and effective solid waste management. In addition, through the adoption and enforcement of housing and building codes, a municipality may mandate the provision of utilities such as individual household piped water supplies or sewerage connections, and rainwater (stormwater) run-off control for new housing developments, or forbid open surface wells.

Role of the Ministry of Education

The Ministry of Health should work closely with the Ministry of Education to develop a health education (health communication) component targeted at school children, and devise and communicate appropriate health messages. Health education models can be jointly developed, tested, implemented and evaluated for various age groups. Research programmes in universities and colleges can be encouraged to include components that produce information of direct importance (e.g. vector biology and control, case management) or indirect importance (e.g. improved water supply, educational interventions to promote community sanitation, waste characterization studies) to dengue control programmes.

Role of the ministry responsible for the environment

The Ministry of Environment can help the Ministry of Health collect data and information on ecosystems and habitats in or around cities at high risk of dengue. Data and information on local geology and climate, land usages, forest cover, surface waters, and human populations are useful in planning control measures for specific ecosystems and habitats. The Ministry of Environment may also be helpful in determining the beneficial and adverse impacts of various *Ae. aegypti* control tactics (chemical, environmental and biological).

Role of the ministry responsible for information, communication and the mass media

Information directed at the community at large is best achieved through the mass media, such as television, radio and newspapers. Therefore, the ministry responsible for information, communication and the mass media should be approached to coordinate the release of messages on the prevention and control of dengue developed by public health specialists.

Role of nongovernmental organizations (NGOs)

NGOs can play an important role in promoting community participation and implementing environmental management for dengue vector control. This will most often involve health education, source reduction, and housing improvement related to vector control. Community NGOs may be informal neighbourhood groups or formal private voluntary organizations, service clubs, churches or other religious groups, or environmental and social action groups.

After proper training by the Ministry of Health staff in source reduction methods, NGOs can collect discarded containers (tyres, bottles, tins, etc.), clean drains and culverts, fill depressions, remove abandoned cars and roadside junk, and distribute sand or cement to fill treeholes. NGOs may also play a key role in the development of recycling activities to remove discarded containers from yards and streets. Such activities must be coordinated with the environmental sanitation service.

NGOs may also be able to play a specific, but as yet unexplored, role in environmental management during epidemic control. Under guidance from the Ministry of Health, NGOs could concentrate on the physical control of locally identified, key breeding sites such as water drums, waste tyre piles, and cemetery flower vases.

Service clubs such as Rotary International have supported DF/DHF prevention and control programmes in the American Region for over 15 years[57]. In Asia and the Pacific, programmes have been initiated in Sri Lanka, Philippines, Indonesia and Australia to provide economic and political support for successful community-based campaigns. A new grant from the Rotary Foundation of Rotary International has been awarded to study the possibility of upscaling this project to a global programme. Women's clubs have contributed to *Ae. aegypti* control by conducting household inspections for foci and carrying out source reduction. There are many opportunities, mostly untapped, for environmental organizations and religious service groups to play similar roles in each *Ae. aegypti*-infested community.

9.3 Model Development

Model development for dengue control through a community participation approach should be initiated in order to define potential prime movers in the communities and to study ways to persuade them to participate in vector control activities. Social, economic and cultural factors that promote or discourage the participation of these groups should be

intensively studied in order to gain more participation from the community. Model development focusing on school children has been studied in several countries and this strategy should be modified and introduced into each country.

9.4 Social Mobilization

Advocacy meetings should be conducted for policy makers to attain political commitment for mass clean-up campaigns and environmental sanitation. Intersectoral coordination meetings should be conducted to explore possible donors for mass antilarval control campaigns and measures and to help finance the programme. Reorientation training of health workers should be conducted to improve their technical capability and ability to supervise prevention and control activities. A "DHF month" should be identified twice a year, during the pre-transmission season and during the peak transmission period.

9.5 Health Education

Health education is very important in achieving community participation. It is a long-term process to achieve human behavioural change, and thus should be carried out on a continuous basis. Even though countries may have limited resources, health education should be given priority in endemic areas and in areas at high risk for DHF. Health education is conducted through the different channels of personal communication, group educational activities, and mass media. Health education can be implemented by women's groups, school teachers, formal and informal community leaders, and health workers. Health education efforts should be intensified before the period of dengue transmission as one of the components of social mobilization. The main target groups are school children and women.

9.6 Legislative Support

Legislative support is essential for the success of dengue control programmes. All countries of the Region have legislation addressing control of epidemic diseases which authorize health officers to take necessary action within the community for the control of epidemics. On a continuous and sustainable basis, various municipalities have adapted legislation for the prevention of "nuisance mosquitoes", however they lack specific provision related to dengue and/or *Ae. aegypti* control. At the national level, all countries are signatories to the International Health Regulations which have a specific provision for the control of *Ae. aegypti* and other disease vectors around international sea/airports.

The formulation of legislation on dengue/*Ae.aegypti* control should, therefore, take into consideration the following points:

(1) Legislation should be a necessary component of all dengue/*Ae.aegypti* prevention and control programmes.

(2) All existing decrees and resolutions on dengue/*Ae.aegypti* prevention and control must be reviewed, and their effectiveness evaluated in terms of structural, institutional and administrative changes. It is also important to add dengue to the list of diseases

that require mandatory notification in each country.

(3) Regulations should be formulated on the basis of existing sanitary codes, a strategy that is most needed in those countries which lack legislation on the subject. In countries where sanitary regulations are primarily the responsibility of agencies other than the Ministry of Health (e.g. municipal governments), a coordinated and cooperative line of action with the ministry should be developed.

(4) Legislation should incorporate municipal authorities from affected regions as the central element for implementation and enforcement. Where national legislation is weak or absent, municipal governments may consider the adoption of local ordinances for *Ae. aegypti* control.

(5) Legislation should contemplate intersectoral coordination among the ministries involved in national development in order to prevent isolated implementation of individual programmes and harmful environmental changes that could create potentially hazardous public health conditions. Ministries should be advised on the best ways to encourage disease prevention.

(6) Legislation should cover all aspects of environmental sanitation in order to effectively contribute to the prevention of all transmissible diseases.

(7) Laws should contemplate the existing judicial administrative framework in the context of national public administration. Importance should also be placed on norms aimed at developing human resources within the institutional framework.

(8) In developing legislation, the social component must be considered. Legislation should seek support based on justice and justification: individuals and the community must be persuaded that the law is good and that it is intended to protect them and their families, and that compliance with it is one of the most important components for dengue control (Box 20).

Box 20
Examples of other enforcement methods that may be considered

- Ordinances that require mosquito-proofing of cisterns, water-storage tanks, wells and septic tanks.
- Ordinances that authorize the removal of junk cars and other scrap, after proper notification.
- Ordinances that authorize the posting of “No Dumping” and “No Littering” signs and civil penalties for violators.
- Ordinances that require house-owners to keep their yards free of junk, litter, and potential foci, under threat of civil penalty.
- Ordinances requiring mandatory household collection of solid wastes for all neighbourhoods.
- Statutes/laws that require certification of imported tyres as being dry and pest-free upon arrival at ports.

10

Evaluation of DF/DHF Prevention and Control Programmes

IT is essential to monitor and evaluate the progress of DF/DHF prevention and control programmes. They enable the programme manager to assess the effectiveness of control initiatives and must be continuous operational processes. The specific objectives of programme evaluation are:

- to measure progress and programme achievements,
- to detect and solve problems,
- to assess programme effectiveness and efficiency,
- to guide the allocation of programme resources,
- to collect information needed for revising policy and replanning interventions, and
- to assess the sustainability of the programme.

10.1 Types of Evaluation[58]

There are two types of evaluation:

(1) Monitoring
(2) Formal evaluation

Monitoring

Monitoring or concurrent evaluation involves the continuous collection of information during programme implementation. It allows immediate assessment and identification of deficiencies that can be rectified without delaying the programme's progress. Monitoring provides the type of feedback which is important to programme managers.

Most monitoring systems follow the quantity and timings of various programme elements such as activities undertaken, staff movements, service utilization, supplies and equipment, and budgeting. Focus should also be made on the process of implementation of the dengue control strategy in time and space and the quality of implementation, seeking reasons for successes and failures.

Monitoring should be undertaken by persons involved in the programme at various levels. This exercise by programme managers will give a better and deeper understanding of the programme's progress, strengths and weaknesses. The information collected should

help programme managers strengthen the weaker links and optimize output.

Formal evaluation

In addition to regular monitoring, which is generally built-in, there is also a need for more formal evaluation at different intervals to obtain a precise picture of programme progress. This type of evaluation is even more essential when the programme is failing to achieve its targets or goals or when it has become static. This type of special evaluation should be done systematically and should take into account all programme elements. The main idea of such a study is to determine whether the programme is moving towards its targets and goals, to identify new needs, particularly for increased inputs (e.g. additional manpower, money, materials, IEC activities, capacity building), and to identify operational research areas for maximum operationalization.

Formal evaluation, therefore, should systematically assess the elements outlined below. However, the evaluation can cover one or more other processes depending on the objectives of the evaluation.

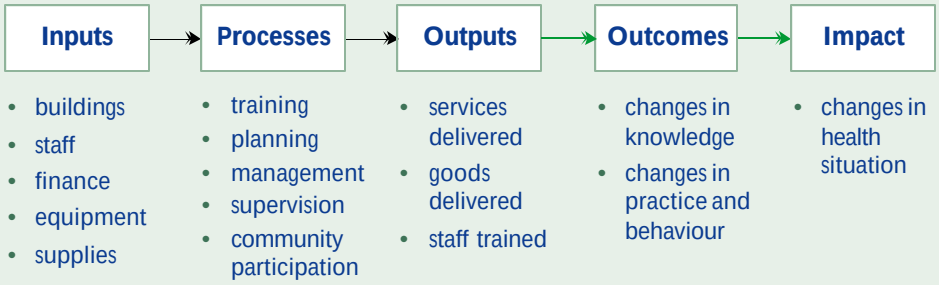
- Evaluation of need, i.e. evaluation of the relative need for the programme.
- Evaluation of plans and design, i.e. evaluation of the feasibility and adequacy of programme plans or proposals.
- Evaluation of implementation, i.e. evaluation of the conformity of the programme to its design. Does the programme provide the goods and services laid down in the plan, in both quality and quantity?
- Evaluation of outcomes, i.e. evaluation of the more immediate and direct effects of the programme on relevant knowledge, attitudes and behaviour. For training activities, for example, outcome measures might relate to the achievement of learning objectives and changes in staff performance.
- Evaluation of impact, i.e. evaluation of the programme's direct and indirect effects on the health and socioeconomic status of individuals and the demography of the community.

10.2 Evaluation Plans

An evaluation plan should have realistic and assessable targets. With this proviso, the development of an evaluation plan consists of the following steps:

- Clarification of the objectives of the evaluation – these must be agreed upon by all concerned.
- Identification of the resources available – there must be sufficient resources to collect the data on the scale envisaged and turn them into useful information.
- Selection of the type of evaluation – once the purpose of the evaluation is clear, it is necessary to decide the type of evaluation and the depth of information required.
- Selection of indicators – a good indicator is directly related to programme activities and anticipated outcomes. Therefore, indicators chosen should be limited in number, readily and uniformly interpretable, and operationally useful. For comparison purposes, use of standard

Box 21
Aspects of a programme that can be evaluated



indictors will introduce consistency into programme reviews and allow comparison over time and among countries. Although there are many ways of classifying indicators, one useful way is according to the programme structure outlined in Box 21. Thus, there can be input, process, outcome and impact indicators.

- Formulation of the detailed evaluation plan – the detailed plan should include the objectives, methods, sampling procedures, source of data, and methods of data analysis to be used, as well as budgeting and administrative arrangements. It should also give details of staff responsibilities for each activity, the reporting mechanism, and the strategies for ensuring that results are used for programme replanning and implementation.
- Collection of data – the objective of this step is to ensure that procedures are followed in such a way that data are collected in a reliable and timely manner.
- Interpretation and analysis of data – decisions about the main approaches to data analysis will have been made when the indicators were selected and the detailed plan was formulated.
- Replanning – at this step the results of the evaluation are fed back into the managerial process. Unfortunately, it is often this replanning step that is done the least well.

10.3 Cost-Effective Evaluation

In most countries of the Region, it is difficult to estimate how much money dengue prevention and/or control programmes spend annually. Often, dengue or *Aedes* control programmes function as branches of malaria control programmes and/or operate sporadically in response to real or perceived emergencies. Supplies, equipment and personnel are not continuously available. In emergencies, or under public pressure, expenditures of national funds or donations

can be very high, especially for insecticides, while little money is available for routine operations at other times.

As a result, substantial funds are spent on unstructured activities, the results of which are difficult or impossible to evaluate. It is therefore important that economic factors be considered during the reorganization or strengthening of dengue control programmes. Information of this nature is essential for planning, for evaluating the cost-effectiveness of individual control measures, for comparing different control measures, and for evaluating new methods. Examples of types of cost estimates that should be obtained are described below.

(a) Vector control costs

Operational costs

It is not enough to merely estimate the quantities of insecticide required. Costing should begin with the size of the population to be protected and the number of premises or the area to be treated, as well as the personnel requirements (at all levels) based on the frequency of application. Personnel costs include expenditures on training, safety equipment, and per diem or overtime where applicable. Initial capital costs for equipment, depreciation and/or shared usage with other programmes must also be considered. Operational costs, especially for ULV space spraying, should include machinery and vehicle maintenance, regular calibration of pumps, as well as the costs of monitoring vector populations, penetration of droplets, and the level of compliance by the local population, depending on the control measures employed. The compilation and analysis of data also involve costs.

Environmental management

Source reduction programmes are often considered less expensive alternatives to chemical control measures. However, this may only be true for short-term "clean up" campaigns. Long-term success in environmental management requires health education, public health communication, and development of community cooperation. Educational materials, promotion through the media, introduction of sanitary concepts into school curricula, training of teachers, etc. may involve considerable costs. Some of these costs can be covered by other sectors such as education, municipal or private, and such collaboration should be encouraged.

Environmental management campaigns, especially clean-up campaigns, may fail from lack of transport and facilities for solid waste disposal. Communities, especially cities, need either to invest in such equipment or make arrangements to rent or borrow it from other sources. As with chemical control, environmental management programmes must be evaluated and the vector and disease data organized and analysed. All of these activities involve costs.

(b) Laboratory surveillance

Most national laboratories that perform serology or virus isolation for other agents (measles, polio, etc.) can also include dengue. The cost of the dengue component must be

adequately assessed based on an analysis of the number of samples processed, the cost of reagents, and the equipment required. Long-term investment must be made and accounted for in the training of professionals and technicians. Refresher training sessions need to be routinely scheduled.

(c) Coordination with hospitals and medical supplies

In addition to coordination among its component parts, the programme requires coordination between curative and preventive services and these expenses should be recognized. An information exchange network is also required. In order to meet the potential for epidemic situations, hospital supplies and equipment must be readily available and be replaced and/or updated regularly.

Each country should estimate the costs associated with individual case management. Through cooperation with and information from neighbouring countries and international organizations, it should estimate its requirements on an annual or biennial basis.

(d) Surveillance

Guidelines for entomological and epidemiological surveillance methods are given in the chapter on surveillance. These can be used as a framework for estimating the size of the required surveillance system in a given city, state, province or country, as well as the cost of the surveillance that, in addition to laboratory costs and information exchange, includes expenditures for collecting and processing samples in the field.

(e) Community participation, health education and communication costs

In addition to the costs that have already been mentioned, liaison must be established with community groups. This is in order to provide technical assistance where required and to determine how the health authorities can assist these groups with their individual and collective efforts. Health education and communication activities will play a significant role in community participation efforts. Consequently, it is extremely important to estimate their cost. The calculation of actual costs of health education, communication and community participation should also be made on an annual basis.

(f) Social and economic impact

The social and economic burden of DF/DHF is another element to be considered when determining the cost-effectiveness of DHF control. In a 1995 study carried out by the Faculty of Tropical Medicine of Mahidol University in Thailand[59], in collaboration with the Faculty of Economics of Chulalongkorn University in Bangkok, Thailand, several parameters [treatment-seeking behaviour, direct impact, i.e. cost of the illness of patients (average 7.9 days) and time-cost spent by parents/caretakers (average 9.5 days), and indirect impact due to disruption of family life resulting in increased expenses] were identified. From the provider side, expenditures for the hospitalization of DHF patients included drug, laboratory and nursing costs and the cost of prevention and control.

The estimated costs for these items are provided in Box 22.

Another approach is to measure the disability-adjusted life years (DALYs) associated with dengue infection. A recent study in Puerto Rico showed a constant increase in the DALYs associated with dengue infection from 1984 to 1994[60,61]. Surprisingly, the DALYs associated with dengue infection in Puerto Rico were of the same order of magnitude as the DALYs caused by a number of other infectious diseases in Latin America, including malaria, tuberculosis, sexually transmitted diseases (excluding HIV/AIDS), hepatitis, the childhood cluster and the tropical cluster.

(g) Other costs

Each national programme will have additional cost elements depending on the governmental structure and the requirements of their accounting systems. These may include depreciating capital investments (vehicles, pumps, etc.), shared use of facilities (warehouses, administrative services, etc.), and in-country purchase and delivery of supplies (insecticides).

Box 22
Costs of DHF control in Thailand[58]

			US$
1.	**Cost due to morbidity (per patient)**		
	User cost : total patient cost	Child	113.0
		Adult	154.6
	Provider cost : hospitalization		44.0
	Total morbidity cost	***Child***	***157.0***
		Adult	***198.6***
2.	**Cost due to mortality (per patient)**		
	Funeral cost	Child	395.0
		Adult	648.0
	Potential income loss (50 working years)		120,000.0
	Total mortality cost	***Child***	***120,395.0***
		Adult	***120,648.0***
3.	**Cost for prevention and control in 1994**		
	Ministry of Public Health annual budget		1,868,968.0
	Bangkok Municipality Administration annual budget		112,000.0
	Ministry of Interior (75 provinces, est. 0.25 million/province)		2,891,400.0
	Total prevention and control cost		**4,872,368.0**

Note: Costs from providers do not include salaries, administration and supportive expenditures.

Once the costs of the components of individual dengue control projects have been determined, it will not only be possible to estimate total costs, but also to identify where savings may be gained through collaboration with other government agencies and the private sector. The cost data collected, along with the epidemiological and entomological data, provide an initial framework for conducting cost-effectiveness studies of the different interventions used in the national programme. New methods and improvements of existing methods can be more effectively evaluated for operational use when their economic benefits or limitations are fully understood. The benefits to dengue control programmes should be considered in the light of social and economic considerations as well as the health impact of epidemics.

11

The Regional Strategy for the Prevention and Control of DF/DHF

11.1 Basic Elements

The basic elements of the regional strategy[62] are to:

(1) Establish effective disease and vector surveillance systems based on reliable laboratory and health information systems.

(2) Undertake disease prevention through selective, stratified and integrated vector control with community and intersectoral participation.

(3) Establish emergency preparedness capacity to prevent and control outbreaks with appropriate contingency plans for vector control, hospitalization, education and adequate logistics.

(4) Ensure prompt case management of DHF/DSS, including early recognition of the signs and symptoms, to prevent case mortality.

(5) Strengthen capacity and promote training, health education, and research on surveillance, vector control, laboratory diagnosis and case management (see Box 23).

Strategy requirements

(1) Recognition of DF/DHF as an important health problem in endemic countries.

(2) Decison to include DF/DHF in the list of reportable diseases for all endemic countries.

(3) Long-term political commitment from governments and multisectoral involvement.

Box 23
Regional Strategy

- Effective disease and vector surveillance
- Selective and stratified integrated vector control through community participation
- Emergency preparedness and response
- Clinical diagnosis and prompt case-management
- Capacity building and promotion of training and research

(4) Sustainable national financial support to DF/DHF prevention and control programmes in the context of health care and overall development.
(5) Development of national plans of action with realistic and clear objectives and targets to reduce DHF mortality and dengue morbidity.
(6) Development of monitoring systems for disease activity and vector distribution and density, through improved surveillance which must include clinical, laboratory and entomological components.
(7) Support to health services to ensure early diagnosis and prompt treatment of DHF/DSS cases.
(8) Development of national capacity for undertaking selective and sustainable vector control and other preventive measures within the health and other sectors, as well as within the community.
(9) Development of national capacity to undertake research related to the vector and the epidemiology and laboratory diagnosis of the infection.

In order to ensure their sustainability, national strategies for the prevention and control of DF/DHF should be made a part of the existing infrastructure of infectious disease control programmes and should be based on larval source reduction. The community should actively participate in control activities, particularly in eliminating vector breeding sources.

11.2 National Dengue Control Programmes in South-East Asian Countries

In several countries of the Region where DHF is prevalent, national dengue control programmes have existed for several decades. In others, where DHF is a newly emergent disease, there are no existing control programmes.

In Indonesia, the national dengue control programme started in 1974 and gradually expanded to become an integral part of general health services in the context of primary health care. In Thailand, control measures were confined to high-risk areas until 1974, when a national campaign for dengue control was established. The vertically structured programme was later integrated into local health services with logistics supplied at the central level. Community-based vector control has now been developed in the country, focusing particularly on school children. In Myanmar, prevention and control measures have been focused mainly on source reduction measures by active community participation and intersectoral coordination with the education sector for prevention and control in primary schools. Local NGOs have also actively participated.

In the wake of the increasing incidence of DHF and its geographic spread, it is desirable to organize national dengue control programmes in each country within the framework outlined below with modifications as needed for the local situation.

11.3 Planning a Dengue Control Programme

A dengue control programme is aimed at reducing morbidity and mortality due to DHF. In the absence of a safe, effective and economic vaccine against DF/DHF, vector control is the only method available to prevent and control the disease.

Source reduction (elimination of *Ae. aegypti* larval habitats) through community participation is the most promising method for a sustainable, long-term control programme, and is the fundamental control strategy of DF/DHF. However, it is realized that full participation of communities will require considerable time, since it is based on behavioural change. Meanwhile, outbreaks of DHF accompanied by deaths continue to occur in many countries. Therefore, emergency preparedness plans to prevent and control DHF epidemics should also be developed, especially in high-risk areas. The planning of DF/DHF control programmes requires the collection and evaluation of basic epidemio-logical, entomological and other relevant information to determine which control measures should be combined in an integrated manner for the success of the programme. The desired goal must first be clearly defined. The information collected should be analysed for the formulation of a sound and feasible control strategy which will best meet the local conditions, needs and resources.

Preparatory phase

Various basic data to be collected include those on metereology, geography, epidemiology and entomology. Data are also required on the sociocultural characteristics of communities as well as on the feasibility and extent of community participation, intersectoral action, degree of awareness of the problem in the community, and the community's expectations from the proposed control programme. Such data can be collected through knowledge, attitude and practice (KAP) surveys and other formative research.

Planning phase

Based upon basic data and epidemiological information collected in target areas, a working plan should be prepared once feasibility is confirmed. The plan of action should be formulated in the light of administrative support from various departments and agencies. An intersectoral committee should be established to develop a collaborative plan and formulate policy, defining objectives, targets, budgeting, logistics, technical guidance, evaluation, training, intersectoral linkages, etc. The items listed below should be clearly mentioned in the plan of action.

Objectives

The main purpose and desired goals of the control programme should be defined.

Targets

The degree of desired outcomes of the programme with reference to a time line should be determined. Each country should set up its own targets according to its epidemiological situation, available manpower and financial resources.

Strategy

The intervention methods to achieve the goals and targets of the programme should be outlined. Area prioritization should be included in order to emphasize high-risk areas. Such prioritization of areas should be reviewed periodically to ensure that the limited resources are effectively allocated. Case management should be emphasized in all areas, but especially in those with high

Table 7. **Plan of action for the control of dengue fever/ dengue haemorrhagic fever vectors**

Control method	Agent	Activities	Ways and means of approach
Source reduction	Community	1. Removal/reduction of non-essential water containers receptive to mosquito breeding 2. Protection of water containers from larval breeding	1. Health education 2. Mass media (radio, TV, films) 3. School children/ housewives 4. Volunteers 5. PHC workers 6. Community leaders
	Government	1. Disposal of refuse 2. Provision of reliable piped water 3. Legislation 4. Monitoring and assessment	1. Set up core working committee for inter- and intrasectoral coordination
Larval control	Community	1. Larviciding 2. Release of larvivorous fish/copepods	Same as for source reduction
	Government	1. Supply of control materials (larvicides, copepods, fish) and equipment as needed	Same as for source reduction

Source: Bang and Tonn 1993 – Vector Control and Intervention Regional Publication: SEARO No.22 [51]

mortality. Control measures should be focused on source reduction by community participation. Decentralization down to local health services and intersectoral coordination should be introduced to the control programmes in order to sustain vector control activities.

Activity Plan

Details of activities to support the strategy, including responsible organizations/agencies and timeframe, should be defined (Table 7).

Logistic support

Routine requirements for DF/DHF control should be estimated and calculated based on the past experience of the country concerned. Additional requirements for unexpected or emergency requirements of supplies, equipment and insecticides should be planned for well in advance. The WHO Technical Advisory Committee has recommended the following items for treating a town covering a 20 sq km area during an emergency:

- technical malathion – 1000 litres (for two applications),
- one vehicle-mounted aerosol generator, and
- five mist blowers and ten swing fogs.

The total number of DHF/DSS cases should be estimated and essential supplies, equipment and beds needed for case management should be obtained. Disease surveillance and information systems should be established and strengthened for early detection of DHF/DSS, for early referral to hospitals, and for effective transmission control. Intensive care units in children's hospitals should develop contingency plans to accommodate an increased patient load, including diagnostic facilities, drugs and other requirements. Development of IEC materials should be considered a priority for allocation to high-risk areas.

Implementation phase

After basic information has been collected and working plans drawn up for each task, a plan should be developed for the efficient operation of the programme. The programme should then be formally inaugurated by the leaders of the concerned villages/towns with the participation of community leaders from different localities. These prominent citizens should appeal to the public to accept the programme in the interest of their families, and to extend their full cooperation for its success. The function should be widely publicized by the mass media.

In a community-based programme, it is important to give feedback to the community about the successes, failures, and benefits of the programme. Such feedback helps in retaining continued support from communities, and thus in sustaining the programme, as experienced in Singapore.

Monitoring and evaluation

The plan should include (a) periodic operational assessments to determine the progress of work and actual inputs received by the programme in terms of materials and manpower, and (b) periodic entomological assessments to determine the success or failure of the control measures applied to the vector population and/or epidemiological analysis. When the bulk of the work is being carried out through community participation, it is desirable to have a built-in mechanism to cross check the work.

12

Emergency Preperedness and Effective Response

12.1 Predictive Indicators

Despite ongoing control programmes in some countries of the Region, dengue epidemics are being reported with ever-increasing frequency and greater numbers of DHF cases. There is therefore an urgent need to establish an early-warning, predictive capability of epidemic transmission, and a rapid emergency response capability to contain outbreaks.

Prediction of impending epidemics

Identification of high-risk areas has been described in Chapter 2. The ability to predict an impending epidemic depends on the following factors.

Receptivity for dengue epidemic

Geographical reconnaissance of towns and cities should be carried out to identify and map all permanent foci of *Aedes* breeding. The maps should be updated each year before the rainy season.

Vulnerability of the area

All places where people congregate, which act as centres of transmission, should be identified and the pattern of both intra and inter-city movements of the human population should be determined.

Active surveillance

Active surveillance of suspected, probable and confirmed cases of DF/DHF should be maintained as described in Chapter 6. The location of cases by number and serotypes should be actively monitored. Other cities and countries in the region should also be monitored, with regular exchange of surveillance data among public health counterparts. Sentinel hospitals, clinics and physicians, and fever-alert surveillance systems should be implemented to provide current data on the location of virus serotypes and the severity of illness in the catchment areas. The objective is to detect, without delay, the introduction of a new strain or serotype of

dengue virus or to detect any unusual increase in the spread of dengue transmission (see section on Surveillance).

Routine monitoring of DF/DHF cases

The most effective method of predicting a DHF epidemic is the active monitoring of DF/DHF cases on a weekly basis in the commu-nity. In addition to seasonal transmission patterns and the number of reported cases, the disease severity, seropositivity rate, virus serotype and geographic clustering of cases can provide early warning information on which the prediction of epidemic activity can be based. This can be done at the health-centre level. Impending epidemics can be detected by comparing the DHF cases of a given month/fortnight/week with either those of the same period of the previous year (last month, fortnight, week) or with the average number of cases during that month over the last 3-5 years.

12.2 DF/DHF Epidemic Management

DF/DHF outbreaks can cause high morbidity and mortality in a short span of time, and may create panic among the people who expect urgent action from the government. At such times it becomes essential to have a rapid, emergency response plan and to have administrative flexibility at the central, provincial and local government levels[63]. Such a response requires the setting of priorities for the control of DF/DHF/DSS epidemics.

Administrative actions

Emergency Action Committee (EAC) and Rapid Action Team (RAT)

For contingency planning of DHF epidemic control, it is essential that a mechanism is embodied at national, state and local levels for creation of a multidisciplinary Emergency Action Committee (EAC) and a Rapid Action Team (RAT). The EAC is entrusted with all administrative actions and coordinates all activities aimed at emergency interventions. The RAT undertakes epidemiological investigations and control measures[62,63] (see Annex XIII).

Establishment of emergency control centres

Emergency control centres are under the administrative control of a technical manager appointed by the EAC. The control centre monitors the progress of the epidemic on a 24-hour basis throughout the emergency.

Declaration of dengue as a notifiable disease

DF/DHF should be made a notifiable disease to ensure that individual medical practitioners, clinics and hospitals report all suspected cases to the government. This facilitates the identification and appropriate management of cases in hospitals, and ensures that measures are taken to keep hospitals free of *Ae. aegypti*.

Reporting system

- Peripheral health units should report to the District/Municipal Health Officer.

- Confirmation of the epidemic is made by the Chief, MOD/DOH.
- Reports should be made to the Chief Administrator of the District/Municipal Corporation.
- Reports should be made to the provincial and national level.

Activities

Contingency plans require the following:

- Delimitation of epidemic areas.
- Mobilization of adequate human and financial resources, materials and equipment.
- Intersectoral meeting at district/city level to inform the local authorities of plans.
- Provision of information to communities through the mass media.
- Implementation of the action plan for control measures.

Case management

Adequate provision of hospital beds, diagnostic facilities, fluids, drugs, equipment and other requirements must be ensured.

Vector control

Vector control should be based on: (i) insecticide space spraying, (ii) application of larvicides, (iii) source reduction, and (iv) health education to ensure involvement of communities, school children and NGOs.

Intersectoral collaboration

This requires (i) political commitment and financing, (ii) constitution of intersectoral committees for joint activities, and (iii) maximizing the use of the mass media for health education and urgent implementation of plans by communities, school children and NGOs.

Role and functions of public information, media and community

Public information

Public information is vital to allay the fears of the community when an epidemic occurs. Public information, therefore, should be exhaustive and clear and should explain how the disease is caused, how it spreads, how it is controlled, the responsibility of the citizens of the community, and where to get treatment. Comprehensive communication guidelines on the treatment and control of dengue epidemics, including the "Do's and Don'ts" should be developed to inform the public. This includes information generated by other sources.

Role of the media

It is acknowledged that the media can play an important role in epidemic prevention and control. To be effective, the media should be given accurate information quickly and comprehensively. Such information should be provided only through an authorized media spokesperson of the Ministry of Health or via the municipal/district health officer. The Ministry of Health should provide addresses of authorized information outlets to ensure the reliability of information. It is important to provide consistent messages. Press releases are the recommended means of communication.

Community participation

The prevention and control of dengue epidemics cannot be achieved without the cooperation and involvement of the community. Health managers must understand the social and cultural beliefs of the population regarding dengue fever, including whether they understand the role of the mosquito in dengue transmission and the benefit they perceive from vector control. An intensive campaign to implement community participation should be initiated as part of the emergency response. In DF/DHF emergency epidemic control campaigns, members of the community are encouraged to undertake source reduction measures, such as emptying water containers, removing solid waste material including used tyres, preventing breeding in man-made breeding places (e.g. cisterns and wells), and undertaking personal protection methods (e.g. using mosquito nets and coils, etc.) to prevent mosquito bites.

For the success of any campaign, the community should understand the importance of DF/DHF and that sustainability is enhanced by linking the programme with existing well-organized programmes and by mobilizing societal forces and organizations, both within and outside the health sector, to initiate and maintain dengue control activities. Details about community actions during epidemics are included in Annex XIV.

Management of DF/DHF/DSS and laboratory services in hospitals during epidemics

Appointment of coordination committee

During an epidemic of DHF there will be a large number of patients with DF. As an epidemic becomes known to the public, large numbers of patients, both dengue and non-dengue, may overwhelm outpatient and inpatient facilities, rapidly exhausting the medical care staff. It is essential, therefore, to establish a coordination committee within the hospital to facilitate interdisciplinary and interagency communication.

Outpatient medical services – special DHF OPD

Since the prognosis of DHF depends on early diagnosis and proper management, and since during the early febrile phase DHF resembles DF and numerous other viral, bacterial and parasitic infections, patients with high fever and a positive tourniquet test should be suspected of having DHF. They should be tested for thrombocytopenia and plasma leakage which are constant findings in DHF. A CBC with platelet count and haematocrit should be done in the hospital outpatient department or clinic. Since only about one-third of DHF patients will develop shock and the critical period is reached about the time of defervesence, patients who are suspected to have DHF can stay at home during the febrile phase, with regular follow up every 24 hours to monitor whether there is significant leakage of plasma. Patients who live far away from hospitals or whose parents or relatives cannot be relied upon to observe clinical changes, should be kept for observation as outpatients. An observation unit of approximately 10-20 beds should be set up to accommodate these patients. Dengue fever and some mild cases of DHF can be treated at outpatient departments and clinics. This observation unit will help to avoid

overcrowding of hospital wards and ensure that persons who have DHF and genuinely require hospital care are admitted. It is essential that the observation unit is well staffed and that it has clinical laboratory capability (see below).

Inpatient services – special DHF treatment unit

A special DHF treatment unit should be established for providing care to DHF/DSS patients. Those in shock require intensive nursing and medical care, and the unit should be staffed with well-trained nurses. There should be about 20-30 beds with adequate equipment and supplies needed for taking care of DSS patients. Paramedical workers or parents can assist by giving oral fluid therapy or by monitoring the rate of intravenous administration and the general status of the patient.

Clinical laboratory support

Laboratory studies necessary for clinical diagnosis include total white blood count, platelet count and haematocrit determination. The ability to conduct these laboratory tests should be available at outpatient departments at all times. A microcentrifuge for haematocrit determination and a microscope for platelet estimation should be available at all institutions providing care to DHF patients.

Equipment and medications

A blood pressure manometer with different sizes of arm cuffs for children in different age groups is required for tourniquet testing and blood pressure measurements. It is estimated that about 20-30% of DHF patients will progress to shock, that about half of the grade I-II patients will require intravenous therapy with isotonic salt solution, and that about 10% of the patients may require blood transfusion. Based on these assumptions, the estimates in Box 24 can be made for materials needed.

Training

(a) Hospital staff, doctors and nurses should be trained (short course/seminar) to diagnose cases of DHF, to recognize shock, and to

Box 24
Estimated DF/DHF materials required in hospitals

100 cases of DHF	–	200-300 litres of normal saline or Ringers acetate solution.
30 cases with shock	–	30 litres of volume expander, e.g. Dextran 40 or plasma.
10 cases of DHF with significant haemorrhage	–	Approximately 10-20 units of fresh whole blood. Oral electrolyte solution as used in diarrhoea. Solutions for volume replacement: 5% dextrose in normal saline, 5% dextran in 1/2 normal saline. Ringer's lactate or acetate, plasma expander, Dextran 40.

provide proper management using WHO criteria and guidelines.

(b) Laboratory workers should be trained to do haematocrits, CBCs and platelet counts or estimation by examination of peripheral blood smears and coagulogramme. They should also be trained to collect blood specimens for serological diagnosis and/or virus isolation.

Prevention of death

Prevention of death can be achieved by early diagnosis, hospital admission, good nursing care and proper case management. Since only about one-third of DHF cases develop shock, the parents or attendants of patients should be given thorough instructions for taking care of the patient at home during the febrile phase and to recognize the early warning signs of shock.

Management of dengue haemorrhagic fever

The major pathophysiologic hallmarks that distinguish DHF from DF and other diseases are abnormal haemostasis and increased vascular permeability that leads to leakage of plasma. The clinical features of DHF are rather stereotypical, with the acute onset of high fever, haemorrhagic diathesis (most frequently on the skin), and circulatory disturbance (in the most severe form as dengue shock syndrome). Hepatomegaly is usually present, but not always. Thrombocytopenia and concurrent haemoconcentration, which represent abnormal haemostasis and plasma leakage respectively, are constant findings. It is thus possible to make an early and accurate clinical diagnosis of DHF before the critical stage of shock occurs.

The management of DHF is entirely supportive and symptomatic and is directed towards the replacement of plasma losses. Survival depends on early clinical recognition and frequent monitoring of patients for plasma leakage. Early volume replacement when the haematocrit rises can prevent shock and/or modify disease severity. In shock cases, satisfactory results have been obtained with the regimen described in Box 25.

At the Children's Hospital in Thailand,[64] where a large number of DHF cases are treated every year, this regimen (without using steroids or vasopressors) has resulted in a steady decline in the case fatality rate of shock cases, from about 5% in 1971 to 2% in 1984 and to 0.2% in 1991. The results of studies

Box 25
Recommended regimen for shock cases of DHF

- Immediately and rapidly replace the plasma loss with isotonic salt solution and plasma or plasma expander (in cases of profound shock).
- Continue to replace further plasma losses to maintain effective circulation for a period of 24-48 hours.
- Correct metabolic and electrolyte disturbances (metabolic acidosis, hyponatraemia, hypoglycaemia or hypocalcaemia).
- Give blood transfusion in cases with significant bleeding.

from various places on the use of corticosteroids in treating DSS showed no benefit, either in reducing the fatality rate, or reducing the volume or duration of fluid therapy.

Vector control for containment of epidemics

Development of emergency vector control programmes

DF/DHF outbreaks often evolve quickly, requiring emergency actions to immediately control infected mosquitoes in order to interrupt or reduce transmission and to reduce or eliminate the breeding sites of *Ae. aegypti*. In order to meet such emergencies, it is essential that persons at all levels, including individuals, the family, the community and the government, contribute to preventing the spread of the epidemic. In the following sections, an attempt is made to highlight emergency actions that can be taken to prevent or contain an incipient epidemic.

Self-reliance actions for vector control and personal protection

At household level

- Kill adult mosquitoes by making use of commercially-available safe aerosols (pyrethroid-based). Spray bedrooms including closets, bathrooms and kitchens for a few seconds and close the rooms for 15-20 minutes. The timing of spray should coincide with the peak biting times of early morning or late afternoon.
- Intensify efforts to reduce actual or potential larva habitats.
- Cover water containers in the house to prevent fresh egg-laying.
- Have infants sleep under bed nets during the day.
- Wear protective clothing, preferably sprayed with a repellent.
- Use commonly-available repellents during the day time and also make liberal use of mats and coils, etc. during night and day (including all family members – whether they stay at home or go to work).

At school level

School children should be provided with health education on all aspects of dengue fever, i.e. what it is, how it spreads, the role of mosquitoes, how they breed, and how they can be controlled. Following health education, school children should be trained on how to detect and eliminate the breeding of *Ae. aegypti* in and around schools, in their homes and in the neighbourhood.

At community level

At the community level, people should form groups to supplement and reinforce efforts at the household level. Such groups can identify commercial activities such as traders dealing in used tyres, which may be contributing larval habitats for the vector. They can create awareness about dengue and seek cooperation for the removal of breeding places (see also Annex XIV).

Action by local health authorities

For the control of epidemics, chemical control of the adult mosquito vector is considered an

important strategy in an attempt to interrupt or reduce transmission. It should be emphasized, however, that rapid and effective source reduction will achieve the same results. Moreover, larval control is more economical and provides sustainable control by eliminating the source of newly-emergent adult mosquitoes. Under most conditions, chemical space sprays are not effective and it is rare that an epidemic will be controlled using these methods. Because of their visibility, however, people think the government is doing something. This often creates a false sense of security and prevents the implementation of the community as well as the individual efforts outlined above[53].

There are two main methods of space spraying for adult mosquito control: (i) cold aerosol ULV, and (ii) thermal fogging. The guidelines for space spraying with adulticides, and equipment which has been experimentally shown to be effective in the control of caged adult mosquitoes, are included in Annexes VII and VIII.

12.3 Post-DHF Epidemic Management

The evaluation of prevention and control measures implemented during an epidemic are an important learning tool to improve effectiveness during subsequent epidemics. A retrospective study of an outbreak provides essential material for case studies as well as for teaching purposes.

Retrospective study of epidemics – lessons learned

A retrospective study should cover all aspects of hospital care and case management, any variation in clinical signs and symptoms from the known management successes, and all administrative aspects relative to the adequacy of hospital management to meet such emergencies. The evaluation study should cover all aspects of the agent-host-vector interaction and all morbidity and mortality data including prevalence of the infection by age, sex, occupation and sociocultural factors which may have promoted outbreaks, vector prevalence, types of containers promoting breeding, evaluation of all *Ae.aegypti* control measures, factors related to cost-effectiveness and sustainability, degree of community participation, degree of governmental preparedness to respond to and control such epidemics, and all other factors which will enhance the future capabilities of all those involved in epidemic control.

13

WHO Support Activities

The WHO Regional Office for South-East Asia (SEARO) is committed to:

- Together with Member States, continue to support implementation of the regional strategy for the prevention and control of DF/DHF.
- Cooperate with Member States to coordinate and strengthen surveillance activities on a regular basis, in order to analyse trends, provide feedback to Member States, and exchange information.
- Lay special emphasis to support countries' efforts in selective, effective, stratified and integrated vector control with community and intersectoral participation.
- The establishment of emergency preparedness capacity to control dengue epidemics and development of contingency plans for vector control, including timely hospitalization of DHF cases, education and adequate logistics.
- Provide a Regional Rapid Response Team to cooperate with Member States in the emergency management of DHF as requested or required by countries.
- Provide continued support to the development of training modules, guidelines and other training/educational materials for case management of DF/DHF/DSS including audio/video materials on the prevention and control of DF/DHF/DSS.
- Facilitate the organization of workshops and seminars on vector control, laboratory diagnosis, production of diagnostic reagents, and clinical case management of DF/DHF/DSS.
- Together with WHO collaborating centres and national reference laboratories, support the establishment of a surveillance network in the Region.
- Continue to support basic research to understand the epidemiological complexities of the disease and operational research to develop cost-effective control strategies.
- Make an inventory of dengue viruses isolated from DF/DHF cases in each country and coordinate genotyping studies at WHO collaborating centres.
- Continue to support the development and field testing of live attenuated tetravalent dengue vaccine at the Mahidol University, Bangkok, Thailand, and future implementation of this vaccine for mass vaccination in dengue-endemic countries in the South-East Asia Region.

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Annex I

List of National Programmes and WHO Collaborating Centres

Institutions

Bangladesh

- Malaria and Other Parasitic Control,
  Directorate-General of Health Services,
  Mohakhali, Dhaka,
  Bangladesh
  Tel. (8802)606326 Fax (8802)863247
- Institute of Epidemiology, Disease Control and Research,
  Directorate-General of Health Services,
  Dhaka, Bangladesh

Bhutan

- National Malaria Control Programme,
  Gaylegphu, Bhutan
  Tel. (975)-3-51115

India

- Director, National Malaria Eradication Programme
  22 Sham Nath Marg,
  Delhi 110052
  Tel. (91-11)2918576, 2927108
  Fax 2518329
- Director, National Institute of Communicable Diseases
  22 Sham Nath Marg,
  Delhi 110052
  Tel. (91-11)2913148

Indonesia

- Directorate of Vector Borne Disease Control
  Directorate General of Communicable Disease Control
  Ministry of Health and Environmental Health, Jl. Percetakan Negara No.29
  Jakarta, Indonesia
  Tel. 4247573
  Fax 62-21 424 7573

Maldives

- Programme Manager
  Department of Public Health, Male
  Republic of Maldives
  Tel. 322488 Fax 314653

Myanmar

- Vector Borne Disease Control
  Division of Control of Communicable Diseases, Department of Health
  Ministry of Health,
  Yangon, Myanmar

Nepal

- Epidemiology and Disease Control Division,
  Department of Health Services,
  Teku, Panchali,
  Kathmandu
  Tel. 227268

Sri Lanka

- Epidemiology Unit, Department of Health, Ministry of Health
  Colombo
  Sri Lanka
  Tel. (00-94-1)501110
- Director, Anti Malaria Campaign
  P.O. Box 1472, Colombo 5
  Sri Lanka
  Tel. (00-94-1)581918
- Medical Research Institute
  Colombo 8
  Sri Lanka
  Tel. (00-94-1)693532, Fax 691495

Thailand

- Division of General Communicable Disease Control, Department of Communicable Disease Control
  Ministry of Public Health
  Soi Bamrajnaradul, Tivanond Road
  Nonthaburi 11000
  Thailand
  Tel. (66-2)9659182, 5903160-1
  Fax (66-2)5918432

WHO Collaborating Centres

SEARO

India

- National Institute of Virology (NIV)
  20-A Dr Ambedkar Road
  P.O. Box 11, 411001
  Poona
  Fax (+91-212) 622669
- Vector Control Research Centre (VCRC)
  Pondicherry
  Tel. (91-413)72784, 72396
  Fax (91-413)72422, 72041

Indonesia

- Vector Control Research Station
  Health Ecology Research Centre
  National Institute for Health Research and Development
  Salatiga, Semarang
  Indonesia
  Tel. (0298)27096, Fax (0298)22604
- U.S. Naval Medical Research Unit No.2
  NAMRU-2 Laboratory, Kotak Pos 226
  Jakarta Pusat 10570
  Fax (+62)21 4244507

Thailand

- Queen Sirikit's National Institute of Child Health, 420/8 Rajvithi Road
  Bangkok, 10400
  Thailand
  Fax (+66-2) 2457580

WPRO

Australia

- Queensland University of Technology
  2 George Street, GPO Box 2434
  Brisbane Queensland 4001
  Fax (+61)7 8641534

Japan

- Institute of Tropical Medicine
  Department of Virology
  Nagasaki University
  12-4 Sakamoto-Machi
  852 Nagasaki
  Fax (+81)958 476607

Malaysia

- Department of Medical Microbiology
  University of Malaya,
  59100 Kuala Lumpur
  Fax (+60)3 7557740

Australia

- University of Western Australia
  Queen Elizabeth II Medical Centre
  Nedlands
  Western Australia 6090
  Fax (+61) 7 33654620

Other Regions

Brazil

- Instituto Evandro Chagas
  c/o Fundacao SESP
  Caixo Postal 1530
  Belem
  Fax (+55) 91 2661284

Canada

- Laboratory Center for
  Disease Control
  Health Protection Branch
  Tunney's Pasture
  Ottawa, Ontario, K1A 0L2
  Fax (+1)613 9540207

Finland

- Department of Virology
  Haartman Institute
  University of Helsinki
  P.O. Box 21
  Helsinki
  Fax (+358)0 94346491

France

- Centre National de
  Reference pour les
  Fievres Hemorragiques et les
  Arbovirus, Institut Pasteur
  25 rue du Dr Roux
  75724 Paris
  Cedex 15
  Fax (+33)1 40613151

Italy

- Laboratory of Virology
  Arbovirus Unit
  Istituto Superiore de Sanita
  299 Viale Regina Elena
  00161 Rome
  Fax (+39)6 49902082

Netherlands

- Department of Virology
  Erasmus University Rotterdam
  P.O. Box 1738
  3000 DR Rotterdam
  Fax (+31)10 4365145

Russian Federation

- Ivanovsky Institute of Virology
  Department of Arboviruses
  16 Gamaleya Street
  123098 Moscow
  Fax (+7)095 1907485

United Kingdom

- Division of Pathology
  Public Health Laboratory Service
  Centre for Applied Microbiology and
  Research
  Porton Down, Salisbury
  Wiltshire SP4 0JG
  England
  Fax (+44)1980 612731

USA

- Division of Vector-borne Infectious
  Diseases
  Centers for Disease Control and
  Prevention
  P.O. Box 2087
  Fort Collins
  CO 80522
  Fax (+1)303 2216428

- Department of Epidemiology and Public Health
  Yale University School of Medicine
  60 College Street
  P.O. Box 208034
  New Haven, CT 06520-8034
  Fax (+1)203 7854782
- Special Pathogens Branch
  Division of Viral and Rickettsial Diseases
  National Center for Infectious Diseases
  Centers for Disease Control and Prevention
  1600 Clifton Road NE
  Atlanta, GA 30333
  Fax (+1)404 6391118
- Center for Tropical Diseases
  University of Texas Medical Branch, Galveston
  TX 77555-0609
  Fax (+1)409 7472429

Annex II

Arbovirus Laboratory Request Form

Name of patient _____ Hospital No. _____
Address _____ Hospital _____
_____ Age _____ Sex _____ Physician _____
Date of admission _____ Admission complaint _____
Date of onset _____

Clinical findings: 1. Fever _____ ^{0}C or ^{0}F (max). Duration _____ days
2. Tourniquet test _____ Petechiae _____ Epistaxis _____
Haematemesis/melaena _____ Other bleeding (describe) _____
3. Hepatomegaly _____ (cm at right costal margin). Tenderness _____
4. Shock _____ blood pressure _____ (mmHg) Pulse _____ (per min.)
Restlessness/Lethargy _____ Coldness of extremities/body _____

Clinical laboratory findings:

Platelets (X103) _____/mm3 (on _____ day of illness).
Haematocrit (%) _____ (max) _____ (min)

Blood specimens

(Acute) Hospital admission Date_____	Hospital discharge Date_____	Convalescent Date_____
○	○	○

Instructions: Fill the form completely with all clinical findings in duplicate. Saturate the filter-paper discs completely so that the reverse side is saturated and clip them to the form. Obtain admission and discharge specimens from all patients. If the patient does not return for a convalescent sample, mail promptly.

Source: Dengue Haemorrhagic Fever: Diagnosis, treatment, prevention and control, second edition, WHO, Geneva, 1995.

Annex III

Relative Sensitivity and Interpretation of Serological Tests

(1) Haemagglutination Inhibition (HI)

- Ideal for seroepidemiology; sensitive; easy to perform; minimal equipment; reliable; best for most flaviviruses, well-standardized.
- Remove non-specific inhibitors and agglutinins from serum; lack of specificity of serotypes; usually paired serum samples.

(2) Complement Fixation (CF)

- More specific
- More difficult, longer, less widely used; requires highly-trained personnel.

Interpretation of Dengue Haemagglutination-Inhibition Antibody Response[a]

Antibody response	Convalescent S1-S2 interval[b]	Convalescent Titre[c] < < 1	Convalescent titre[c]	Interpretation
>4-fold rise	≥7 days		≤1:1280	Acute flavivirus infection, primary
≥4-fold rise	Any specimen		≥1:2560	Acute flavivirus infection, secondary
>4-fold rise	≥7 days		≤1:1280	Acute flavivirus infection, either primary or secondary
No change	Any Specimen		≥1:2560	Recent flavivirus infection, secondary
No change	≥7 days		≤1:1280	Not dengue
No change	≤7 days		≤1:1280	Uninterpretable
Unknown	Single specimen		≤1:1280	Uninterpretable

Clarke OH, Casals J. -American Journal of Tropical Medicine and Hygiene, 1958, 7:561-573.

(a) These criteria were derived empirically from data accumulated at the US Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. Individual laboratories should assess the sensitivity of their assay with standard sera from WHO collaborating centres. Laboratories should also establish baseline data for the population they serve during a period of little or no flavivirus transmission. Test results should be transformed to reduce variance. Results that are two standard deviations greater than the geometric mean may be presumed to indicate recent dengue infection.

(b) Interval in days between acute (S1) and convalescent (S2) specimens.

(c) Against any dengue antigen.

(3) Neutralization Test (NT)

- Most specific and sensitive; mostly used PRNT
- Detects past infection
- Expensive and time-consuming

(4) IgM-capture (Mac-ELISA)

- New, simple, RAPID, IgM, only one sample needed, screening many samples.
- Less sensitive than HI

(5) IgG-EIA (Indirect IgG ELISA)

- Insensitive

(6) Dot Blot Immunoassay

- Reagents and test procedures are evolving
- Needs standardization

Interpretation of MAC-ELIZA Results[a]

IgM antibody response	S1-S2 interval[b]	IgM to IgG ratio	Interpretation[c,d]
Increase in molar fraction	2-14 days	High	Acute flavivirus infection, primary
		Low	Acute flavivirus infection, secondary
Elevated, no change or decrease in molar fraction	2-14 days	High	Recent flavivirus infection, primary
		Low	Recent flavivirus infection, secondary
Elevated	Single specimen	High	Recent flavivirus infection, primary
		Low	Recent flavivirus infection, secondary

(a) These criteria were derived empirically from the data accumulated at the US Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. Individual laboratories should assess the sensitivity of their assay with standard sera from WHO collaborating centres. Test results should be transformed to reduce variance. Results that are two standard deviations greater than the geometric mean may be presumed to indicate elevated levels of anti-dengue IgM or IgG.

(b) Guidelines do not apply to intervals between acute (S1) and convalescent (S2) specimens greater than 14 days.

(c) In order to infer whether the dengue virus elicited anti-flavivirus IgM, laboratories must test with a regionally appropriate panel of flavivirus antigens. Laboratories must also determine appropriate criteria for categorizing primary and secondary sero responses.

(d) Sera for standardization of the assay are available from the Chief, Department of Virology, US Armed Forces Research Institute of Medical Sciences, 315/6 Rajvithi Road, Bangkok 10400, Thailand (fax 66-2-247-6030).

Source: Management of Dengue Epidemic, WHO/SEARO, May, 1997

Annex IV

Sample Size in *Aedes* Larval Surveys

For *Aedes* larval surveys, the number of houses to be inspected in each locality depends on the level of precision required, level of infestation, and available resources. Although increasing the number of houses inspected leads to greater precision, it is usually impractical to inspect a large percentage of houses because of limited human resources.

Table 1. **Number of houses that should be inspected to detect *Aedes* larval infestation**

Number of houses in the locality	True house index		
	>1%	>2%	>5%
100	95	78	45
200	155	105	51
300	189	117	54
400	211	124	55
500	225	129	56
1,000	258	138	57
2,000	277	143	58
5,000	290	147	59
10,000	294	148	59
Infinite	299	149	59

Table 1 shows the number of houses that should be inspected to detect the presence or absence of infestation. For example, in a locality with 5,000 houses, in order to detect an infestation of >1%, it is necessary to inspect at least 290 houses. There is still a 5% chance of not finding any positive houses when the true house index = 1%.

Table 2 shows the number of houses that should be inspected in a large (>5 000 houses) positive locality, as determined by the expected house index and the degree of precision desired. For example, if the preliminary sampling has indicated that the

Table 2. **Precision of the *Aedes* house index in large localities (>5,000 houses)**

House index (%)	95% confidence interval of the house index			
	Number of houses inspected			
	100	200	300	1,000
2	0.2-7.0	0.5-5.0	0.7-4.3	1.2-3.1
5	2-11	2-9	3-8	4-7
10	5-18	6-14	7-14	8-12
20	13-29	16-26	16-25	18-23
50	40-60	43-57	44-56	47-53
70	60-79	62-76	64-75	67-73

expected house index is approximately 10%, and a 95% confidence interval of 8%-12% is desired, then 1,000 houses should be inspected. If there are only sufficient resources to inspect 200 houses, the 95% confidence limits will be 6%-14%. In other words, there is a 5% chance that the true house index is less than 6% or greater than 14%.

In small localities, the same precision may be obtained by inspecting fewer houses. For example, if the expected house index is 50% and a 95% confidence interval of 44%-56% is acceptable, then in a large locality it would be necessary to inspect 300 houses (Table 2). However, as seen in Table 3, if the locality consists of only 1,000 houses, the same precision will be obtained by inspecting 231 houses.

Table 3. **Number of houses to inspect in small localities**

Total number of houses in the locality	Number of houses to be inspected for desired precision if this were a large locality (from Table 2)			
	100	200	300	1,000
50	33	40	50	50
100	50	66	75	100
200	67	100	120	170
300	77	122	150	230
400	80	134	171	290
500	83	142	189	330
1,000	91	166	231	500
5,000	100	200	285	830
10,000	100	200	300	910
20,000	100	200	300	950
30,000	100	200	300	1,000
40,000	100	200	300	1,000
100,000		200	300	1,000

Source: Scientific Publication No.548, PAHO, 1994

Annex V

Pictorial Key to *Aedes (Stegomyia)* Mosquitoes in Domestic Containers in South-East Asia*

Adults

Postspiracular setae absent

Lower mesepimeral setae present

Culex quinquefasciatus Say

Lower mesepimeral setae absent

Polynesian feral species

Postspiracular setae present

Erect forked scales numerous, not restricted to occiput

Polynesian feral species

Erect forked scales not numerous, restricted to occiput

Scutum with lyre-shaped white markings

Aedes aegypti

Scutum with a long median longitudinal white stripe extending from anterior margin to about level of wing root

Aedes albopictus

* Adapted from Yiau-Min Huang. The mosquitoes of Polynesia with a pictorial key to some species associated with filariasis and/or dengue fever. Mosquito Systematics, 1977, 9: 289-322.

Annex V (contd.)

Larvae

Siphon with 4 pairs of subventral tufts

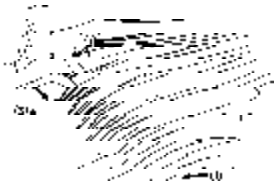
(1) Mental plate with at least 10 teeth on each side of median tooth

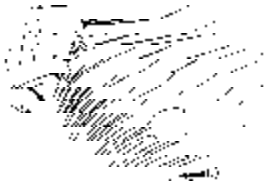
(2) Seta 1-C very slender, filamentous distally, usually very lightly pigmented

Culex quinquefasciatus Say

Siphon with a single pair of subventral tufts

(1) Ventral brush with 4 or 5 pairs of setae and (2) no precratal tufts





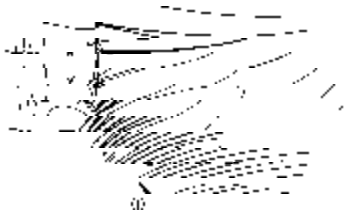
(1) Comb in a patch of several rows of scales, those of distal row elongate and varied in development

Polynesian feral *Aedes* species

(1) Comb in a single row

Annex V (contd.)

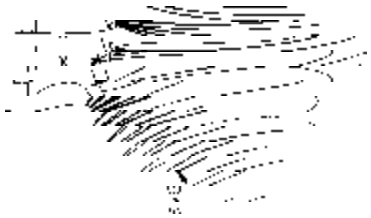
(1) Ventral brush with 5 pairs of setae



(2) Comb scale with very strong denticles at base of apical spine

Aedes aegypti (Linnaeus)

(1) Ventral brush with 4 pairs of setae



Saddle complete

Saddle incomplete

Polynesian feral *Aedes* spp.

Seta 4a, b-X single

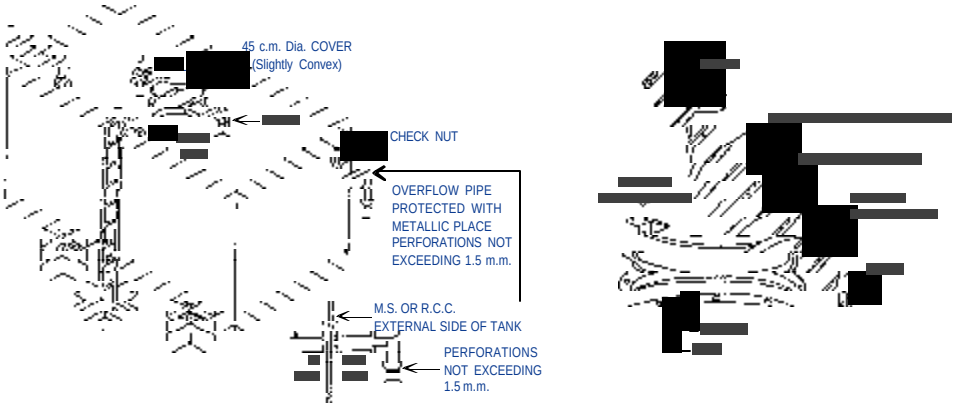
Aedes albopictus (Skuse)

Seta 4a, b-X branched

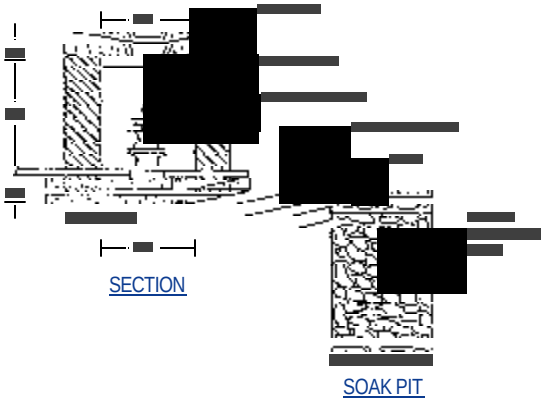
Polynesian feral *Aedes* species

Annex VI

Standard Design for Mosquito Proofing of Overhead Tanks and Cisterns



Standard Design for Overhead Tank with Cover Design for Mosquito Proofing of Overhead Tank/Wells/Cisterns



Design for Masonry Chamber and Soak Pit for Sluice Valve and Water Meter

Source: R.S.Sharma, G.K.Sharma and G.P.S.Dhillon, Epidemiology and control of malaria in India - 1996. Dte. of NMEP, 22 Sham Nath Marg, Delhi 110 054, India

Annex VII

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 area to be treated

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

Area of one end = 107 x 150
= 16,050 cm^2
Area of one side = 200 x 150
= 30,000 cm^2
Area of top = 107 x 200
= 21,400 cm^2

The sides and ends need to be multiplied by 2:

2(16,050 + 30,000) = 92,100 + 21,400
(end) (side) (top)
= 113,500 cm^2
If 10,000 cm^2 = 1m^2 then
113,500/10,000 = 11.35 m^2 area of net

(b) Determine how much insecticide is needed

Assume that 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 the net size by the dosage:

11.35 x 0.5 = 5.67 grams of insecticide needed.

(c) Determine the amount of liquid required to saturate a net

In order to determine the percentage solution to be used for 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 the net to the solution till it is thoroughly wet and then remove it. Allow the drips to fall into a bucket for 15 to 30 seconds. Set the net aside. Repeat the process with two other nets. Cotton nets can be lightly squeezed but not the synthetic ones. Measure the water or solution remaining in the 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 percentage

concentration required for dipping is calculated as follows:

$$\frac{\text{grams required per net}}{\text{ml solution retained per net}} = \frac{5.67}{280} = 2\%$$

(d) Preparation of dipping solutions to treat bulk quantities of mosquito nets or curtains

The general formula is:

X = (A/B) - 1

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

A = concentration of the emulsifiable concentrate (%)

B = required concentration of the final solution (%)

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

X = (25/2.0) - 1

= 12.5 - 1 = 11.5

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

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

X = (50/2) - 1 = 24

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

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

X = (25/.3) - 1

= 83.3 - 1 = 82.3

or 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 dipping cotton mosquito nets or curtains is to be prepared from a 50% concentrate.

X = (50/.3) - 1

= 166.6 - 1 = 165.6

or rounded to 166.

Therefore, 166 parts of water to 1 part of concentrate are required, or one litre of concentrate to 166 litres of water, or one-half litres of concentrate to 83 litres of water to accommodate a smaller container.

(e) Preparation of 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 a container (with premeasured marks to indicate volume)

Add 1 litre (1 bottle) concentrate to the container
Total volume : 12.5 litres
Grams permethrin : 250
% concentration : 2%

For 50% concentrate:

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

(f) Preparation of a 0.3% dipping solution using a one-litre bottle of 25% or 50% permethrin emulsifiable concentrate for soaking cotton nets or curtains

For 25% concentrate:

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

For 50% concentrate:

Add 166 litre of water to a container
Add 1 litre (1 bottle) concentrate to the container
Total volume : 167 litres
Grams permethrin : 500
% concentration : 0.3%

(g) Drying of nets

Polyethylene and synthetic nets are dried in a horizontal position. Do not hang to dry. Drying the nets on mats removed from houses has proved to be convenient and acceptable. The nets should be turned over about once every hour for up to three or four hours. If the weather is good, the nets can be dried outside in the sun but for 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 finish drying. 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 (soaking)

As shown in (a) above, if it is assumed that the net size is 11.35 m^2, 5.67 grams of 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:

grams required x 100
= 5.67 x 100
= 22.68 ml
rounded to 23 ml
% concentrated used: 25

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

The 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 metre of permethrin on polyethylene, polyester, nylon or other type of synthetic fiber net or curtain. The residual effect lasts for six 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 with 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 gram per square metre) 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 can easily learn the technique 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 litre of solution can treat four to five 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 the nets outside in open air. With one dipping station, about 150 nets or curtains can be treated in two hours or less.

(3) About 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 so as to reduce the vector density. When used in this 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.

Source: WHO/Western Pacific Region. Background Document No.16, 1995

Annex VIII

Quantities of 1% Temephos (Abate) Sand Granules Required to Treat Different-size Water Containers to Kill Mosquito Larvae

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

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.

Source: WHO/Western Pacific Region Background Document No. 16, 1995

Annex IX

Procedure, Timing and Frequency of Thermal Fogging and ULV Space Spray Operations

Basic steps

The steps listed below are to be followed in carrying out the space spraying of a designated area:

- The street maps of the area to be sprayed must be studied carefully before the spraying operation begins.
- The area covered should be at least 300 metres within the radius of the house where the dengue case was located.
- Residents should be warned before the operation so that food is covered, fires extinguished, and pets are moved out together with the occupants.
- Ensure proper traffic control when conducting outdoor thermal fogging since it can pose a traffic hazard to motorists and pedestrians.
- 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

- Doors and windows of houses and buildings in the area to be sprayed should be opened.
- 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 stationery.
- When possible, spraying should be carried out along streets that are at right angles to the wind direction. Spraying should commence on the downwind side of the target area and progressively move upwind.
- In areas where streets run parallel as well as perpendicular to the wind direction, spraying is only done when the vehicle travels upwind on the road parallel to the wind direction.
- 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 be driven close to the edge of the road.
- In areas where the roads are narrow, and houses are close to the roadside, the spray head should be pointed directly towards the back of the vehicle.
- In dead-end roads, the spraying is done only when the vehicle is coming out of the dead-end, not while going in.

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

Portable thermal fogging

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

Basic points

- Each spray squad consists of four spraymen and one supervisor.
- Each sprayman sprays for 15-30 minutes and then is relieved by the next sprayman. For reasons of safety, he must not spray when tired.
- The supervisor must keep each sprayman in his sight during actual spraying in case he falls or needs help for any reason.
- Do not directly spray humans, birds or animals that are in front of spray nozzles and less than five metres away.
- 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.

House spraying technique

- Do not enter the house. House spraying means spraying in the vicinity of the house.
- Stand 3-5 metres in front of the house and spray for 10 to 15 seconds, directing the nozzle towards all open doors, windows and eaves. If appropriate, turn away from the house and, standing in the same place, spray the surrounding vegetation for 10 to 15 seconds.
- If it is not possible to stand three metres from the house due to the closeness of houses and lack of space, the spray nozzle should be directed towards house openings, narrow spaces and upwards.

- While walking from house to house, hold the nozzle upwards so that particles can drift through the area. Do not point the nozzle towards the ground.
- Spray particles drift through the area and into houses to kill mosquitoes which become irritated and fly into the particles. The settled deposits can be residual for several days to kill mosquitoes resting inside houses and on vegetation not exposed to the rain.
- This technique permits treatment of a house with an insecticide ranging from 1 to 25 grams in one minute. The dosage depends on the 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 two grams per square metre to 150 square metres of sprayable surface.

Information to be given to inhabitants

- Time of spraying, for example 0630 to 1000 hours.
- All doors and windows should be open.
- Dishes, food, fish tanks, and bird cages should be covered.
- Stay away from open doors and windows during spraying, or temporarily leave the house and/or the sprayed area until the spraying is completed.
- Children or adults should not follow the 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:

For optimum spraying conditions, please note

- 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* mosquitoes are most active at these hours.

	Most favourable conditions	Average conditions	Unfavourable conditions
Time	Early morning (0630-0830 hrs) or late evening	Early to mid-morning or late afternoon, early evening	Mid-morning to mid-afternoon
Wind	Steady, between 3-13 km/hr	0-3 km/hr	Medium to strong, over 13 km/hr
Rain	No rain	Light showers	Heavy rain
Temperature	Cool	Mild	Hot

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 mosquitoes are flying or resting, thus rendering the spray ineffective.

- An optimum wind speed of between 3 and 13 hm/hr enables the spray to move slowly and steadily over the ground, allowing for maximum exposure of mosquitoes 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.
- In heavy rain, the spray generated loses its consistency and effectiveness. When the 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.
- Spraying is permissible during light showers. Also, mosquito activity increases when the relative humidity reaches 90, especially during light showers.

Frequency of application

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

- 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.
- At least one treatment should be carried out within each breeding cycle of the mosquitoes (seven to ten days for *Aedes*). Therefore, a repeat spraying is carried out within seven to ten days after the first spraying. Also, the extrinsic incubation period of dengue virus in the mosquito is 8 to 10 days.

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. However, 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 should also be accompanied by a reduction in hospitalized cases after the incubation period of the disease in humans (about 5 - 7 days) has elapsed. The spraying should be repeated at seven day intervals to eliminate the possibility of infected mosquitoes.

Source: WHO/Western Pacific Region. Background Document No.16, 1995

Annex X

Guidelines For Chemical Space Spraying

Aedes aegypti is the main vector of DF/DHF and has been responsible for all urban epidemics of this disease. *Ae. albopictus* is also involved in dengue transmission, mainly in the South-East Asia and the Western Pacific Regions. *Ae. aegypti* has a close association with man and it is a highly domestic species, with more than 90% resting on non-sprayable surfaces in houses. Indoor residual treatment of houses is therefore not generally recommended. Chemical control using insecticides generally has very little impact for long-term control of DF/DHF. Thus use of insecticides should be discouraged for long-term prevention and control. However, experiments on the control of *Ae. aegypti* in several countries in the Region has shown that thorough treatment at an interval of 1-2 weeks with portable fogging applicators together with truck-mounted applicators yielded control of *Ae. aegypti*. Space spraying with insecticides should be considered an epidemic contingency measure. Total coverage should be targeted for, however attention should be focused inside houses and in places where high vector densities have been recorded. Space spraying should be implemented in a compact community and should be within a radius of 400-500 metres of the affected houses.

Suitable insecticides for thermal and cold aerosols are indicated in the table below.

Insecticides suitable as cold aerosol sprays and for thermal fogs for mosquito control

Insecticide	Chemical[a]	Dosage of ai.[b] (g/ha)		Toxicity: oral LD_{50} of ai.[b] for rats(mg/kg body weight)
		Cold	Thermal	
Chlorpyrifos	OP	10-40	150-200P	135
Cyfluthrin	PY	1-2	-	500
Cypermethrin	PY	1-3	-	7180
Cyphenothrin	PY	2-5	-	2250-2640
Deltamethrin	PY	0.5-1.0	-	>2940[c,d]
D-phenothrin	PY	5-10	-	>10,000
Etofenprox	PY	10-20	10-20	>40,000
Fenitrothion	OP	250-300	270-300	503
Fenthion	OP	150	-	330[d]
Malathion	OP	112-693	500-600	>4000
Naled	OP	56-280	-	430
Permethrin[e]	PY	5-10	-	>4000[c,d]
Pirimiphos-Methyl	OP	230-330	180-200	2018
Propoxur	C	100	-	95
Zeta-Cypermethrin	PY	1-3	-	86

[a] PY = Synthetic pyrethroid, OP = organophosphorus, and C = Carbamate

[b] ai. = active ingredient

[c] Because of their low dermal toxicity and on the basis of experience with their use, these products have been classified as Class III in the WHO Hazard Classification, Table 5 (WHO/PCS/94.2).

[d] Dermal toxicity

[e] Also used in mixtures with knock-down agents or synergists

Source: WHO (1997), WHO/CTD/WHOPES/97.2

Annex XI

Safety Measures For Insecticide Use

Safety measures for 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 exposure to insecticides, prevent accidental poisoning, monitor sub-acute poisoning, and provide adequate treatment for acute poisoning. These measures can be broken down into the four broad categories listed in the box below.

Four Issues for Safety Measures

- the choice of insecticides to be used;
- the safe use of insecticides;
- the monitoring of sub-acute insecticide poisoning, and
- the treatment of insecticide poisoning.

The human population exposed to insecticide treatment is of prime importance. It must be ensured that health hazards are not a problem.

1. Choice of insecticides to be used

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

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

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

- An effective and/or cheap insecticide should not be used if the chemical is highly toxic to humans and other non-target organisms.
- Pyrethroids, generally, have very low mammalian toxicity when compared to other groups of insecticides such as carbamates.
- The liquid formulation of an insecticide is usually more dangerous than a solid formulation of the same strength. Certain solvents in liquid formulation facilitate skin penetration.
- With regard to occupational exposure, dermal exposure is more important than gastrointestinal or respiratory exposure.

Thus, an insecticide with low dermal toxicity is preferred.

- The latest information on the safety aspect of insecticides being considered must be available before a wise choice can be made.

2. The safe use of insecticides

The key to the safe 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 many factors, as outlined in the box below.

Level of Exposure Depends 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.

In order to minimize the 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 - Keey 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 stocks are getting low.
- Stocks received first must be used first. This avoids prolonged storage of any batch of insecticide.

Steps 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 anti-dotes for the insecticides being used.

During mixing and spraying/fogging with insecticides:

- Do not drink, eat or smoke while working. This prevents accidental inhalation or ingestion of insecticides.
- Mix insecticides in a well-ventilated area, preferably outdoors.
- Mix only as much insecticide as is needed for each application. This will reduce the problem of storing and disposing of excess insecticide.
- Do not smell or inhale insecticides.
- Never mix insecticides directly with bare hands.
- Stand with the wind blowing from behind when mixing insecticides.
- Do not clear blocked spray nozzles by blowing with the mouth.
- Make sure that the spray equipment does not leak; check all joints regularly.
- Keep all unconcerned people away from where insecticides are being mixed.
- Exposure to spraying normally should not exceed five hours a day.
- 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.
- Those applying insecticides should always wear long-sleeved shirts and trousers.
- 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.
- Mixers and baggers should wear rubber boots, gloves, aprons and masks, since they come in contact with technical material and concentrated formulations.
- Those engaged in thermal fogging and ULV spraying should be provided with overalls, goggles, hats and masks.
- Those engaged in larviciding (e.g. with temephos) need no special protective clothing because the risk of toxicity is low.
- To protect yourself and your family, never work with insecticides in your street clothes.
- Do not wear unwashed protective clothing. Make sure your gloves and boots have been washed inside and outside before you put them on.
- Take heed of the wind direction to avoid drift.

Steps after spraying/fogging of insecticides:

- Wash all spray equipment thoroughly and return to the storeroom. It is important to maintain equipment in good working order after usage.
- 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 should be punctured so that they cannot be reused.

- 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.
- All workers must wash thoroughly with soap and water. This removes deposits of insecticides on the skin.
- All protective clothing should be washed after each use.
- All usages of insecticides must be recorded.
- Eat only after a thorough washing with soap and water.

3. 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.

- Mixers, baggers, and spraymen should be instructed to detect and report any early signs and symptoms of mild intoxication.
- Any undue prevalence of illness not associated with well recognized signs and symptoms of poisoning by a particular insecticide should be noted and reported.
- 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:

DDT and other organochlorines

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

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 diarrhoea, convulsions, coma, loss of relaxes, 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).

Carbamates

Headache, nausea, vomiting, bradycardia, diarrhoea, tremors, convulsive seizures of muscles, increased secretion of bronchial, lacrimal, salivary and sweat glands.

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 could be 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 lambdacyhalothrin, can cause eye and skin irritation if adequate precautions are not taken.

Bacterial 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.

4. Treatment of acute insecticide poisoning

- Know the symptoms of poisoning due to different insecticides.
- Call a physician.
- Begin emergency treatment in the field. This treatment is continued during transport and ends in a medical centre.
- Provide supportive treatment for the patient. This may include:
  - Artificial respiration if spontaneous respiration is inadequate.
  - A free airway must be maintained. Excess vomitus and secretions should be removed.
  - Oxygen therapy for cyanosis (a blue or purplish discoloration of the skin due to insufficient oxygen).
- Decontaminate the patient as soon as possible. This may involve:
  - Removal of contaminated clothing.
  - Thorough washing of the skin and hair with soap and water.
  - Flushing contaminated eyes with water or saline solution for 10 minutes.
  - Evacuation to fresh air.
- Eliminate the poison. Determine whether the insecticide is in water emulsion or petroleum solution, if possible.
  - If the insecticide is dissolved in a water emulsion, induce vomiting by putting a finger or spoon down the throat. If this fails, give one tablespoon of salt in a glass of warm water until vomitus is clear.
  - If the insecticide is dissolved in a petroleum product, have the doctor or nurse perform gastric lavage, sucking the insecticide out of the stomach with a tube to prevent the possibility of the petroleum product entering the lungs and causing pneumonia.
  - Administer a laxative such as epsom salts or milk of magnesia in water to eliminate the insecticide from the alimentary tract. Avoid oily laxatives, such as caster oil, which might increase the absorption of insecticide.
- Administer an antidote, where possible. This involves the following steps:
  - 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 hydro-

carbon, an organosphosphate, a carbamate, a pyrethroid or a bacterial insecticide.

– If the insecticide is an organophosphate, either airopine sulphate or a 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 the severity of the poisoning. The dose of 2-PAM chloride is 1 gm for an adult and 0.25 gm for an infant.
– If the insecticide is a carbamate, atropine sulphate is used as an antidote. 2-PAM and other oximes are not to be used.

Source: WHO/Western Pacific Region. Background Document No.16, 1995

Annex XII

Requirements for Sustainable Community Participation in Vector-borne Disease Control

Requirements				
Technologies	**Approaches**	**Inputs**	**Processes**	**Outputs**
Proven needs (down to earth) visible	Holistic Sustainable	Health Education Seed money/material	Participatory Harmonious	Address felt needs
Supported by				
Inter- and intra-sectoral cooperation	Incentive/ income-linked schemes	Voluntary agencies Community leaders	Research Training Health Education	
Influencing factors				
Cultural Socioeconomic	Support structures Quality of input	Political and social will Community motivation	Disease Prevalence	
Result				
Cost-effective and sustainable vector-borne disease control				
Impact				
Vector control, parasite control, and improved economy and environment				

Source: WHO, Geneva, Technical Report Series, 857.

Annex XIII

Functions of Emergency Action Committee (EAC) and Rapid Action Team (RAT)

(A) Emergency Action Committee (EAC)

Constitution

The EAC will comprise administrators, epidemiologists, entomologists, clinicians and laboratory specialists, school health officers, health educators, and representatives of other related sectors.

Functions

(a) To take all administrative actions and to coordinate activities aimed at the management of serious cases in all medical care centres and undertake emergency vector control intervention measures.

(b) To draw urgent plans of action and resource mobilization in respect of medicines, intravenous fluids, blood products, insecticides, equipment and vehicles.

(c) To liaise with intersectoral committees in order to mobilize resources from non-health sectors, namely Urban Development; Ministry of Education; Ministry of information; Legal Department;Water Supply; Waste Disposal, and Information for the elimination of Breeding Potential of *Aedes aegypti*.

(d) To interact with the news media and NGOs for dissemination of information related to health education and community participation.

(B) Rapid Action Team (RAT)

Constitution

The RAT at state/provincial levels will comprise epidemiologists, entomologists, and a laboratory specialist at state and local levels.

Local Levels

Medical officer, public health officer, non-health staff, local government staff.

Functions

- Undertake urgent epidemiological and entomological investigations.
- Provide required emergency logistical support, e.g. delivery of medical and laboratory supplies to health facilities.
- Provide on-the-spot training in case management for local health staff.
- Supervise the elimination of breeding places and application of vector control measures.
- Carry out health education activities.
- Sample the collection of serum specimens.

Source: Management of Dengue Epidemic, WHO/SEARO, May 1997

Annex XIV

Community Actions for *Aedes* Control during Epidemics

Community activities against larvae and adult mosquitoes can include:

- Cleaning and covering water storage containers;
- Keeping surroundings clean and improving basic sanitation measures;
- Burning mosquito coils to kill or repel mosquitoes;
- Burning coconut shells and husks to repel mosquitoes and also eliminate these potential outdoor breeding sites;
- Screening houses, particularly bedrooms;
- Making available hand aerosols for killing mosquitoes;
- Clearing weeds and tall grass to reduce the available outdoor resting places for adult mosquitoes near houses, and
- Using mosquito nets to protect infants and small children from bites during the day time, and also insecticide-treated mosquito nets and curtains to kill mosquitoes attempting to bite through the nets or resting on the nets or curtains.

Specific activities for controlling larvae are:

- Collection, removal, disposal, burying or burning of all unusable tin cans, jars, bottles, tyres, coconut shells and husks, cocoa pods and other items that can collect and hold water.
- Keeping 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.
- Arranging 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 potential breeding sites in and around houses.
- Turning 200-litre water drums and small earthen jars upside down once a week. This emptying and cleaning procedure is easier when the water level is low.
- Periodically scrubbing the inside of water containers to destroy *Aedes* eggs at the time of container cleaning.

- Regularly emptying the water in flower vases in houses and offices at least once a week.
- Properly covering 200-litre water drums with burlap bags or other material which allows rainwater, but not mosquitoes to enter .
- Covering large volume (500 litres+) water storage tank inlets and overflow outlets with mosquito wire mesh.
- Shredding or cutting old tyres into flat pieces and disposing of them in properly constructed and managed landfills away from populated areas.
- Turning canoes and small fishing boats upside down.
- Cleaning roof gutters and placing salt in ant traps.
- Construction of rectangular cement water tanks with a plug at the bottom to allow easy drainage for weekly cleaning.
- Puncturing holes in tyres used for recreational purposes by children in schools, parks and beaches.
- Draining waterlogged tree holes.
- Turning tin cups used to collect sap from rubber trees in rubber plantations upside down when not in use.
- Levelling or filling-in the tops of bamboo fences to prevent the accumulation of water and breeding sites.
- Filtering water from one container to another through cloth in order to trap and dislodge larvae and pupae.
- Pouring boiling water into small earthen-ware jars to kill larvae when the water level is low.
- Scrubbing down the sides of jars to kill mosquito eggs.
- Removing small copepod crustaceans of the genus *Mesocyclops* from ponds or lakes and placing several of them in water storage containers to kill mosquito larvae.
- Removing small larvivorous fish from a pond, stream or canal and placing one or two of them in water storage containers to kill larvae.

Source: WHO/Western Pacific Region. Background Document No.16, 1995