Dengue haemorrhagic fever

Diagnosis, treatment, prevention and control

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Preface

Dengue fever, and especially the more severe manifestation dengue haemorrhagic fever, ranks highly among new and newly emerging infectious diseases in public health significance and is considered to be the most important of the arthropod-borne viral diseases. Since the early 1970s, the World Health Organization (WHO) has been actively involved in developing and promoting strategies for the treatment and control of dengue. In 1986, WHO published a guide to the diagnosis, treatment and control of dengue haemorrhagic fever which has enjoyed popularity and been internationally recognized as an authoritative reference.

In resolution WHA46.31 the Forty-sixth World Health Assembly in 1993 confirmed that dengue prevention and control should be among the priorities of WHO. Global and regional strategies emphasizing the need for effective prevention, active surveillance and outbreak preparedness have since been developed. Three WHO Regional Offices have recently issued publications on dengue: in 1993, the Regional Office for South-East Asia (SEARO) published *Monograph on dengue/dengue haemorrhagic fever*; in 1994, the Regional Office for the Americas (PAHO) published *Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control*; and in 1995, the Regional Office for the Western Pacific (WPRO) published *Guidelines for dengue surveillance and mosquito control*.

This second edition of the 1986 book has been produced to make widely available to health practitioners, laboratory personnel, those involved in vector-control efforts and public health officials a concise publication of worldwide relevance containing practical information about dengue and dengue haemorrhagic fever. If offers in-depth, proven and easy-to-follow recommendations for the diagnosis and treatment of dengue and dengue haemorrhagic fever and provides a global perspective on the history, prevention, surveillance and control of dengue. While retaining key features of the original, the present edition furnishes some new information, particularly with respect to methods of laboratory diagnosis and vector surveillance and control.

Like the WHO regional publications, this book has benefited from the review of numerous experts within and outside WHO, as well as from the work of several international meetings addressing aspects of the global dengue problem. In particular, Dr Natth Bhamarapravati, Dr Duane Gubler, Dr Scott

Halstead, Dr Bruce Innis, Dr Suchitra Nimmanitya and Dr David Vaughn should be acknowledged for their kind assistance. It is hoped this publication will contribute to prevention and control of the morbidity and mortality due to dengue, and continue to serve as an authoritative reference for workers and researchers in the field.

General considerations

Dengue fever (DF) is an acute febrile viral disease frequently presenting with headaches, bone or joint and muscular pains, rash and leukopenia as symptoms. Dengue haemorrhagic fever (DHF) is characterized by four major clinical manifestations: high fever, haemorrhagic phenomena, often with hepatomegaly and, in severe cases, signs of circulatory failure. Such patients may develop hypovolaemic shock resulting from plasma leakage. This is called dengue shock syndrome (DSS) and can be fatal.

Dengue[1] or dengue-like epidemics were reported throughout the nineteenth and early twentieth centuries in the Americas, southern Europe, North Africa, the eastern Mediterranean, Asia and Australia, and on various islands in the Indian Ocean, the south and central Pacific and the Caribbean. As discussed below, DF and DHF have steadily increased in both incidence and distribution over the past 40 years, and in 1996, 2500–3000 million people lived in areas potentially at risk for dengue virus transmission. Annually, it is estimated that there are 20 million cases of dengue infection, resulting in around 24 000 deaths. Annex 1 lists countries or territories by WHO Region in which DF or DHF is known to have occurred between 1975 and 1996. Figure 1.1 is a map illustrating the same information. Reported cases of DF and DHF for the period 1956–1995 are shown in Table 1.1.

Dengue in the South-East Asia and Western Pacific Regions of WHO

The disease now known as DHF was first recognized in the Philippines in 1953. The syndrome was etiologically related to dengue viruses when serotypes 2, 3 and 4 were isolated from patients in the Philippines in 1956; 2 years later dengue viruses of multiple types were isolated from patients during an epidemic in Bangkok, Thailand. During the next three decades, DHF/DSS was recognized in Cambodia, China, India, Indonesia, the Lao People's Democratic Republic, Malaysia, Maldives, Myanmar, Singapore, Sri Lanka, Viet Nam, and several Pacific Island groups.

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[1] In this book "dengue" refers to the entire spectrum of dengue viral disease; abbreviations (i.e. DF, DHF, DSS) are used to refer to specific gradations of dengue.

Fig. 1.1

The general distribution of dengue fever and/or dengue haemorrhagic fever, 1975–1996

* Countries which have experienced DHF outbreaks
△ Imported cases (probable or confirmed)

Table 1.1
Global reports of dengue and dengue haemorrhagic fever, 1956–1995[a]

Time interval	No. years	No. cases	Mean no. cases per year
1956–1980	25	1547760	61910
1981–1985	5	1304305	260861
1986–1990	5	1776140	355228
1991–1995	5	1704050	340810

[a] Figures compiled from reports in WHO Regional Offices (AMRO, SEARO & WPRO).

During the 1960s and 1970s, DHF/DSS progressively increased as a health problem, spreading from its primary location in major cities to smaller cities and towns in endemic countries. It established seasonal and cyclical epidemic patterns, with large outbreaks occurring at 2–3 year intervals. During this period, 1070207 cases and 42808 deaths were reported, mostly in children. During most of the 1980s, in the endemic countries of China, Indonesia, Malaysia, Myanmar, Philippines, Thailand, and Viet Nam, DHF/DSS spread peripherally, affecting even rural villages. Exceptionally large outbreaks occurred in Viet Nam (354517 cases in 1987) and Thailand (174285 cases in 1987). The total number of people contracting and dying from DHF/DSS reported in all countries of the Western Pacific and South-East Asia Regions for the decade of the 1980s was 1946965 and 23793, respectively. Epidemiologically important new introductions of DHF/DSS were reported in China (1985), Maldives (1985), India (1988), New Caledonia (1988), Sri Lanka (1989) and Tahiti (1989). The experiences in India and Sri Lanka are particularly interesting, because virological surveillance documented the endemic transmission of all four dengue serotypes accompanied by DF cases, but not by DHF/DSS prior to the above-mentioned outbreaks.

In each country of these Regions where DHF has become endemic, the sequence has been more or less the same; frequent transmission of dengue virus, first associated with sporadic cases of DHF, followed by DHF epidemics which progressively become more frequent, until DHF cases are seen virtually every year, with major epidemics occurring at 3–5 year intervals. All four dengue serotypes are present in these two Regions, and increasing international travel serves to introduce new virus strains and serotypes rapidly into susceptible populations. In many countries, DF and DHF are primarily diseases of children, since they represent the largest segment of susceptible individuals within the population at risk. Increasingly, DF, and occasionally DHF, are also seen among travellers. DHF is now a significant public health problem in most of the countries in the tropical areas of the South-East Asia and Western Pacific Regions. The disease is among the ten leading causes of hospitalization and death in children in at least eight tropical Asian countries.

Dengue outbreaks in the Americas

Until 1981, only sporadic suspected cases of DHF had been reported in the Americas, although epidemics of classic DF occurred in the Caribbean and northern South America in 1963–64, 1968–69, 1972–75 and 1977–78. However, in 1981 an outbreak of DHF/DSS occurred in Cuba that marked the start of DHF in the Region of the Americas. During this epidemic, 344 203 cases of dengue were reported, including 10 312 patients classified as severely ill according to the WHO criteria (grades III and IV; see Chapter 2). During the same epidemic, 158 deaths, of which 101 were in children, were reported. In a 3-month period, 116 143 persons were hospitalized. The second largest outbreak of DHF/DSS in the Region occurred in Venezuela from October 1989 to April 1990. Moreover, the epidemic reappeared in the second half of 1990 and in each of the subsequent years up to and including 1993. A total of 11 260 cases of DHF and 136 deaths were reported in Venezuela during the period 1989–1993. Dengue virus serotypes 1, 2 and 4 were isolated during these outbreaks.

Cases of DHF or DHF-like disease have been reported in the Americas nearly every year since 1981. The countries or territories affected include Aruba, Barbados, Brazil, Colombia, the Dominican Republic, El Salvador, French Guiana, Guadeloupe, Guatemala, Honduras, Jamaica, Mexico, Nicaragua, Panama, Puerto Rico, Saint Lucia, Suriname and Venezuela. Dengue has been recorded in virtually all Latin American countries, with the possible exceptions of Argentina, Chile and Uruguay, and it appears that DHF/DSS is gradually becoming endemic in several countries of the Americas, following the trend observed in Asia. The marked increase in DHF/DSS noted in several Asian countries during the past 30 years clearly illustrates what the Americas may face.

Dengue in the African and Eastern Mediterranean Regions

All countries with dengue virus transmission should be considered at risk for DHF outbreaks, and while there is comparatively little information on DF and DHF in the African and the Eastern Mediterranean Regions, it is nevertheless clear that they pose a growing threat there. Dengue disease has been prevalent in tropical Africa and has appeared episodically in the temperate regions of North Africa and the Mediterranean region of Europe. Since 1967, dengue virus has been reported in Angola, Burkina Faso, Comoros, Côte d'Ivoire, Democratic Republic of the Congo Djibouti, Ethiopia, Ghana, Guinea, Kenya, Madagascar, Mauritius, Mozambique, Nigeria, Pakistan, Réunion, Saudi Arabia, Senegal, Seychelles, Sierra Leone, Somalia, Sudan and the United Republic of Tanzania. Some outbreaks have involved a large portion of the population, as for example the 1993 outbreak of serotype 1 in the Comoros, in which more than 60 000 people were estimated to have contracted dengue. The appearance of dengue in Pakistan in 1994 constituted the first epidemic of DHF in these Regions.

Economic impact of dengue

Few studies of the economic impact of DF and DHF/DSS have been conducted. Children most frequently suffer from DHF/DSS, with average hospital stays of 5–10 days for severe cases. Intensive care is required for severely ill patients, including intravenous fluids, blood or plasma transfusion and medicines, and adults can miss work in order to attend to their children's illness. Consequently, there are both direct and indirect costs for each dengue patient, ranging from inconvenience due to a sick child (or adult) with uncomplicated DF, to substantial costs for hospitalization and significant disruption of earning potential. In addition, there are costs to local municipalities for vector control activities, and often revenue lost through reduced tourism. The cost of the 1981 Cuban epidemic of DHF/DSS was estimated to be approximately US$ 103 million, which includes the cost of control measures (US$ 43 million) and medical services (US$ 41 million). As another example, DF and DHF/DSS epidemics in Puerto Rico since 1977 are estimated to have cost US$ 150–200 million. The direct costs that were estimated for the 1987 epidemic of DHF/DSS in Thailand, including hospitalization and mosquito control, were US$ 16 million. A 1995 report estimated that the annual economic burden due to DHF in Thailand ranges from US$ 19 million to US$ 51 million per year, depending on whether low or high levels of transmission occur. While the exact cost of each epidemic is difficult to calculate, it is clear that DF and DHF/DSS represent a significant economic burden on the societies affected.

Characteristics of dengue haemorrhagic fever outbreaks

Although the early outbreaks of DHF seem to have appeared suddenly in the Philippines and in Thailand, retrospective studies indicate that they were probably preceded by a decade or so in which cases occurred but were not recognized. In Thailand, outbreaks first occurred in Bangkok in a pattern with a 2-year cycle, then subsequently in irregular cycles as the disease spread throughout the country. DHF then became endemic in many large cities of Thailand, eventually spreading to smaller towns and villages during periods of epidemic transmission. A similar pattern was observed in Indonesia, Myanmar and Viet Nam.

During the 40 years' experience with dengue in the Western Pacific and South-East Asia Regions, two important epidemiological patterns have been recognized. First, DHF/DSS has appeared most frequently in areas where multiple dengue serotypes are endemic. The usual pattern is that of sporadic cases or small outbreaks in urban areas that steadily increase in size until there is an explosive outbreak that brings the disease to the attention of public health authorities. The disease then usually establishes a pattern of epidemic activity every 2–5 years. In addition, DHF/DSS is typically confined to children, with a modal age at hospitalization of 4–6 years. A second pattern is observed in areas of low endemicity. Multiple dengue serotypes may be transmitted at

relatively low rates of infection (below 5% of the population per year). In these areas, previously uninfected adults are susceptible to dengue infection, and children and young adults, with a modal age of 6–8 years, are also vulnerable.

A cyclical pattern of increased transmission coinciding with the rainy season has been observed in some countries. The interactions between temperature and rainfall are important determinants of dengue transmission, as cooler temperatures affect adult mosquito survival, thus influencing transmission rates. Furthermore, rainfall and temperature may affect patterns of mosquito feeding and reproduction, and hence the population density of vector mosquitos.

Although DHF may affect persons of all ages in dengue endemic areas, most DHF cases occur in children less than 15 years of age. Since 1964, the trend in Bangkok has been towards progressively lower attack rates (constant hospital admission rates despite an increasing population), with the modal age of hospitalized children being 6–7 years throughout Thailand. Surveillance data from some areas have suggested a slight excess of infected girls over boys, while other areas have shown an almost even distribution.

A retrospective evaluation of the impact of DHF during an outbreak in Bangkok/Thon Buri in May–November 1962 indicated that in a population of 870 000 children under 15 years of age, an estimated 150 000–200 000 minor febrile illnesses were caused by dengue and occasionally by chikungunya viruses; 4187 patients were hospitalized with DHF, and 4000 additional patients were treated in private clinics or at home. Moreover, shock occurred in about one-third of the hospitalized DHF patients. In the more recent large epidemic in Thailand in 1987, the attack rate of DHF/DSS was 320 cases per 100 000 population for all ages. In southern Viet Nam between 1975 and 1992, the attack rate of DHF/DSS ranged from 30 to 380 per 100 000 population, with mortality rates from 0.39 to 6.42 per 100 000 population, while the incidence of DHF in Indonesia for 1991 and 1992 was 11.56 and 9.45 per 100 000, respectively.

Transmission of dengue viruses

Dengue viruses are transmitted to humans through the bite of infected *Aedes* mosquitos, principally *Aedes aegypti*, and are therefore considered to be arboviruses (arthropod-borne viruses). Once infected, a mosquito remains infected for life, transmitting the virus to susceptible individuals during probing and feeding. Infected female mosquitos may also pass the virus to the next generation of mosquitos by transovarian transmission, but this occurs infrequently and probably does not contribute significantly to human transmission. Humans are the main amplifying host of the virus, although studies have shown that monkeys in some parts of the world may become infected and perhaps serve as a source of virus for feeding mosquitos. The virus circulates in the blood of infected humans at approximately the time that they have fever, and uninfected

mosquitos may acquire the virus if they feed on an individual when he or she is viraemic. The virus then develops in the mosquito for a period of 8–10 days before it can be transmitted to other humans during subsequent probing or feeding. The length of time required for this extrinsic incubation depends in part on environmental conditions, especially ambient temperature.

The virus

Dengue virus belongs to the family *Flaviviridae*. The four serotypes of dengue virus (designated DEN-1, DEN-2, etc.) can be distinguished by serological methods. Infection in humans by one serotype produces life-long immunity against reinfection by that same serotype, but only temporary and partial protection against the other serotypes. Dengue viruses share many characteristics with other flaviviruses, having a single-stranded RNA genome surrounded by an icosahedral nucleocapsid and covered by a lipid envelope. The virion is approximately 50 nm in diameter. The flavivirus genome is approximately 11 kb (kilobases) in length, and the complete genome sequence is known for isolates of all four serotypes of dengue virus. The genome is composed of three structural protein genes, encoding the nucleocapsid or core protein (C), a membrane-associated protein (M), an envelope protein (E) and seven non-structural (NS) protein genes. The domains responsible for neutralization, fusion and interactions with virus receptors are associated with the envelope protein. The order of proteins encoded is 5′-C-prM(M)-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3′.

The vectors

Ae. aegypti is a tropical and subtropical species of mosquito found around the globe, usually between latitudes 35°N and 35°S, approximately corresponding to a winter isotherm of 10 °C as shown in Figure 1.2. Although *Ae. aegypti* has been found as far north as 45°N, such invasions have occurred during the warm season, and the mosquitos have not survived the winters. Distribution of *Ae. aegypti* is also limited by altitude. It is usually not found above 1000 m but has been reported at 2121 m in India, at 2200 m in Colombia, where the mean annual temperature is 17 °C, and at 2400 m in Eritrea. *Ae. aegypti* is one of the most efficient mosquito vectors for arboviruses, because it is highly anthropophilic and thrives in close proximity to humans and often lives indoors. Dengue outbreaks have also been attributed to *Ae. albopictus, Ae. polynesiensis*, and several species of the *Ae. scutellaris* complex. Each of these species has its own particular geographical distribution; however, they are less efficient epidemic vectors than *Ae. aegypti*. While vertical (possibly transovarian) transmission of dengue viruses has been demonstrated in both the laboratory and the field, the significance of this to maintenance of the virus has not been established. A factor complicating eradication of the vector is that *Ae. aegypti*

Fig. 1.2
Approximate actual and potential distribution of *Aedes aegypti*[a]
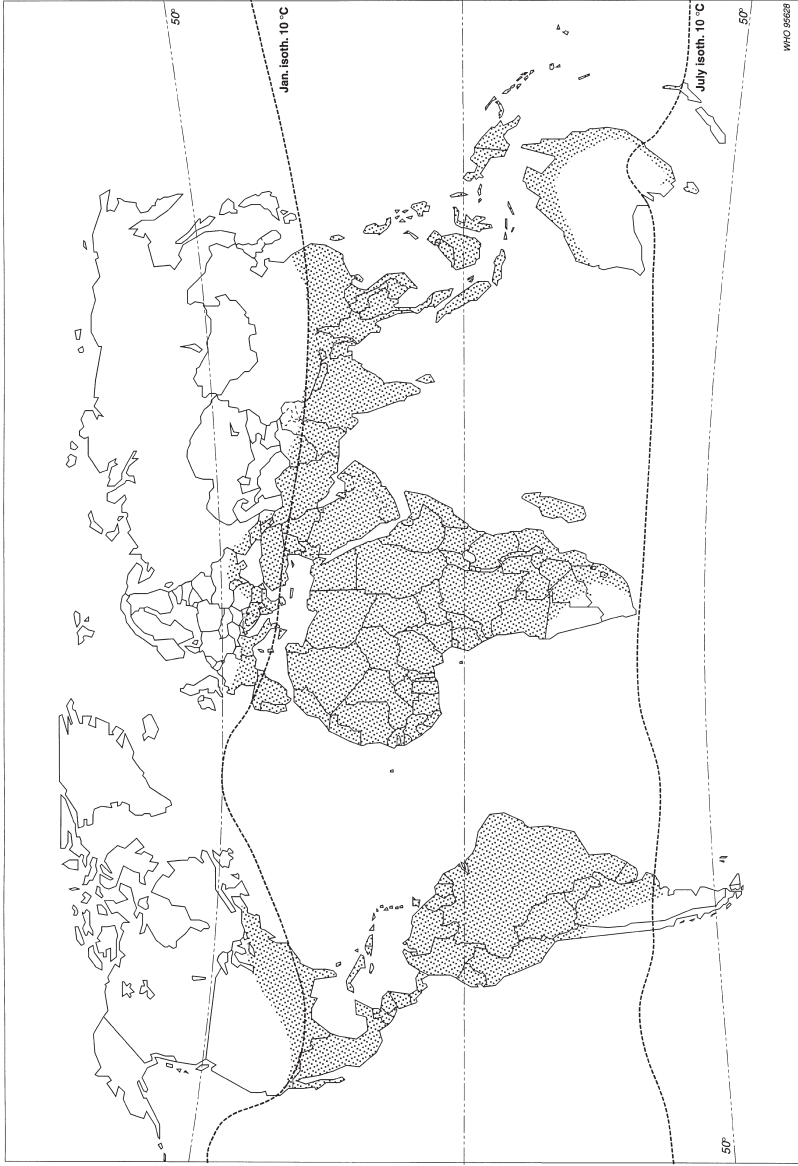
[a] The band between the 10 °C isotherms represents potential distribution.

eggs can withstand long periods of desiccation, sometimes for more than a year.

The host

In humans, each of the four dengue virus serotypes has been associated with DF and with DHF. Studies in Cuba and Thailand have shown a consistently high association between DEN-2 infection and DHF/DSS, but in the 1976–1978 Indonesia, 1980–1982 Malaysia, and 1989–90 Tahiti epidemics, and from 1983 onwards in Thailand, DEN-3 was the predominant serotype recovered from patients with severe disease. In the 1984 Mexico, the 1986 Puerto Rico, and the 1989 El Salvador outbreaks, DEN-4 was most often isolated from DHF patients. DSS occurs with higher frequency in two immunologically defined groups: children who have experienced a previous dengue infection, and infants with waning levels of maternal dengue antibody. The acute phase of infection, following an incubation of 3–14 days, lasts about 5–7 days and is followed by an immune response. The first infection produces life-long immunity to the infecting serotype but only temporary and partial protection against the other three serotypes, and secondary or sequential infections are possible after a short time. Transmission of dengue virus from infected humans to feeding mosquitos is determined by the magnitude and duration of viraemia in the human host; persons with high viraemia provide a higher infectious dose of virus to the feeding mosquito, normally leading to a greater percentage of feeding mosquitos becoming infected, although even very low levels of virus in blood may be infectious to some vector mosquitos.

Pathology

At autopsy, all patients who have died of DHF show some degree of haemorrhage; in order of frequency, haemorrhage is found in the skin and subcutaneous tissue, in the mucosa of the gastrointestinal tract, and in the heart and liver. Gastrointestinal haemorrhage may be severe, but subarachnoid or cerebral haemorrhage is rarely seen. Serous effusion with a high protein content (mostly albumin) is commonly present in the pleural and abdominal cavities, but is less common in the pericardial cavity.

Light microscopy of blood vessels shows no significant changes in vascular walls. Capillaries and venules in the affected organ systems may show extravascular bleeding by diapedesis and perivascular haemorrhage, with perivascular infiltration by lymphocytes and mononuclear cells. Morphological evidence of intravascular clot formation in small vessels has been recognized in patients with severe haemorrhage.

In most fatal cases, lymphocyte tissue shows an increased activity of the B-lymphocyte system, with active proliferation of plasma cells and lymphoblastoid cells, and active germinal centres. There is evidence indicating that

proliferation of large immunoblasts and considerable turnover of the lymphocytes occur. The latter is manifested by a reduction of white splenic pulps, lymphocytolysis, and marked lymphocytic phagocytosis.

In the liver, there is focal necrosis of hepatic cells, swelling, appearance of Councilman bodies and hyaline necrosis of Kupffer cells. Proliferation of mononuclear leukocytes, and less frequently polymorphonuclear leukocytes, occurs in the sinusoids and occasionally in the portal areas. Lesions in the liver typically resemble those 72–96 hours after infection with yellow fever virus, when parenchymal cell damage is limited.

At autopsy, dengue virus antigen has been found predominantly in liver, spleen, thymus, lymph node, and lung cells. The virus has also been isolated at autopsy from the bone marrow, brain, heart, kidney, liver, lungs, lymph nodes, and the gastrointestinal tract.

Pathological studies of the bone marrow, kidneys and skin have been made in patients who had non-fatal DHF. In the bone marrow, depression of all haematopoietic cells was observed, which would rapidly improve as fever subsided. Studies in kidneys have shown a mild immune-complex type of glomerulonephritis, which would resolve after about 3 weeks with no residual change. Biopsies of skin rashes have revealed perivascular oedema of the terminal microvasculature of dermal papillae and infiltration of lymphocytes and monocytes. Antigen-bearing mononuclear phagocytes have been found in the vicinity of this oedema. Deposition of serum complement, immunoglobulin and fibrinogen on vessel walls has also been described.

Pathogenesis of DHF/DSS

Two main pathophysiological changes occur in DHF/DSS. One is an increased vascular permeability that gives rise to loss of plasma from the vascular compartment. This results in haemoconcentration, low pulse pressure and other signs of shock, if plasma loss becomes critical. The second change is a disorder in haemostasis involving vascular changes, thrombocytopenia and coagulopathy.

A constant finding in DHF/DSS is activation of the complement system, with profound depression of C3 and C5 levels. The mediators that increase vascular permeability and the precise mechanism(s) of the bleeding phenomena seen in dengue infections have not yet been identified; consequently, further studies are needed. Immune complexes have been described in DHF but their role is not yet clear.

Platelet defects may be both qualitative and quantitative, i.e. some circulating platelets during the acute phase of DHF may be exhausted (incapable of normal function). Therefore, even a patient with a platelet count greater than $100\,000$ per mm³ may still have a prolonged bleeding time.

A mechanism that may contribute to the development of DHF/DSS is enhancement of virus replication in macrophages by heterotypic antibodies. In

secondary infections with a virus of a different serotype from that causing the primary infection, cross-reactive antibodies that fail to neutralize virus may increase the number of infected monocytes as dengue virus–antibody complexes are taken into these cells. This in turn may result in the activation of cross-reactive CD4+ and CD8+ cytotoxic lymphocytes. The rapid release of cytokines caused by the activation of T cells and by the lysis of infected monocytes mediated by cytotoxic lymphocytes may result in the plasma leakage and haemorrhage that occur in DHF.

CHAPTER 2

Clinical diagnosis

Dengue virus infections may be asymptomatic or may lead to undifferentiated fever, dengue fever (DF) or dengue haemorrhagic fever (DHF) with plasma leakage that may lead to hypovolaemic shock (dengue shock syndrome, DSS) (Figure 2.1).

Dengue fever

The clinical features of DF frequently depend on the age of the patient. Infants and young children may have an undifferentiated febrile disease, often with a maculopapular rash. Older children and adults may have either a mild febrile syndrome or the classic incapacitating disease with high fever of abrupt onset, sometimes with 2 peaks (saddle-backed), severe headache, pain behind the eyes, muscle and bone or joint pains, nausea and vomiting, and rash. Skin haemorrhages (petechiae) are not uncommon. Leukopenia is usually seen and thrombocytopenia may be observed. Recovery may be associated with prolonged fatigue and depression, especially in adults.

In some epidemics, DF may be accompanied by bleeding complications, such as epistaxis, gingival bleeding, gastrointestinal bleeding, haematuria, and

Fig. 2.1.
Manifestations of dengue virus infection
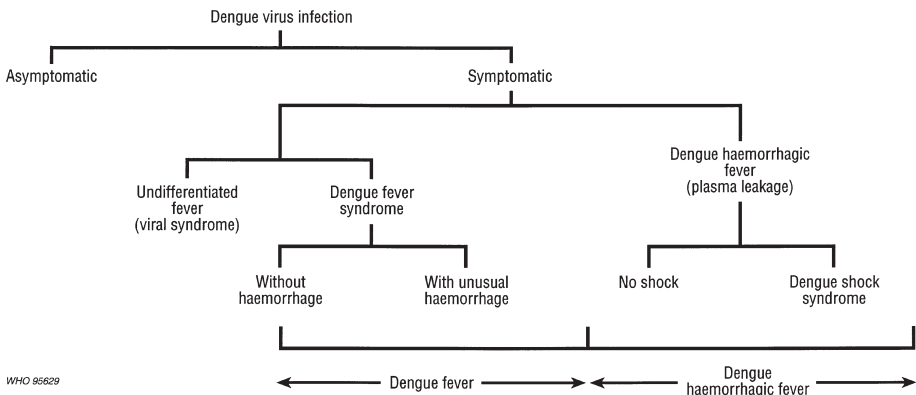
Table 2.1

Observed frequency of findings in classical dengue fever in adults and chikungunya and dengue virus infections in Thai children diagnosed as having haemorrhagic fever[a]

Finding	Classical dengue fever in adults[b]	Chikungunya fever in Thai children	Dengue haemorrhagic fever in Thai children
Fever	++++	++++	++++
Positive tourniquet test	++	+++	++++
Petechiae or ecchymosis	+	++	++
Confluent petechial rash	0	0	+
Hepatomegaly	0	+++	++++
Maculopapular rash	++	++	+
Myalgia/arthralgia	+++	++	+
Lymphadenopathy	++	++	++
Leukopenia	++++	++++	++
Thrombocytopenia	++	+	++++
Shock	0	0	++
Gastrointestinal bleeding	+	0	+

[a] + = 1–25%; ++ = 26–50%; +++ = 51–75%; ++++ = 76–100%.
[b] Modified from Halstead SB et al. *American journal of tropical medicine and hygiene*, 1969, 18: 984–996, and refers mainly to Caucasian adults.

menorrhagia. During outbreaks of DEN-1 infections in Taiwan, China, studies have shown that severe gastrointestinal bleeding may occur in persons with pre-existing peptic ulcer disease. Unusually severe bleeding can cause death in such cases. The case-fatality rate of DF, however, is less than 1%. It is important to differentiate cases of DF with unusual bleeding from cases of DHF with increased vascular permeability, the latter being characterized by haemoconcentration. In many endemic areas, DF must also be differentiated from chikungunya fever, another vector-borne virus disease of similar epidemiology and overlapping distribution in much of Asia and the Pacific (see Table 2.1).

Dengue haemorrhagic fever

Typical cases of DHF are characterized by four major clinical manifestations: high fever, haemorrhagic phenomena, and often, hepatomegaly and circulatory failure. Moderate to marked thrombocytopenia with concurrent haemoconcentration is a distinctive clinical laboratory finding of DHF. The major pathophysiological change that determines the severity of disease in DHF—and differentiates it from DF—is the leakage of plasma, as manifested by an

Table 2.2

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

Criteria	DHF(%)	Chikungunya Fever (%)
Injected pharynx	96.8	90.3
Vomiting	57.9	59.4
Constipation	53.5	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
Rhinitis	12.8	6.5
Maculopapular rash	12.1[b]	59.4[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	6.3[c]	3.1[c]
Coma	3.0	0.0

[a] Modified from Nimmannitya S et al. *American journal of tropical medicine and hygiene*, 1969, 18: 954–971.
[b] Statistically significant difference.
[c] Infants under 6 months.

elevated haematocrit[1] (i.e. haemoconcentration), a serous effusion or hypo-proteinaemia.

Children with DHF commonly present with a sudden rise in temperature accompanied by facial flush and other non-specific constitutional symptoms resembling DF, such as anorexia, vomiting, headache, and muscle or bone and joint pain. Some patients complain of sore throat, and an injected pharynx is frequently evident on examination, but rhinitis and cough are infrequent. Mild conjunctival injection may be observed (see Table 2.2). Epigastric discomfort, tenderness at the right costal margin, and generalized abdominal pain are common. The temperature is usually high (>39 °C) and remains so for 2–7 days. Occasionally, temperature may be as high as 40–41 °C; febrile convulsions may occur, particularly in infants.

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[1] Haematocrit = erythrocyte volume fraction, i.e. the percentage of the volume of a blood sample occupied by red blood cells.

The most common haemorrhagic phenomenon is a positive tourniquet test, easy bruising and bleeding at venepuncture sites. Present in most cases are discrete fine petechiae scattered on the extremities, axillae, face and soft palate, which are usually seen during the early febrile phase. Epistaxis and gingival bleeding occur infrequently; mild gastrointestinal haemorrhage may be observed during the febrile period.

The liver is usually palpable early in the febrile phase and varies in size from just palpable to 2–4 cm below the costal margin. Although liver size is not correlated with disease severity, an enlarged liver is observed more frequently in shock than in non-shock cases. The liver is tender, but jaundice is not usually observed. Splenomegaly is rarely observed in infants; however, the spleen may be prominent on X-ray examination.

The critical stage of the disease course is reached at the end of the febrile phase. After 2–7 days of fever, a rapid fall in temperature is often accompanied by signs of circulatory disturbance of varying severity. The patient may sweat, be restless, have cool extremities and show some changes in pulse rate and blood pressure. In less severe cases, these changes are minimal and transient, reflecting a mild degree of plasma leakage. Many patients recover spontaneously, or after a short period of fluid and electrolyte therapy. In more severe cases, when plasma loss is critical, shock ensues and can progress rapidly to profound shock and death if not properly treated.

The severity of the disease can be modified by early diagnosis and replacement of plasma loss. Thrombocytopenia and haemoconcentration are usually detectable before the subsidence of fever and the onset of shock.

Dengue shock syndrome

The condition of patients who progress to shock suddenly deteriorates after a fever of 2–7 days' duration. This deterioration occurs at the time of, or shortly after, the fall in temperature—between the third and the seventh day of the disease. There are the typical signs of circulatory failure: the skin becomes cool, blotchy, and congested; circumoral cyanosis is frequently observed; the pulse becomes rapid. Patients may initially be lethargic, then become restless and rapidly enter a critical stage of shock. Acute abdominal pain is a frequent complaint shortly before the onset of shock.

DSS is usually characterized by a rapid, weak pulse with narrowing of the pulse pressure (<20 mmHg (2.7 kPa), regardless of pressure levels, e.g. 100/90 mmHg (13.3/12.0 kPa)) or hypotension with cold, clammy skin and restlessness. Patients in shock are in danger of dying if appropriate treatment is not promptly administered. Patients may pass into a stage of profound shock, with the blood pressure or pulse becoming imperceptible. However, most patients remain conscious almost to the terminal stage. The duration of shock is short: typically the patient dies within 12–24 hours, or recovers rapidly following

appropriate volume-replacement therapy. Pleural effusion and ascites may be detected by physical examination or radiography. Uncorrected shock can give rise to a complicated course, with the development of metabolic acidosis, severe bleeding from the gastrointestinal tract and other organs, and a poor prognosis. Patients with intracranial haemorrhages may convulse and enter a coma. Encephalopathy, reported occasionally, can occur in association with metabolic and electrolyte disturbances or intracranial bleeding.

Convalescence in patients with corrected DSS is short and uneventful. Even in cases of profound shock, once shock is overcome, surviving patients recover within 2–3 days, although pleural effusion and ascites may still be present. Good prognostic signs are adequate urine output and the return of appetite.

Common findings during the convalescence of DHF patients are sinus bradycardia or arrhythmia and the characteristic confluent petechial rash with small round areas of normal skin. Maculopapular or rubella-type rashes are less common in DHF than in DF and may be observed either early or late in the disease. The course of DHF is approximately 7–10 days. In general, there is no prolonged fatigue.

Laboratory findings

Thrombocytopenia and haemoconcentration are constant findings in DHF. A drop in the platelet count to below 100 000 per mm^3 is usually found between the third and eighth day of illness, often before or simultaneous with changes in the haematocrit. A rise in the haematocrit level, indicating plasma leakage, is always present, even in non-shock cases, but is more pronounced in shock cases. Haemoconcentration with an increase in the haematocrit of 20% or more is considered to be definitive evidence of increased vascular permeability and plasma leakage. It should be noted that the haematocrit level may be affected either by early volume replacement or by bleeding. The time-course relationship between a drop in the platelet count and a rapid rise in the haematocrit appears to be unique for DHF; both changes occur before defervescence and before the onset of shock.

In DHF, the white-blood-cell count may be variable at the onset of illness, ranging from leukopenia to mild leukocytosis, but a drop in the total white-blood-cell count due to a reduction in the number of neutrophils is virtually always observed near the end of the febrile phase of illness. Relative lymphocytosis, with the presence of atypical lymphocytes, is a common finding before defervescence or shock. A transient mild albuminuria is sometimes observed, and occult blood is often found in the stool. In most cases, assays of coagulation or fibrinolytic factors show a reduction in fibrinogen, prothrombin, factor VIII, factor XII, and antithrombin III. A reduction in α-antiplasmin (α-plasmin inhibitor) has been noted in some cases. In severe cases with marked liver dysfunction, reductions are observed in the levels of the prothrombin factors

that are vitamin-K dependent, 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 patients, respectively. Thrombin time is prolonged in severe cases. Platelet function has also been found to be impaired. Serum complement levels, particularly that of C3, are reduced.

The other common findings are hypoproteinaemia (due to a loss of albumin), hyponatraemia, and elevated levels of serum aspartate aminotransferase. Metabolic acidosis may frequently be found in prolonged shock. Blood urea nitrogen is elevated at the terminal stage of shock.

X-ray examination of the chest reveals pleural effusion, mostly on the right side, as a constant finding, and the extent of pleural effusion is correlated with the severity of disease. In shock, bilateral pleural effusion is a common finding.

Complications and unusual manifestations

As dengue infections have become more common, an increasing number of cases of DF or DHF-like disease have been associated with unusual manifestations. These include such central nervous system phenomena as convulsions, spasticity, changes in consciousness and transient pareses. A subtle form of seizure is occasionally observed during the febrile phase in infants. This may be only a simple febrile convulsion, since the cerebrospinal fluid has been found to be normal in such cases. Water intoxication resulting from the excessive administration of hypotonic solution to treat DHF/DSS patients with hyponatraemia may lead to encephalopathy. Patients with encephalopathy as a complication of disseminated intravascular coagulation have also been reported.

Patients with neurological manifestations who have died have been reported in India, Indonesia, Malaysia, Myanmar, Puerto Rico and Thailand. While there have been a few reports of isolation of the virus or of anti-dengue IgM from cerebrospinal fluid, to date there is no evidence of the direct involvement of dengue virus in neuronal damage. Intracranial bleeding may occur, and brain-stem herniation due to cerebral oedema has been observed. In general, patients who have died with neurological signs or symptoms have not been subjected to an autopsy study. Both gross and microscopic studies are essential to establish the nature and etiology of any neurological manifestations accompanying a fatal DHF/DSS-like disease.

Great care must be taken to prevent iatrogenic complications in the treatment of DHF/DSS, to recognize them quickly if they occur and not to mistake preventable and treatable iatrogenic complications for normal DHF/DSS findings. Such complications include sepsis, pneumonia, wound infection and overhydration. The use of contaminated intravenous lines or fluids can result in Gram-negative sepsis accompanied by fever, shock and severe haemorrhage; pneumonia and other infections can cause fever and complicate convalescence.

Overhydration can cause heart or respiratory failure, which may be mistaken for shock (see Chapter 3).

Liver failure has been associated with DHF/DSS, particularly during the epidemics in Indonesia in the 1970s and the 1987 epidemic in Thailand. This may be due either to the successful resuscitation of patients with severe circulatory failure, or to an unusual liver tropism of certain viral strains. Dengue virus serotypes 1, 2 and 3 have been isolated from patients dying from liver failure, with both primary and secondary dengue infections. Necrosis of hepatocytes was found to be extensive in some of these cases. Dengue antigen was detected in hepatocytes, in Kupffer cells and occasionally in acute inflammatory cells. The histopathological findings were distinct from those seen in Reye syndrome. Whether liver injury is due to the direct effect of dengue infection or to the host's response to infection remains to be determined. Encephalopathy associated with acute liver failure is commonly observed, and renal failure is a common terminal event.

Other unusual reported manifestations include acute renal failure and haemolytic uraemic syndrome, sometimes in patients with underlying conditions, e.g. glucose-6-phosphate dehydrogenase (G6PD) deficiency and haemoglobinopathy. Simultaneous infections, such as leptospirosis, viral hepatitis B, typhoid fever, chickenpox and melioidosis, have been reported and could contribute to unusual manifestations of DHF/DSS.

Case definition for dengue fever

Given the variability in the clinical illness associated with dengue infection, it is not appropriate to adopt a detailed clinical definition of dengue fever. Rather, the need for laboratory confirmation is emphasized.

The following classifications are proposed:

* *Probable*—an acute febrile illness with two or more of the following manifestations:
  — headache
  — retro-orbital pain
  — myalgia
  — arthralgia
  — rash
  — haemorrhagic manifestations
  — leukopenia;
and
  — supportive serology (a reciprocal haemagglutination-inhibition antibody titre ≥1280, a comparable IgG enzyme-linked immunosorbent assay (ELISA, see Chapter 4) titre or a positive IgM antibody test on a late acute or convalescent-phase serum specimen);

or
  — occurrence at the same location and time as other confirmed cases of dengue fever.
- *Confirmed*—a case confirmed by laboratory criteria (see below).
- *Reportable*—any probable or confirmed case should be reported.

Laboratory criteria for confirmation of dengue fever are (see Chapter 4):

- Isolation of the dengue virus from serum or autopsy samples; or
- 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; or
- Demonstration of dengue virus antigen in autopsy tissue, serum or cerebrospinal fluid samples by immunohistochemistry, immunofluorescence or ELISA; or
- Detection of dengue virus genomic sequences in autopsy tissue serum or cerebrospinal fluid samples by polymerase chain reaction (PCR).

Case definition for dengue haemorrhagic fever

The following must all be present:

- Fever, or history of acute fever, lasting 2–7 days, occasionally biphasic.
- Haemorrhagic tendencies, evidenced by at least one of the following:
  — a positive tourniquet test[1]
  — petechiae, ecchymoses or purpura
  — bleeding from the mucosa, gastrointestinal tract, injection sites or other locations
  — haematemesis or melaena.
- Thrombocytopenia (100 000 cells per mm³ or less).[2]
- Evidence of plasma leakage due to increased vascular permeability, manifested by at least one of the following:
  — a rise in the haematocrit equal to or greater than 20% above average for age, sex and population;

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[1] The tourniquet test is performed by inflating a blood pressure cuff on the upper arm to a point midway between the systolic and diastolic pressures for 5 minutes. A test is considered positive when 20 or more petechiae per 2.5 cm (1 inch) square are observed. The test may be negative or mildly positive during the phase of profound shock. It usually becomes positive, sometimes strongly positive, if the test is conducted after recovery from shock.

[2] This number represents a direct count using a phase-contrast microscope (normal is 200 000–500 000 per mm³). In practice, for outpatients, an approximate count from a peripheral blood smear is acceptable. In normal persons, 4–10 platelets per oil-immersion field (100×; the average of the readings from 10 oil-immersion fields is recommended) indicates an adequate platelet count. An average of ≤3 platelets per oil-immersion field is considered low (i.e. <100 000 per mm³).

— a drop in the haematocrit following volume-replacement treatment equal to or greater than 20% of baseline;

— signs of plasma leakage such as pleural effusion, ascites and hypo-proteinaemia.

Case definition for dengue shock syndrome

All of the above four criteria for DHF must be present, plus evidence of circulatory failure manifested by:

• Rapid and weak pulse, and
• Narrow pulse pressure (<20 mmHg (2.7 kPa))

or manifested by:

• Hypotension for age,[1] and
• Cold, clammy skin and restlessness.

The spectrum of dengue haemorrhagic fever is shown in Figure 2.2.

Fig. 2.2
The spectrum of dengue haemorrhagic fever

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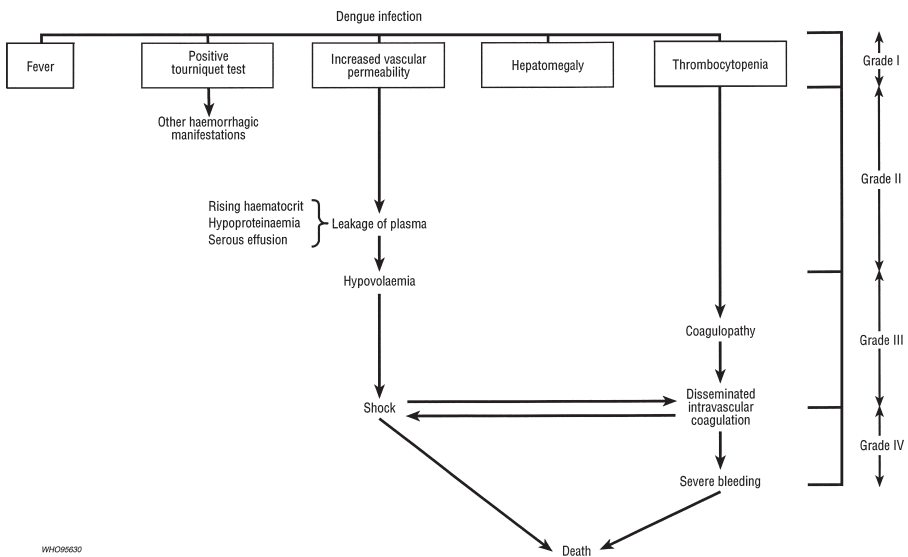
[1] See p. 21, bottom, for the definition of hypotension.

Guidance for diagnosis of DHF/DSS

The following manifestations have been selected as indicating a provisional diagnosis of DHF/DSS. They are not intended to be substitutes for the above case definitions. The use of these criteria may help clinicians to establish an early diagnosis, ideally before the onset of shock, as well as to avoid overdiagnosis.

Clinical

The following clinical observations are important indicators of DHF/DSS:

- High fever of acute onset
- Haemorrhagic manifestations (at least a positive tourniquet test)
- Hepatomegaly (observed in 90–96% of Thai and 67% of Cuban children with DHF)
- Shock.

Laboratory

These laboratory findings support the above clinical observations:

- Thrombocytopenia (100 000 cells per mm^3 or less)
- Haemoconcentration (haematocrit elevated at least 20% above average for age, sex and population).

The first two clinical observations, plus one of the laboratory findings (or at least a rising haematocrit), are sufficient to establish a provisional diagnosis of DHF. In monitoring haematocrit, one should bear in mind the possible effects of pre-existing anaemia, severe haemorrhage or early volume-replacement therapy. Moreover, pleural effusion observed on a chest X-ray, or hypoalbuminaemia, can provide supporting evidence of plasma leakage, the distinguishing feature of DHF. For a patient with a provisional diagnosis of DHF, if shock is present, a diagnosis of DSS is supported.

Reportable cases of DHF or DSS

Patients with a provisional diagnosis of DHF or DSS should be reported to the health authorities as cases of DHF or DSS if there is:

- Virological or serological evidence of acute dengue infection, or

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[1] Hypotension is defined to be a systolic pressure <80 mmHg (10.7 kPa) for those less than 5 years of age, or <90 mmHg (12.0 kPa) for those greater than or equal to 5 years of age. Note that narrow pulse pressure is observed early in the course of shock, whereas hypotension is observed later, or in patients who experience severe bleeding.

• History of exposure in a dengue endemic or epidemic area (during a period of epidemic transmission, or significant levels of endemic transmission, it is unlikely that many cases will have laboratory confirmation).

Grading severity of dengue haemorrhagic fever

DHF is classified into four grades of severity, where grades III and IV are considered to be DSS. The presence of thrombocytopenia with concurrent haemoconcentration differentiates grades I and II DHF from DF.

Grade I: Fever accompanied by non-specific constitutional symptoms; the only haemorrhagic manifestation is a positive tourniquet test and/or easy bruising.

Grade II: Spontaneous bleeding in addition to the manifestations of Grade I patients, usually in the forms of skin or other haemorrhages.

Grade III: Circulatory failure manifested by a rapid, weak pulse and narrowing of pulse pressure or hypotension, with the presence of cold, clammy skin and restlessness.

Grade IV: Profound shock with undetectable blood pressure or pulse.

Grading the severity of the disease at the time of discharge has been found clinically and epidemiologically useful in DHF epidemics in children in the WHO Regions of the Americas, South-East Asia and the Western Pacific, and experience in Cuba, Puerto Rico and Venezuela suggests that grading is also useful for adult cases.

Table 2.3
Criteria for differential diagnosis of dengue haemorrhagic fever and chikungunya fever[a]

Criteria	Dengue haemorrhagic fever (%)	Chikungunya fever (%)
Duration of fever:		
2–4 days	23.6	62.5
5–7 days	59.0	31.2
>7 days	17.4	6.3
Haemorrhagic manifestations:		
positive tourniquet test	83.9	77.4
scattered petechiae	46.5	31.3
confluent petechial rash	10.1	0.0
epistaxis	18.9	12.5
gum bleeding	1.5	0.0
melaena/haematemesis	11.8	0.0
Hepatomegaly	90.0	75.0
Shock	35.2	0.0

[a] Modified from Nimmannitya S et al. *American journal of tropical medicine and hygiene*, 1969, 18: 954–971.

Differential diagnosis of dengue haemorrhagic fever

Early in the febrile phase, the differential diagnosis for DHF/DSS includes a wide spectrum of viral, bacterial and parasitic infections. Chikungunya fever may be difficult to differentiate clinically from DF and mild or early cases of DHF (see Tables 2.2 and 2.3). A record sheet for documenting the symptoms of patients suspected of having DHF is presented in Annex 2. By the third or fourth day, laboratory findings may establish a diagnosis before shock occurs. Shock virtually rules out a diagnosis of chikungunya fever. Marked thrombocytopenia with concurrent haemoconcentration differentiates DHF/DSS from diseases such as endotoxin shock from bacterial infection or meningococcaemia.

Treatment

Loss of plasma volume

The major pathophysiological abnormality seen in DHF/DSS is an acute increase in vascular permeability leading to loss of plasma from the vascular compartment. Studies reveal a reduction in plasma volume of more than 20% in severe cases. The evidence that supports the existence of plasma leakage includes findings of pleural effusion and ascites by examination or radiography, haemoconcentration, hypoproteinaemia and serous effusion (at post mortem). The fact that no destructive or inflammatory vascular lesions are observed suggests that transient, functional vascular changes due to short-acting mediators occur. Plasma leakage can lead to shock, which, if uncorrected, leads to tissue anoxia, metabolic acidosis and death.

The haemostatic changes in DHF include three elements: vascular changes, thrombocytopenia and disorders of coagulation. All patients demonstrate an increase in capillary fragility, reflected by positive tourniquet tests and easy bruising. Most patients with DSS and some non-shock patients exhibit disseminated intravascular coagulation, as evidenced by concomitant thrombocytopenia, prolonged partial thromboplastin time, a decreased fibrinogen level and increased levels of fibrinogen degradation products. In cases of prolonged uncontrolled shock, disseminated intravascular coagulation can cause bleeding and may play an important role in the development of lethal shock. About one-third of patients who experience shock, mostly those in whom shock is refractory, manifest bleeding, mainly from the gastrointestinal tract. In the majority of patients who die, gastrointestinal haemorrhage is observed.

Early and effective replacement of plasma losses with plasma expander or fluid and electrolyte solution results in a favourable outcome in most cases. With adequate and appropriate fluid administration, DSS is rapidly reversible. Early and rapid resuscitation from shock and the correction of metabolic and electrolytic disturbances will prevent disseminated intravascular coagulation. The prognosis depends mainly on the early recognition and treatment of shock, which depend on careful monitoring and prompt action.

It is not necessary to hospitalize all patients with suspected DHF, since shock develops in only about one-third. The finding of a continuing drop in the platelet count concurrent with a rise in the haematocrit is an important indicator of the onset of shock. So that early signs of shock can be recognized,

patients should have repeated platelet and haematocrit determinations. Parents and other persons caring for patients should be advised to watch for signs of deterioration or warning signs of shock such as restlessness or lethargy, acute abdominal pain, cold extremities, skin congestion or oliguria. The critical period is usually on the day of defervescence, typically after the third day of illness.

Dengue haemorrhagic fever

Thirst and dehydration result from high fever, anorexia and vomiting; thus fluid intake by mouth should be ample. An electrolyte replacement solution or fruit juice is preferable to plain water. Oral rehydration solution, as for the treatment of diarrhoeal disease, is recommended.[1]

During the acute febrile phase there is some risk of convulsions. Antipyretics may be indicated in patients with hyperpyrexia, particularly those with a history of febrile convulsions. Salicylates should be avoided since they may cause bleeding and acidosis, or precipitate Reye or Reye-like syndrome. Paracetamol is preferable to reduce fever but should be used with caution, in the following doses:

<1 year	60 mg/dose
1–3 years	60–120 mg/dose
3–6 years	120 mg/dose
6–12 years	240 mg/dose.

A dose should be administered when body temperature is greater than 39 °C, but no more than 6 doses should be administered in a 24-hour period.

Patients should be closely observed for signs of shock. The critical period is the transition from the febrile to the afebrile phase of illness, which usually occurs after the third day. Haematocrit determinations are an essential guide to therapy at that stage, since they indirectly indicate the degree of plasma leakage and the corresponding need for intravenous fluid. A rising haematocrit usually precedes changes in blood pressure and pulse. The haematocrit should be determined daily from the third day of illness until the patient's fever has

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[1] If oral rehydration solution is to be given to children under 2 years of age, additional fruit juice or water should be given in the proportion of one volume for every two volumes of oral rehydration solution. Oral rehydration solution consists of the following, dissolved in 1 litre of potable water:

Sodium chloride	3.5 g
Trisodium citrate dihydrate	2.9 g
or 2.5 g sodium bicarbonate	
Potassium chloride	1.5 g
Glucose	20.0 g

It is important to give oral rehydration solution in small amounts at a steady rate (a teaspoonful every 1–2 minutes).

subsided for 1 or 2 days. If determination of the haematocrit is not possible, haemoglobin determination may be used, although it is less sensitive.

Parenteral fluid therapy can be given in an outpatient rehydration unit for patients in whom fever, vomiting or anorexia produce dehydration. The fluid used to correct dehydration is chosen according to the nature of the fluid loss. In cases of isotonic dehydration, 5% glucose (50 g/l) diluted 1:2 or 1:1 in physiological (normal) saline should be used. Bicarbonate-containing solutions should not be used for the initial intravenous management of dehydration in DHF, and should be reserved for cases where there are persistent fluid losses from diarrhoea. The necessary volume of replacement fluid is equivalent to the amount of fluid and electrolyte lost: thus, 10 ml/kg should be administered for each 1% of normal body weight lost. Maintenance fluid requirements, calculated according to the Halliday & Segar formula (Table 3.1), should be added to the replacement fluid volume. 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 replacement is necessary to avoid overhydration.

Example of volume replacement

A 2-year-old child (normal body weight, 10 kg) has Grade-II DHF with the following indications;

- High fever for 3 days
- Symptoms worsen on day 4 when temperature drops
- Physical examination finds: temperature 37°C, pulse rate 120/minute, blood pressure 100/70 mmHg (13.3/9.3 kPa), petechiae, a positive tourniquet test and the liver enlarged by 2 cm

Table 3.1
Calculations for maintenance 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. Reproduced by permission of *Pediatrics*.

- Laboratory examination finds: 0–1 platelets/oil-immersion field (100×), haematocrit 45% (baseline 35%).

Administration of intravenous fluid is necessary as the patient has a >20% increase in haematocrit and early signs of circulatory disturbance (i.e. rapid pulse and worsening condition).

The following steps should be taken:

- Calculate the intravenous fluid needed, on the assumption of 5% isotonic dehydration:
  — replacement fluid: 10 × 50   = 500 ml
  — daily maintenance fluid: 10 × 100   = 1000 ml
  — total fluid needed: 500 + 1000 = 1500 ml/day
- Order 500 ml of 5% glucose (50 g/l) diluted 1:2 or 1:1 in physiological saline (fluid volume should not exceed 500 ml per order, *or* should not be for a period longer than 6 hours; orders should specify type of solution and rate of administration).
- Check vital signs every 1–2 hours and haematocrit every 3–4 hours; monitor urine output and patient's condition.
- Adjust intravenous fluid administration according to vital signs, haematocrit and urine output (see Fig. 3.1).

Indications for hospitalization

Hospitalization for bolus intravenous fluid therapy may be necessary where significant dehydration (>10% of normal body weight) has occurred and rapid volume expansion is needed. Signs of signficant dehydration include:

- Tachychardia
- Increased capillary refill time (>2 s)
- Cool, mottled or pale skin
- Diminished peripheral pulses
- Changes in mental status
- Oliguria
- Sudden rise in haematocrit or continuously elevated haematocrit despite administration of fluids
- Narrowing of pulse pressure (<20 mmHg (2.7 kPa))
- Hypotension (a late finding representing uncorrected shock).

Dengue shock syndrome

Shock is a medical emergency. The immediate administration of intravenous fluid to expand plasma volume is essential. Children may go in and come out of shock during a 48-hour period. Consequently close observation round the clock by qualified nursing staff is imperative.

Fig. 3.1

Volume replacement flow chart for a patient with DHF and a >20% increase in haematocrit


```
                        ┌─────────────┐
                        │   5% fluid  │
                        │   deficit   │
                        └─────────────┘

              Initiate intravenous therapy:
              5% glucose in physiological saline
                    (6–7 ml•kg⁻¹•h⁻¹)

   ┌─────────────────────┐            ┌─────────────────────┐
   │     IMPROVEMENT     │            │   NO IMPROVEMENT    │
   │ Haematocrit falls,  │            │ Haematocrit or pulse│
   │ pulse rate and blood│            │ rate rises, pulse   │
   │ pressure stable,    │            │ pressure falls below│
   │ urine output rises  │            │ 20 mmHg (2.7 kPa),  │
   └─────────────────────┘            │ urine output falls  │
                                      └─────────────────────┘
```

5% fluid deficit

Initiate intravenous therapy:
5% glucose in physiological saline
(6–7 ml•kg^{-1}•h^{-1})

IMPROVEMENT
Haematocrit falls, pulse rate and blood pressure stable, urine output rises

NO IMPROVEMENT
Haematocrit or pulse rate rises, pulse pressure falls below 20 mmHg (2.7 kPa), urine output falls

Reduce intravenous therapy
(5 ml•kg^{-1}•h^{-1})

VITAL SIGNS OR HAEMATOCRIT WORSEN

Increase intravenous therapy
(10 ml•kg^{-1}•h^{-1})

IMPROVEMENT

IMPROVEMENT

NO IMPROVEMENT

Reduce intravenous therapy
(3 ml•kg^{-1}•h^{-1})

Increase intravenous therapy
(15 ml•kg^{-1}•h^{-1})

FURTHER IMPROVEMENT

UNSTABLE VITAL SIGNS
Urine output falls, signs of shock

Discontinue intravenous therapy after 24–48 h

Establish central venous access and urinary catheter, administer rapid fluid bolus
(see fig. 3.2)

VITAL SIGNS AND HAEMATOCRIT STABLE
Adequate diuresis

HAEMATOCRIT RISES
(or distress)

HAEMATOCRIT FALLS

Intravenous colloid therapy

Blood transfusion

IMPROVEMENT

WHO95632

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Immediate replacement of plasma loss

Fluids used for rapid volume expansion include the following:

- Physiological saline
- Ringer's lactate or Ringer's acetate
- 5% glucose solution diluted 1:2 or 1:1 in physiological saline
- Plasma, plasma substitutes (e.g. dextran 40) or 5% albumin (50 g/l)

Ringer's lactate, Ringer's acetate or 5% glucose diluted in physiological saline should be administered as a rapid (<20 minutes) intravenous bolus (10–20 ml/kg). Another bolus bringing the fluid dose to 20–30 ml/kg can be administered if necessary. If shock persists, oxygen should be given and the haematocrit should be checked. If the haematocrit is rising, plasma, plasma substitutes or 5% albumin (10–20 ml/kg) should be administered as a rapid bolus, repeated if necessary for a total dose of 20–30 ml/kg of colloidal solution. If shock still persists, haematocrit values should be reviewed for evidence of decline, which may indicate internal bleeding. Fresh whole-blood transfusion (10 ml/kg, if the haematocrit is still above 35%) may be needed in such cases. When shock ceases, the intravenous infusion rate should be reduced and adjusted according to the haematocrit level, urine output and vital signs (see Fig. 3.2).

Continued replacement of further plasma loss

Plasma loss may continue for 24–48 hours, requiring continued fluid administration. Determination of central venous pressure may be necessary in the management of refractory shock.

The administration of intravenous fluids should be discontinued when the haematocrit level drops to approximately 40%, with stable vital signs. Good urine flow indicates sufficient circulating fluid. In general, intravenous fluid therapy should not be needed for more than 48 hours after the termination of shock. Reabsorption of extravasated plasma occurs (manifested by a further drop in haematocrit after intravenous fluid has been stopped), and hypervolaemia, pulmonary oedema or heart failure may be caused if more fluid is given. *It is extremely important that a drop in haematocrit at this later stage is not interpreted as a sign of internal haemorrhage.* Strong pulse and blood pressure and adequate diuresis are good signs during this phase. They rule out the likelihood of gastrointestinal haemorrhage, which is found mostly during the shock stage. The return of the patient's appetite is also a sign of recovery.

Correction of electrolyte and metabolic disturbances

Hyponatraemia and metabolic acidosis can occur in severe cases. Electrolyte levels and partial pressures of blood gases should be determined periodically in severely ill patients and in patients who do not seem to respond as promptly as

Fig. 3.2
Volume replacement flow chart for a patient with DSS

```
                    ┌─────────────────────────────┐
                    │     UNSTABLE VITAL SIGNS     │
                    │      urine output falls,     │
                    │        signs of shock        │
                    └─────────────────────────────┘
                                  │
        Immediate, rapid volume replacement*: 10–20 ml•kg⁻¹
         Ringer's lactate, Ringer's acetate or 5% glucose diluted in
         physiological saline, as intravenous bolus (repeat if necessary)

          ┌─────────────────────┐        ┌─────────────────────┐
          │     IMPROVEMENT      │        │    NO IMPROVEMENT    │
          └─────────────────────┘        └─────────────────────┘
                    │                                │
         Adjust intravenous therapy               Oxygen
             (follow Fig. 3.1.)

                        ┌────────────────────┐  ┌────────────────────┐
                        │  HAEMATOCRIT FALLS │  │  HAEMATOCRIT RISES │
                        └────────────────────┘  └────────────────────┘
                                 │                        │
                          Blood transfusion      10–20 ml•kg⁻¹ plasma,
                           (10 ml•kg⁻¹,          plasma substitutes or 5%
                      if haematocrit is still >35%) albumin, as intravenous bolus
                                                     (repeat if necessary)
```

In cases of acidosis, hyperosmolar or Ringer's lactate solution should not be used.　　　WHO 95633

expected. These indicators 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 uncorrected, may lead to disseminated intravascular coagulation and to a more complicated course. In general, early volume replacement and the early correction of acidosis with sodium bicarbonate result in a favourable outcome.

Sedatives

Sedative therapy is needed in some cases to restrain an agitated child. Restlessness may be associated with insufficient tissue perfusion, which may require rapid volume replacement, and agitation may also be an early sign of hepatic failure. Hepatotoxic drugs and long-acting sedatives should be avoided. A single dose of chloral hydrate (12.5–50 mg/kg), orally or rectally, is recommended (the total dose not exceeding 1 g).

Oxygen therapy

Oxygen therapy should be given to all patients in shock, but the nursing staff involved should be aware that an oxygen mask or a tent may increase the anxiety of the patient and should be prepared to manage this eventuality.

Blood transfusion

Blood grouping and matching should be carried out as a routine precaution for every patient in shock, but blood transfusion is only indicated in cases with significant clinical bleeding. Internal bleeding may be difficult to recognize in the presence of haemoconcentration. A drop in haematocrit, e.g. from 50% to 40%, with no clinical improvement despite adequate fluid administration, indicates a significant internal haemorrhage. Transfusion with fresh whole blood is preferable, and the amount given should be such that the normal red-blood-cell concentration is not exceeded. Fresh frozen plasma or concentrated platelets may be indicated in cases where coagulopathy causes massive bleeding. Disseminated intravascular coagulation is usual in severe shock and may play an important part in the development of massive bleeding or lethal shock. Invasive devices and procedures should be limited to those that are strictly necessary as they may lead to severe bleeding in the presence of coagulopathy. The results of haematological tests (prothrombin time, partial thromboplastin time and thrombin time) should be studied in all patients with shock in order to document the onset and severity of disseminated intravascular coagulation.

Essential laboratory tests

In assessing a patient's condition, the following tests are recommended:

- Haematocrit
- Serum electrolytes and blood gas studies
- Platelet count, prothrombin time, partial thromboplastin time and thrombin time
- Liver function tests—serum aspartate aminotransferase, serum alanine aminotransferase and serum proteins.

Monitoring patients in shock

Frequent recording of the vital signs and determination of the haematocrit are important in evaluating the results of treatment. If patients show signs of shock, vigorous therapy should be instituted promptly. Patients should then be under constant and careful observation until there is a reasonable certainty that the danger has passed. The following measures should be taken routinely in such instances:

- Pulse, blood pressure and respiration should be recorded every 30 minutes (or more often) until shock is overcome.
- Haematocrit or haemoglobin levels should be determined every 2 hours for the first 6 hours, then every 4 hours until stable.
- A fluid balance sheet should be kept, recording the type of fluid and the rate and volume of its administration in order to evaluate the adequacy of fluid replacement. The frequency and volume of urine output should also be recorded, and a urinary catheter may be needed in cases of refractory shock.

Unusual manifestations of dengue haemorrhagic fever

The management of DHF patients with acute hepatic failure poses a difficult problem. The early detection of highly elevated levels of serum alanine aminotransferase in patients who exhibit an unusual change in consciousness or abnormal neurological signs (e.g. hyperreflexia) will, if acted upon, have an impact on prognosis and survival. These patients should be given intravenous fluid with extreme caution in order to avoid the excessive fluid replacement that can cause brain oedema and encephalopathy. Colloidal solution should be used early to correct plasma loss. Fluid and electrolyte replacement therapy may prevent mild hepatic coma. In severe cases with a progressive change in consciousness, exchange blood transfusion has been tried and appears to increase survival rate. Most patients with acute liver failure die from severe haemorrhage, renal failure, brain oedema (and sometimes herniation), pulmonary oedema or a superimposed infection.

Outpatient and inpatient flow charts

Outpatient and inpatient flow charts are included in Annexes 3 and 4 to provide guidance on the diagnosis and treatment of DHF/DSS. Physicians may use these charts to become familiar with the decisions involved in providing appropriate medical care to these patients. They may also be useful for training nurses, medical students and paramedical personnel in the identification and treatment of severe cases of dengue virus infection. They are designed for primary and secondary health units where sophisticated electronic monitoring

equipment is not available. If highly technical intensive care is available, judgement must be used to determine the best but least invasive treatment programme for each patient. Additional guidance may be sought from the WHO Collaborating Centre for Case Management of Dengue/DHF/DSS (see Annex 6).

Criteria for discharging inpatients

The following criteria should be met before patients recovering from DHF/DSS are discharged:

- Absence of fever for at least 24 hours without the use of antifever therapy (cryotherapy or antipyretics)
- Return of appetite
- Visible clinical improvement
- Good urine output
- Stable haematocrit
- Passing of at least 2 days after recovery from shock
- No respiratory distress from pleural effusion or ascites
- Platelet count of more than $50\,000$ per mm^3.

Laboratory diagnosis

The two basic methods for establishing a laboratory diagnosis of dengue infection are detection of the virus (e.g. culture) or detection of anti-dengue antibodies (serology). Until recently, detection of the virus implied solely the recovery of the virus by culture; however, current procedures can detect dengue virus RNA and specific dengue virus antigens. Consequently, these procedures are likely to become routine as the necessary reagents and instrumentation become more widely available. An understanding of the kinetics of dengue virus replication and host responses, as well as of the collection and handling of specimens, will help clarify the strengths and weaknesses of the two laboratory methods for diagnosing dengue infection.

Kinetics of dengue virus replication and host response

By the time a person infected with dengue virus develops fever, the infection is widely disseminated. The virus is found in serum or plasma, in circulating blood cells and in selected tissues, especially those of the immune system, for approximately 2–7 days, roughly corresponding to the period of fever. Dengue virus usually infects the peripheral blood mononuclear cells within a few days of the infective mosquito bite, and the infection rate revealed by antigen staining is usually 1–10 infected cells per 10 000 cells. Detectable levels of anti-dengue antibodies appear after several days of fever. Two patterns of immune response are distinguished: primary and secondary (anamnestic) (see Figure 4.1).

Persons never previously infected with a flavivirus, nor immunized with a flavivirus vaccine (e.g. yellow fever, Japanese encephalitis, tick-borne encephalitis), mount a primary antibody response when infected with dengue virus. The dominant immunoglobulin isotype is IgM. Anti-dengue IgM detectable by IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) appears in half of the patients with a primary infection while they are still febrile; in the other half, it appears within 2–3 days of defervescence. In one series of dengue patients (infection confirmed by virus isolation or paired serum serology), 80% had detectable levels of IgM antibody by day 5 of illness, and 99% by day 10. Once detectable, IgM levels rise quickly and appear to peak about 2 weeks after the onset of symptoms; they then decline to undetectable

Fig. 4.1
Primary and secondary immunological response in dengue virus infection
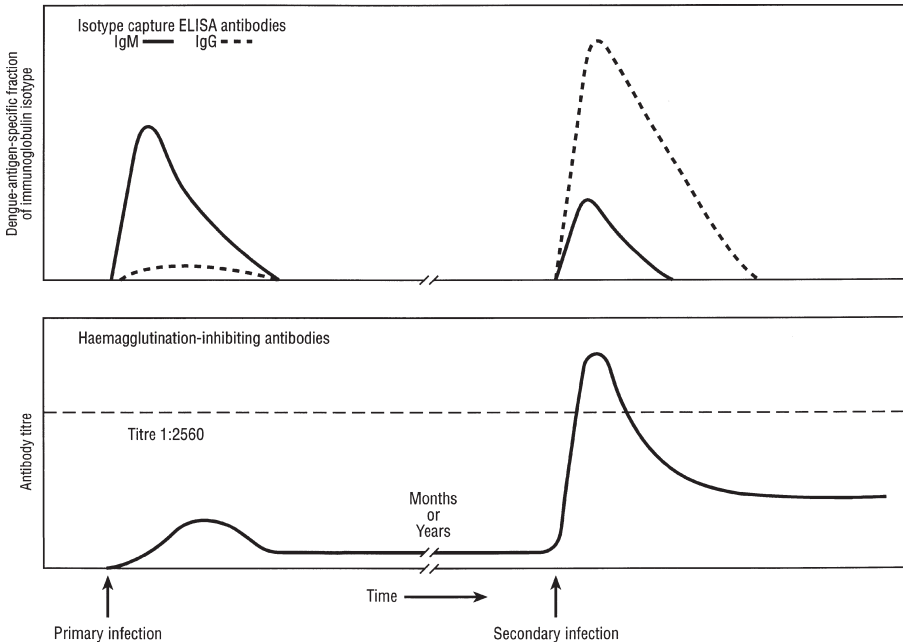
levels over 2–3 months. Anti-dengue antibodies inhibit the haemagglutination of gander red blood cells by dengue virus; haemagglutination-inhibiting antibodies appear simultaneously with the detection of IgM by enzyme immunoassay. Anti-dengue IgG appears shortly afterwards. The physiological definition of a primary infection is therefore one characterized by a high molar fraction of anti-dengue IgM and a low molar fraction of anti-dengue IgG.

In primary infection with dengue virus, serological tests may yield results that indicate a specific dengue serotype with specimens obtained early in the disease. In other cases, cross-reactive antibodies, often apparent in the first 1–2 months after infection, may confound determination of the serotype. In such cases, a monotypic antibody specific for the infecting serotype may be detected 3–6 months after infection. Therefore, specimens obtained during late convalescence from patients with a primary seroresponse pattern may be useful in determining the infecting dengue virus serotype.

Individuals with immunity due to previous flavivirus infection or immunization mount a secondary (anamnestic) antibody response when infected with dengue virus. In secondary flavivirus infections, which account for most cases of DHF, the dominant immunoglobulin isotype is IgG. Anti-dengue IgM

appears in most instances, and while the kinetics of IgM production are similar to those observed in primary infections, the levels of IgM are dramatically lower. In contrast to primary infection, secondary infection with dengue virus results in the appearance of high levels of anti-dengue IgG before, or simultaneously with, the IgM response. Once detected, IgG levels rise quickly, peak about 2 weeks after the onset of symptoms and then decline slowly over 3–6 months. Anti-dengue IgM levels also peak at about 2 weeks, begin to wane thereafter and are detectable in about 30% of patients 2 months after the onset of symptoms. The physiological definition of a secondary infection is one characterized by a low molar fraction of anti-dengue IgM and a high molar fraction of IgG that is broadly reactive to flaviviruses.

Both IgM and IgG anti-dengue antibodies neutralize dengue virus. The neutralizing antibodies rapidly increase as fever subsides and interfere with the recovery of the virus from serum.

Collection and handling of specimens

When collecting blood specimens from patients with suspected dengue infections, health care workers should:

- 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 should allow the collection of at least two serum samples for comparison, and ideally will provide for an adequate interval between sera. Similar practices would apply to outpatients in clinics. 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.

An abbreviated case history, including the following information, should accompany specimens: the patient's name and registration number, address, age, sex, date of onset of illness, date of hospitalization, attending physician's name, date of the collection of the specimen, and concise clinical findings.

Blood may be collected in tubes or vials or on filter-paper. High-quality absorbent paper has been used for many years to facilitate the shipment of blood specimens to central laboratories for serology. Blood or specimens may also be mailed to a laboratory in sterile, plastic specimen vials or tubes, in accordance with pertinent postal regulations. In the absence of microbial

contamination, exposure to ambient temperatures for up to 7 days while in transit will not significantly alter the results of standard dengue serology tests. Many laboratories now prefer to receive blood specimens in vials or tubes rather than blotted on paper, since the latter require special pretest processing. Annex 5 shows a sample request form for laboratory examination and an arbovirus laboratory reporting form, for use with filter-paper discs. If tubes or vials are used, similar information should be provided.

Specimen-collection procedures: tubes or vials

- Aseptically collect 2–5 ml or more of venous blood.
- Use adhesive tape marked with pencil or indelible ink or a typewritten or printed self-adhesive label to identify the container. At a minimum, the name of the patient, the identification number and the date of collection should be indicated.
- Use tubes or vials with screw-caps, if possible. Fix the cap with adhesive tape, wax or other sealing material to prevent leakage during transport.
- If a specimen cannot be analysed or shipped within 24 hours of being drawn, the serum should be separated from the cells and stored frozen.
- Ship specimens for culture or serology on wet ice to a laboratory as soon as possible.

Do not send frozen whole blood, as the resulting haemolysis can interfere with some tests. Specimens intended only for serology may tolerate shipment at ambient temperatures, particularly if an antiseptic is added to each specimen (e.g. sodium azide, thiomersal).

Specimen-collection procedures: filter-paper

- With a pencil, write the patient's initials or number on 2 or 3 discs or strips of standardized absorbent paper.
- Collect sufficient finger-tip blood (or venous blood in a syringe) on the filter-paper to saturate it through to the reverse side.
- Allow the discs or strips to dry in a place protected from direct sunlight and insects. Preferably, the blood-soaked papers should be placed in a stand that allows aeration of both sides. For unusually thick paper, a drying chamber may be useful, e.g. desiccator jar, air-conditioned room, warm-air incubator.
- Place the dried strips in plastic bags and staple them to corresponding laboratory examination request forms. Once dried, the plastic-enclosed strips may be stored at ambient temperature and mailed to the laboratory.

A question frequently posed concerns the minimum volume of serum required for diagnostic tests. Most assays require 0.1 ml of undiluted specimen. Adequate specimen volume, i.e. 0.3 ml to 2.0 ml of serum, should be submitted

to permit tests in several assays or to allow repeated testing if necessary. Filter-papers with an absorbency equivalent to Whatman No. 3 or Nobuto Type 1 should be used.[1]

Handling specimens for virus culture

Because dengue virus is heat-labile, specimens awaiting transport to the laboratory, including anticoagulated whole blood for the culture of leukocytes, should be kept in a refrigerator or packed in wet ice. For storage up to 24 hours, specimens to be used for virus isolation are preferably kept at $+4\,°C$ to $+8\,°C$; for longer storage, serum and tissue specimens should be frozen at $-70\,°C$. In the latter case, they should be so maintained as to prevent thawing. If specimens are frozen with dry ice, they should be placed in a gas-impermeable secondary container (e.g. a heat-sealed plastic bag), since a low pH will inactivate dengue viruses. These storage conditions are to be considered optimal; failure to adhere to them does not result in the complete inactivation of dengue virus in specimens. Dengue virus has been recovered from clinical specimens packed in wet ice or maintained at ambient temperatures for several days.

Diagnostic approach: virus detection versus serology

Detection of dengue virus by culture is the definitive diagnostic test, but practical considerations limit its use. Most importantly, the period when dengue virus can be successfully detected is brief. Within a day or two after the subsidence of fever, rising levels of antibody interfere with virus culture. Furthermore, as noted above, dengue virus is generally heat-labile and special precautions must be taken against the thermal inactivation of specimens. Lastly, since laboratories equipped and staffed to culture viruses are expensive to develop and maintain, their services are not widely available.

Detection of dengue RNA using specific oligonucleotide primers, reverse transcriptase and thermostable polymerase—a test known as the reverse transcription–polymerase chain reaction (PCR) amplification assay—has been successfully employed in several laboratories. The test uses RNA extracted from serum, plasma or cells. Although detection of dengue RNA by this technique is no less complex or expensive than virus culture, it is faster. Without proper precautions, however, contamination can lead to false-positive results. Nevertheless the procedure may be amenable to commercialization as a kit. More-

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[1] To prepare blood samples on filter-paper for ELISA assay (IgG or IgM):

- Elute discs in $1.0\,ml$ of phosphate-buffered saline for 60 minutes at room temperature, or overnight at $4\,°C$.
- Remove discs and centrifuge at $600g$ for 15 minutes to remove any particulate matter.

This results in approximately a $1:20$ final serum dilution. Each laboratory must standardize the filter-paper technique against results with a panel of venous blood specimens.

over, for technical reasons (discussed below), the test procedure may be particularly useful for detecting viraemia after the subsidence of fever, or in situations where sample handling has not been optimal for virus culture.

Dengue RNA or antigen may also be identified in individual cells using *in situ* hybridization or immunocytochemistry. The former method is theoretically more sensitive, but few laboratories have experience in applying the technique to the detection of dengue RNA in infected tissues. In contrast, antigen detection by immunocytochemistry is simpler, has been employed for a number of years and, with new commercially available reagents, may offer the same specificity as virus isolation.

Serological diagnosis is unhindered by the limitations of virus culture and other direct detection methods. The timing of specimen collection can be more flexible because anti-dengue antibody responses last for at least several weeks after the onset of illness. Immunoglobulins are not easily inactivated by the harsh treatment of specimens: a serum is usually suitable for testing even after prolonged exposure to tropical temperatures. The services of serology laboratories are also more readily available, the assay techniques are relatively simple and some reagents are commercially available.

On the other hand, serological tests may produce false-positive results, which may be due to polyclonal B-cell activation or cross-reactive antibodies elicited by certain group, complex and subcomplex epitopes common to flaviviruses. Shared epitopes, especially those of the envelope glycoprotein (the major structural antigen), cause early antibodies elicited by infection with one dengue serotype to cross-react with other serotypes. Moreover, antibodies elicited by other flaviviruses (e.g. Japanese encephalitis virus) may cross-react with dengue virus. Another factor confounding flavivirus serology is a physiological principle termed "original antigenic sin", i.e. many B-cell clones responding to a first flavivirus infection will be restimulated to synthesize early antibody with a greater affinity for the first infecting virus than for the current infecting virus in every subsequent flavivirus infection. False-positive reactions can confound the diagnosis of dengue, especially in areas where other flaviviruses are present. By specifically detecting anti-dengue IgM responses, more accurate diagnosis of flavivirus infections may be achieved, although this may not allow determination of the specific dengue serotype causing the infection.

In summary, serological diagnosis is in general less specific than diagnosis by culture. The serological diagnosis of dengue in populations exposed to additional flaviviruses is very challenging—some degree of uncertainty is inevitable. Greater confidence in serological diagnosis may be gained by using neutralization tests (see below), which are usually able to distinguish between the immune responses in primary infections to different dengue serotypes. All too often, however, the most precise serological diagnosis many laboratories can render is *acute flavivirus infection*, rather than *acute dengue infection*.

The selection of laboratory methods must be tailored to meet the objectives of the clinician and the constraints of the clinical specimens available. More-

Table 4.1
Suitable specimens for culture

Source	Material
Patient	Serum, plasma, leukocytes washed to remove antibody, cerebrospinal fluid[a]
Autopsy	Homogenized or minced tissues, e.g. liver, lung, spleen, lymph nodes, thymus; cerebrospinal fluid, pleural fluid, serum, plasma
Vector mosquito	Homogenized pooled mosquitos

[a] Where clinically indicated.

over, the certainty and speed of diagnosis must be balanced against the cost and availability of the tests. Determinations of both the virus *and* the antibody type are preferable to either approach alone. Moreover, the use of alternative methods to confirm or refute test results—e.g. MAC-ELISA and the haemag-glutination-inhibition (HI) test—further improves the quality of a laboratory diagnosis.

Laboratory safety precautions

The collection and processing of blood and other specimens places health care workers, particularly those in the laboratory, at risk for exposure to potentially infectious materials. To minimize the risk of infection, safe laboratory tech-niques must be practised.[1] Such techniques include the use of personal protec-tive equipment and appropriate containers for collecting and transporting samples and adherence to proper procedures for the separation of serum, the handling of glass and sharp instruments and the decontamination and disposal of potentially infectious materials.

Technical aspects of available assays

Isolation of virus

Since all patients with dengue virus infection have a period of viraemia, the isolation of dengue virus from clinical specimens is frequently possible. Factors favouring the successful isolation of virus are collection of the specimen early in the course of disease (usually within 5 days after the onset of fever) and proper handling and prompt delivery of the specimen to the laboratory. Suitable specimens for culture are shown in Table 4.1.

Different methods of confirming the presence of dengue virus are given in Table 4.2. The choice of method depends on the local availability of mosqui-

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[1] Detailed guidelines are presented in *Laboratory biosafety manual*, 2nd ed. Geneva, World Health Organization, 1993.

Table 4.2
Methods for isolation of dengue virus

Method	Result confirming presence of dengue virus
Inoculation of mosquitos (adults or larvae)	Detection of antigen in head squash by serotype-specific immunofluorescence
Inoculation of various mammalian or insect cell cultures	Detection of antigen by antibody staining
	Cytopathic effect; identification of virus upon subpassage
	Plaque formation; identification of virus upon subpassage
Intracranial inoculation of suckling mice	Presence of antigen in brain detected by antibody staining
	Symptoms or signs indicating encephalitis
	Identification of virus upon subpassage

tos, cell cultures and mice. Precautions must always be taken to prevent the laboratory contamination of specimens.

The inoculation of clinical specimens into adult or larval mosquitos is the most sensitive dengue virus culture technique. Suitable specimens for inoculation include serum, plasma, other normally sterile body fluids (e.g. cerebrospinal fluid, pleural fluid), peripheral blood leukocytes and tissue homogenates. Infection is detected by immunofluorescence of a tissue smear prepared from the crushed head of the mosquito (head squash). Generally, mosquitos in the genus *Toxorhynchites* are used. Their large size facilitates inoculation; moreover, because they are not haematophagous, inoculated mosquitos can be handled safely. Finally, the culture of specimens in mosquitos reduces the risk of laboratory errors due to the cross-contamination of cultures. Nevertheless, because the inoculum volume is small, high-sensitivity culture requires the inoculation of 5–20 mosquitos per specimen. However, the raising of *Toxorhynchites* mosquitos is labour-intensive because the larvae are carnivorous and the insectary must therefore maintain production of a second mosquito species as a food source. Adult *male* mosquitos of *Ae. aegypti* and *Ae. albopictus* species can be inoculated with a sensitivity and safety equal to those obtained with *Toxorhynchites* spp. Although male *Ae. aegypti* and *Ae. albopictus* mosquitos are easier to maintain, they require more delicate inoculation techniques because of their smaller size.

In laboratories where colonized mosquitos are not available, specimens may be inoculated in any of several widely available mosquito cell lines (e.g. C6/36 or AP-61 cells). This approach may be slightly less sensitive than inoculation in live mosquitos, but with a larger possible inoculum volume it has adequate sensitivity for routine virus isolation. Since only some dengue virus isolates induce a cytopathic effect in mosquito cells, cell cultures must be screened for specific evidence of infection by an immunoassay. If inoculated cells can be assayed in their culture vessel, additional efficiency is achieved. Because mos-

quito cell lines may be propagated at ambient tropical temperatures (25–34 °C), it is even possible for mosquito cells to be carried to the bedside or into the field, although a sterile culture environment would still be required. The culture of clinical specimens (e.g. anticoagulated whole blood) at the bedside is a means of virus culture that has yet to be fully exploited.

The culture of specimens in vertebrate cell lines (e.g. VERO, LLC-MK$_2$) and in intracerebrally inoculated newborn mice are the least sensitive methods. On the other hand, the appearance of plaques in these cells lines, or encephalitis in mice, does constitute presumptive evidence of the presence of an arbovirus.

Once a virus has been isolated in culture, serotype-specific anti-dengue monoclonal antibodies[1] are used to examine mosquito head squashes, infected cells, infected cell-culture fluids, or mouse-brain touch preparations for identification. The binding of a specific monoclonal antibody is revealed with a second labelled antibody. Serotype-specific assays may be supplemented with a flavivirus-group-reactive or dengue-complex-reactive monoclonal antibody to serve as a positive control.

Antigen detection in fixed tissues

Flavivirus antigens may be detected in peripheral blood leukocytes from patients with dengue, especially during the febrile phase of illness. Dengue antigen also may be found in the liver and lung at autopsy, and less often in the thymus, lymph nodes, skin, spleen, bone marrow and serosa. Fluorescent antibody, immunoperoxidase and avidin–biotin enzyme assays have been standardized in a number of research laboratories, permitting the visualization of viral antigen in acetone-fixed leukocytes, snap-frozen tissue and even formalin-fixed tissue after limited protease-digestion (to reveal antigens cross-linked by formalin). Tissues should be collected as soon after death as possible, since a delay of even 24 hours compromises antigen staining.

If facilities for virus isolation are available, the tissues should be divided, with a portion placed in fixative (buffered formalin, if not otherwise specified by the immunohistochemistry laboratory) for antigen staining and a portion placed in ice-cold, sterile, isotonic buffer for virus isolation. When possible, heart blood and other body fluids (cerebrospinal fluid, pleural fluid or ascites) should be collected for culture and IgM detection. In the event of the failure of virus isolation, assay of these fluids for IgM and IgG antibodies with a panel of relevant antigens offers the best opportunity to identify the infecting virus.

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[1] Dengue type-specific monoclonal antibodies or hybridomas may be obtained from Division of Vector-borne Infectious Diseases, Centers for Disease Control and Pevention, Fort Collins, CO 80522, USA (fax: (303) 221 6428).

Reverse transcription–PCR amplification of dengue RNA

PCR amplification can be used to amplify RNA if reverse transcription of the target RNA to cDNA is used as the initial step. If dengue-specific oligonucleotide primers are employed, reverse transcription–PCR amplification assays can detect a small number of dengue RNA molecules among millions of other RNA molecules. Unlike more limited biological amplification through culture, million-fold enzymatic amplification can be accomplished in a matter of hours. Moreover, since nucleic acid can be separated from proteins in a specimen-preparation step, dengue RNA can be purified from immune complexes. Thus, reverse transcription–PCR amplification introduces for the first time a technique with the potential to detect dengue virus during convalescence, when circulating antibodies otherwise preclude its detection. These assays demand meticulous technique, however, as they are highly prone to false-positive results due to contamination.

Several laboratories have published reverse transcription–PCR amplification protocols to detect dengue viraemia. These methods feature two strategies for identification of the four dengue serotypes: combination of the four serotype-specific oligonucleotide primer pairs in a single reaction tube, or use of a universal dengue oligonucleotide primer pair, which requires a subsequent step to classify positives with serotype-specific oligonucleotides. Both approaches have proved highly successful in preliminary trials. Nevertheless, reverse transcription–PCR amplification of dengue RNA must still be considered an investigational approach; its fuller application awaits greater experience, consensus on the optimal preparation of specimens and determination of the oligonucleotide primer sequences capable of detecting all or most dengue genotypes in circulation.

Serological tests

Diagnosis of dengue by the recovery of virus or the detection of antigens is preferable to serological diagnosis; however, the latter is used to confirm most dengue infections. Although serological assays can, in many instances, provide a presumptive diagnosis of *recent* infection from a single serum specimen, a conclusive diagnosis of *acute* infection can be made only when rising levels of anti-dengue immunoglobulin are detected in paired sera. The diagnosis of acute dengue infection is possible on this basis because antibody levels are known to rise only for 2–4 weeks following infection. The subsequent decline to baseline levels requires another 6–24 weeks, during which time single serum assays may still reveal elevated anti-dengue IgM or IgG antibody. The most commonly used serological techniques for the diagnosis of dengue infection are MAC-ELISA and the HI test. Descriptions of these and less commonly used serological tests follow.

MAC-ELISA

In primary or secondary dengue infections, MAC-ELISA can measure a rise in dengue-specific IgM, even in sera samples collected at 1-day to 2-day intervals in the acute phase. Specimens collected over an interval of 2–3 days spanning the day of defervescence are also usually diagnostic in MAC-ELISA. In cases where only a single specimen is available, detection of anti-dengue IgM permits the diagnosis of recent dengue infection even in primary infections where the level of HI antibody would not be diagnostic.

Because anti-flavivirus IgM is complex-specific (i.e. IgM elicited by dengue virus can generally be differentiated from IgM elicited by Japanese encephalitis, St Louis encephalitis, Murray Valley encephalitis, West Nile or Kunjin viruses), diagnosis of acute or recent dengue infection can be made by tests against a panel of antigens. This feature assumes importance when evaluating cases where symptoms may be attributable to different viruses (e.g. fever and rash may be caused by infection with dengue virus or West Nile virus; fever, clouded consciousness and neurological deficits may be caused by infection with dengue virus or any virus of the mosquito-borne flavivirus encephalitis complex). It should be emphasized, however, that some flavivirus cross-reactivity occurs,

Table 4.3
Interpretation of MAC-ELISA results[a]

IgM antibody response	S1–S2 interval[b]	IgM to IgG ratio	Interpretation
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, probably secondary

[a] Criteria derived empirically from data collected at the U.S. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. Laboratories should assess the sensitivity of their assay with standard sera available from Department of Virology, U.S. Armed Forces Research Institute of Medial Sciences, 315/6 Rajvithi Road, Bangkok 10400, Thailand (fax: 66-2-664-4760), WHO Collaborating Centres for Arboviruses and/or Haemorrhagic Fever Reference and Research, or WHO Collaborating Centres for New, Emerging and Re-emerging Diseases (see Annex 6). To infer that dengue virus elicited anti-flavivirus IgM, laboratories must test with a regionally appropriate panel of flavivirus antigens. Results expressed as the ratio of optical density (OD) (test sample) : OD (positive reference sample) will reduce inter-assay variation. Results greater than 2 standard deviations from the mean of negative control sera are presumed to indicate elevated levels of anti-flavivirus IgG or IgM. Laboratories should determine appropriate criteria for categorizing primary and secondary seroresponses. Laboratories should also assess their assay's specificity with a serum panel lacking flavivirus antibodies.
[b] Guidelines do not apply to intervals between acute (S1) and convalescent (S2) specimens greater than 14 days.

and the results of other serological, virological and epidemiological tests should also be used to determine conclusively the infecting virus.

A further advantage of MAC-ELISA is that it may be used without modification to detect anti-flaviviral IgM in cerebrospinal fluid. Since IgM does not normally cross the blood–brain barrier, detection of IgM in cerebrospinal fluid is a significant diagnostic finding, implying flavivirus replication within the central nervous system.

MAC-ELISA provides more information, is more efficient than other serological tests and is especially valuable for laboratories that perform a high volume of testing. A barrier to its wider use is the lack of standardized reagents. Several versions of the test that are performed in 96-well plates and read with spectrophotometric plate readers have been described. A common feature is the use of commercially available anti-IgM as a coating on the plates in order to capture a random sample of isotype immunoglobulin molecules from the test serum. The serum specimen may be tested at a single or multiple dilution(s) with an excess of dengue antigen prepared from infected mouse brain or cell culture lysates, and the use of a detector antibody to measure the quantity of dengue antigen bound by the test serum. The performance characteristics of individual assays are determined by the type and amount of dengue antigen, the detector antibody and the positive and negative controls used to set a cut-off for test positivity. The interpretation of MAC-ELISA results is summarized in Table 4.3.

Haemagglutination-inhibition test

The HI test is simple, sensitive and reproducible and has the advantage of using reagents that may be prepared locally. A disadvantage is that sera samples must be pretreated with acetone or kaolin, to remove non-specific inhibitors of haemagglutination, and then absorbed with gander or type O human red blood cells, to remove non-specific agglutinins. Furthermore, the optimal use of the HI test requires paired sera. Paired sera are most easily obtained upon hospital admission (acute) and discharge (convalescent); if the interval between the first and second serum is less than 7 days, an HI test may not afford a diagnosis in a primary infection. It also normally fails to discriminate between infections by closely related flaviviruses, e.g. between dengue virus and Japanese encephalitis virus, or dengue and West Nile virus.

Dengue viruses agglutinate gander erythrocytes and those of certain other species as well as trypsinized type O human red blood cells. The HI test is based on the ability of dengue virus antibodies to inhibit this agglutination. The test is described in most virology manuals.

- Sera should be extracted with kaolin or acetone and absorbed with gander or trypsinized type O human red blood cells.
- All sera from a single patient should be tested in the same assay using 4–8

Table 4.4
Interpretation of dengue haemagglutination-inhibition antibody response[a]

Antibody response	S1–S2 interval[b]	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

[a] These criteria were derived empirically from data collected at the U.S. Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand. Laboratories should assess the sensitivity of their assay with standard sera from WHO Collaborating Centres for Arboviruses and/or Haemorrhagic Fever Reference and Research or WHO Collaborating Centres for New, Emerging and Re-emerging Diseases (see Annex 6). Laboratories should also establish baseline data for the population they serve during a period of little or no flavivirus transmission.
[b] Interval in days between acute (S1) and convalescent (S2) specimens.
[c] Against any dengue antigen.

haemagglutinating units of the four dengue antigens. (As a screening test, a single broadly reactive dengue antigen may be used (usually DEN-1 or DEN-4) with only a slight loss of sensitivity for primary infections. If a screening test of paired sera is negative, the specimens may be retested against all dengue antigens.)
• Negative serum pairs should be tested against chikungunya antigen where this virus is known to be endemic.
• Known positive and negative sera should be included in each test to standardize results and maintain quality control; reference sera may be available from WHO Collaborating Centres for Arboviruses and/or Haemorrhagic Fever Reference and Research or WHO Collaborating Centres for New, Emerging and Re-emerging Diseases (see Annex 6).

The response to a primary dengue infection is characterized by the slow evolution of the haemagglutination-inhibiting antibody. Because the HI assay does not differentiate among immunoglobulin isotypes, the identification of a primary antibody response must be inferred from the low level or absence of detectable antibody in the acute-phase serum drawn before day 5, as well as from the levels of antibody titres elicited (Table 4.4). The secondary antibody response to dengue is characterized by the rapid evolution of haemagglutination-inhibiting antibody. All antibodies are broadly flavivirus-reactive so a specific diagnosis is not possible on the basis of this test alone. In positive tests there are fourfold or greater increases in titre between acute and convalescent sera, with peak titres always exceeding 1:1280 in secondary responses, and generally falling below this ratio in primary responses.

Neutralization tests

Although several neutralization tests have been described for dengue virus, the most sensitive and specific method is the serum dilution, virus-constant, plaque-reduction test. Following primary dengue infection, relatively specific neutralizing antibodies are detected in early convalescence. Following secondary dengue infections, high-titre neutralizing antibody is produced against at least two and usually all four dengue virus serotypes, as well as against other flaviviruses. In many combinations of sequential infections when appropriately timed specimens are tested, the highest neutralizing antibody titre in convalescent serum is directed against the virus with which the patient was previously (not most recently) infected.

Dot-blot immunoassay

Dot-blot immunoassay technology is relatively new, and reagents and test procedures are evolving. At least one dot-blot immunoassay for dengue antibodies is available commercially. As greater interest develops among commercial manufacturers, additional dot-blot immunoassays are likely to enter the market.

Complement-fixation test

The complement-fixation test may also be used in serological diagnosis, although it is the least sensitive serological assay, and other assays have generally replaced this method. Complement-fixing antibody typically appears later than IgM or HI antibody and is usually more specific. Therefore, it can be useful in confirming dengue infection in patients with paired serum samples taken late in the infection. A fourfold rise in complement-fixing antibody, where the interval between the acute and convalescent serum is less than 2 weeks, signifies a secondary seroresponse pattern.

Vector surveillance and control

The most important vector of dengue virus is the mosquito *Aedes aegypti*, which should be the main target of surveillance and control activities. Other species should be considered for vector control only where there is reliable evidence that they play an epidemiologically significant role in the transmission of dengue infections.

Vector surveillance

Entomological surveillance is used to determine changes in the geographical distribution and density of the vector, evaluate control programmes, obtain relative measurements of the vector population over time and facilitate appropriate and timely decisions regarding interventions. It may also serve to identify areas of high-density infestation or periods of population increase. A number of methods are available for detecting or monitoring immature and adult populations. Selection of appropriate sampling methods depends on the surveillance objectives, levels of infestation, available funding and skills of personnel. Guidance on the choice of surveillance methods for *Ae. aegypti* is presented in Table 5.1.

Several indices have been described and are currently used to monitor *Ae. aegypti* populations for dengue virus transmission. Those related to immature populations include the house index, i.e. the percentage of houses infested with larvae or pupae; the container index, i.e. the percentage of water-holding containers infested with larvae or pupae; and the Breteau index, i.e. the number of positive containers per 100 houses inspected. When using the house index or the Breteau index, the definition of a house should be one unit of accommodation and the surrounding premises, irrespective of the number of people residing therein.

The abundance of adult mosquitos is expressed as either the landing rate or the indoor resting density during a fixed period of collection time. Landing or biting collections on humans are a sensitive but labour-intensive means of detecting low-level infestations. Rates of capture are usually expressed in terms of landing–biting counts per person-hour. Resting collections consist of the systematic search for *Ae. aegypti*, which typically spends periods of inactivity in secluded places indoors such as in closets and under furniture. Resting collec-

Table 5.1
Aedes aegypti surveillance methods

Objective	Method					
	Larval survey	Collection on humans	Collection of resting mosquitos	Ovitrap	Tyre larvitraps	Insecticide susceptibility
Baseline infestation surveys	X			X		X
Control programme monitoring low infestation levels: <5% house index	X	X	X[a]	X	X	
Control programme monitoring ≥5% house index level	X	X	X[a]			
Surveillance against reinfestation	X			X	X	
Verification of eradication	X	X	X	X	X	
Evaluation of control methods[b]	X	X	X	X	X	X

[a] Use of backpack aspirator recommended.
[b] Choice depends on intervention used.

tion studies performed with backpack aspirators are an efficient and effective means of evaluating adult densities. Densities are recorded either as the number of adult mosquitos per house (females, males, or both) or the number of adult mosquitos collected per unit of time. Wherever larval surveys indicate low infestations (e.g. when the Breteau index is <5), ovitraps can be used as a complementary surveillance method. They have proved especially useful for the early detection of new infestations in areas from which the mosquito had been eliminated.

Ae. aegypti has a relatively short flight range, but even so, a very large number of catching stations are required to provide accurate monitoring of areas at risk. Since this is not usually feasible, the best alternative is to concentrate monitoring on high-risk areas as determined by experience or environmental conditions. Special attention should also be given to the evaluation of areas where control activities have been carried out, so that remedial measures can be implemented if required.

In areas of high human population density, many people may be exposed, even if the mosquito house index is low. Distances between houses may thus be of epidemiological significance, especially in areas with single-storey dwellings. In multistorey dwellings, the population per unit area is likely to be higher, and thus survey data for single-storey and multistorey dwellings should be kept separate.

Emergency control measures are based primarily on application of insecticides, and it is essential to monitor periodically the vector's susceptibility to the insecticides most widely used, e.g. temephos, malathion, fenthion and fenitrothion. In tropical countries where there are large areas free of the vector, surveillance against infestation is of paramount importance. Special attention should be given to the surveillance of seaports, airports, other points of entry, cemeteries, and used-tyre storage or retreading facilities. Ports that receive vessels from infested areas should have ongoing inspection programmes. Cemeteries where live or artificial flowers are placed in vases and other containers are important *Aedes* loci. Tyre-retreading facilities that receive used tyre shipments from infested areas may be important sites for the introduction of *Aedes* vectors to urban areas. A good surveillance programme designed to avoid infestation is much less costly than an eradication or control programme that must be established after infestation has occurred.

Vector control[1]

The most effective means of vector control is environmental management, which includes planning, organization, carrying out and monitoring activities for the modification or manipulation of environmental factors with a view to

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[1] See also Rozendaal JA. *Vector control: methods for use by individuals and communities*. Geneva, World Health Organization, 1997.

preventing or reducing vector propagation and human–vector–pathogen contact. In Asia and the Americas, *Ae. aegypti* breeds primarily in man-made containers, while in Africa, it breeds both in natural containers, such as tree holes and leaf axils, and in artificial containers. Control of *Ae. aegypti* in Cuba and Panama in the early part of this century was based on forms of environmental management, and many programmes in the Americas are returning to this fundamental tactic. Environmental management is also part of the control measures taken against *Ae. albopictus*, a secondary vector for dengue in the Pacific and Asia, and a potential vector following recent infestations in Africa, southern Europe, and the Americas.

In 1980, the WHO Expert Committee on Vector Biology and Control defined three types of environmental management:[1]

- *Environmental modification*—long-lasting physical transformations of vector habitats.
- *Environmental manipulation*—temporary changes to vector habitat as a result of planned activity to produce conditions unfavourable to vector breeding.
- *Changes to human habitation or behaviour*—efforts to reduce human–vector– pathogen contact.

Methods for environmental management

Environmental management methods to control *Ae. aegypti* and *Ae. albopictus* and reduce human–vector contact include the improvement of water supply and storage, solid waste management and the modification of man-made larval habitats. Table 5.2 summarizes the primary methods of environmental manipulation used to control *Aedes* larval habitats.

Environmental management should focus on the destruction, alteration, disposal or recycling of containers and natural larval habitats that produce the greatest number of adult *Aedes* mosquitos in each community. These programmes should be conducted concurrently with health education programmes and communications that encourage community participation in the planning, execution and evaluation of container-management programmes (e.g. regular household sanitation or clean-up campaigns).

Improvement of water supply and storage

One method for controlling urban *Aedes* vectors, particularly *Ae. aegypti*, is to improve domestic water supplies. The mere delivery of potable water to neigh-

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[1] *Environmental management for vector control. Fourth report of the WHO Expert Committee on Vector Biology and Control.* Geneva, World Health Organization, 1980 (WHO Technical Report Series, No. 649). See also: *Vector control for malaria and other mosquito-borne diseases. Report of a WHO Study Group.* Geneva, World Health Organization, 1995 (WHO Technical Report Series, No. 857).

Table 5.2
Environmental management actions for control of *Aedes aegypti* larval habitats[a]

Larval habitats	Clean	Cover	Store under roof	Modify design	Use EPS[b] beads	Fill (sand/soil)	Collect: recycle or dispose	Puncture or drain
Useful:								
Water storage tank/cistern	+	+		+	+			
Drum (150–200 litres)	+	+		+	+			
Flower vase with water	+					+		
Potted plants with saucers	+							
Ornamental pool/fountain	+							
Roof gutter	+							
Animal water container	+							
Non-essential:								
Used tyres		+	+			+	+	
Discarded large appliances							+	
Discarded buckets (<20 litres)			+				+	+
Tin cans						+	+	+
Natural:								
Tree-holes						+		
Rock holes						+		

[a] Adapted, by permission, from *Dengue and dengue hemorrhagic fever in the Americas: guidelines for prevention and control*, Washington, DC, Pan American Health Organization, 1994 (Scientific Publication No. 548).
[b] EPS = expanded polystyrene.

bourhoods or individual homes is not, however, sufficient to reduce the use of the water storage containers that play a dominant role in *Ae. aegypti* breeding in many urban areas. For example, after piped water had been supplied to households in one municipality in Thailand, approximately eight water storage jars were still kept by each household. Similar situations have been reported elsewhere in Asia and the Caribbean. Households typically continue to store water because water supplies are not reliable. With such water storage comes the concomitant problem of *Ae. aegypti* breeding and the increased risk of dengue infection. Therefore, potable water must be delivered in sufficient quantity, quality and consistency to reduce the use of water storage containers that serve as larval habitats, such as drums, overhead tanks, and jars. Water piped to households is preferable to wells, communal standpipes, rooftop catchments and other delivery systems. If storage tanks, drums and jars are required for water storage, they should be covered with tight lids or screens. Many people fail to cover water containers because lids and screens are not designed in such a way that they will seal containers while nevertheless enabling users to withdraw water easily. Water storage systems, however, can be designed to prevent *Ae. aegypti* oviposition or adult emergence. In Sarawak, Malaysia, for example, mosquito-proof rainwater collection and storage containers made of high-density polyethylene have fibreglass screens in the lids that allow rainwater to enter but prevent adult mosquitos from emerging. Covered containers should be routinely inspected because even the best-designed lids and screens can tear or deteriorate in harsh climates and with age.

Solid waste management

Vector control efforts should encourage effective and environmentally sound waste management by promoting the basic rule of "reduce, reuse, recycle". In some parts of Africa, plastic containers that may serve as potential larval habitats are effectively recycled. Used tyres are another form of solid waste that is of critical importance to urban *Aedes* control; they should be recycled or disposed of by proper incineration in waste transformation facilities (e.g. incinerators, energy-production plants, lime kilns). If cut into halves, shredded, or chipped, tyres can be mixed with other wastes and buried in landfills, as local sanitary regulations allow. Whole tyres should be buried in a separate area of a landfill, to avoid their rising upwards under compaction and disrupting soil cover.

Modification of man-made larval habitats

Common-sense approaches should be employed to reduce the potential for *Ae. aegypti* mosquitos to breed in and around human habitats. For example, fences and fence posts made from hollow stems, such as bamboo, should be cut to the node; tyres and containers stored outside should be covered or placed in a shed,

and buckets and other small containers should be inverted if stored outdoors. Ant traps used to protect food storage cabinets can be filled with oil or salty water instead of fresh water; condensate-collection pans under refrigerators and air-conditioning units should be drained and cleaned regularly. Floor drains should be cleaned and kept covered. Roof gutters, outdoor sinks, laundry basins and similar items that can retain water and serve as larval habitats should be drained and kept free of debris. Ornamental pools and fountains can be either chlorinated or populated with larvivorous fish. Where possible, housing should be designed or modified to reduce opportunities for mosquitos to enter, i.e. without open eaves and with screened doors and windows. These measures and others will help reduce or prevent the breeding of vector mosquitos near humans, and thereby diminish the risk of dengue viral disease.

Chemical control

Chemicals have been used to control *Ae. aegypti* since the turn of the century. In the first campaigns against yellow fever in Cuba and Panama, in conjunction with widespread clean-up campaigns, *Aedes* larval habitats were treated with oil and houses were dusted with pyrethrins. When the insecticidal properties of DDT were discovered in the 1940s, this compound became the principal method for *Ae. aegypti* eradication programmes in the Americas. When resistance to DDT emerged in the early 1960s, organophosphate insecticides, including fenthion, malathion, fenitrothion and temephos, were used for *Ae. aegypti* control. Current methods for applying insecticides include larvicide application, perifocal treatment and space spraying.

Application methods

Larvicidal or "focal" control of *Ae. aegypti* is usually limited to containers maintained for domestic use that cannot be eliminated. Three larvicides can be used to treat containers that hold drinking-water: 1% temephos sand granules, the insect growth regulator methoprene in the form of briquettes, and BTI (*Bacillus thuringiensis* H-14), which is considered below in the section on biological control. All these larvicides have extremely low mammalian toxicity, and properly treated drinking-water is safe for human consumption.

Perifocal treatment involves the use of hand or power sprayers to apply wettable powder or emulsifiable-concentrate formulations of insecticide as a spray to larval habitats and peripheral areas. This will destroy existing and subsequent larval infestations in containers of non-potable water, as well as kill the adult mosquitos that frequent these sites. This method can be used to treat containers that are preferred by *Ae. aegypti*, whether or not they hold water. The internal and external walls of containers are sprayed until they are covered by a film of insecticide; spraying is also extended to cover any wall within 60 cm of the container. The surface of non-potable water in containers is also treated.

The insecticides currently used in perifocal treatment are malathion, fenitrothion, fenthion, and some pyrethroids.

Space spraying is the spreading of microscopic droplets of insecticide in the air to kill adult mosquitos and is used in emergency situations when an outbreak of dengue fever is already in progress. Two forms of space spray are generally used for *Ae. aegypti* control: thermal fog and ultra-low volume (ULV) aerosols (cold fogs) and mists. Thermal fog is produced by equipment in which the insecticide, usually mixed in an oil with a suitably high flashpoint, is vaporized by being injected into a high-velocity stream of hot gas. When discharged into the atmosphere, the oil carrying the pesticide condenses in the form of a fog. Malathion, fenitrothion, fenthion and some pyrethroids are used in thermal fogging operations. ULV aerosols and mists involve the application of a small quantity of concentrated liquid insecticide. An insecticide concentrate application of less than 4.6 litres per ha (0.5 US gal per acre) is usually considered to be a ULV application. Selected insecticides and the dosages suitable for use in cold sprays in the control of *Ae. aegypti* are listed in Table 5.3.

Aerosols and mists may be applied using portable machines, vehicle-mounted generators, helicopters or fixed-wing aircraft. Portable backpack equipment can be used to apply insecticide mists in small areas or where vehicle-mounted equipment cannot be used. An average of 80 houses per day can be

Table 5.3
Selected insecticides and dosages for cold-spray control of *Aedes aegypti*[a]

Insecticide	Dosage (grams of active ingredient per ha)
Organophosphates	
Malathion	112–693
Fenitrothion	250–300
Naled	56–280
Pirimiphos-methyl	230–330
Pyrethroids	
Deltamethrin	0.5–1.0
Resmethrin	2–4
Bioresmethrin	5
Permethrin	5
Cypermethrin	1–3
Lamda-cyhalothrin	1

[a] Source: DC Chavasse, HH Yap, eds. *Chemical methods for the control of vectors and pests of public health importance*, Geneva, World Health Organization, 1997 (unpublished document WHO/CTD/WHOPES/97.2, available on request from Division of Control of Tropical Diseases, World Health Organization, 1211 Geneva 27, Switzerland).

thus treated, but two or three operators are required because the weight of the machine and engine vibrations make it necessary for operators to rest frequently.

Vehicle-mounted aerosol generators can be used in urban or suburban areas with good road systems. One machine can cover 1500–2000 houses per day. It is necessary to monitor environmental conditions and to calibrate the equipment, vehicle speed and swath width to determine the coverage obtained by a single pass. Maps of the areas to be sprayed showing all passable roads are helpful in planning maximum coverage. An educational effort may be required to persuade residents to open their doors and windows in order to improve the effect of the spraying programme. As *Ae. aegypti* routinely rests indoors, the effectiveness of vehicle-mounted aerosol control measures has been questioned, and surveillance of the natural mosquito population, as opposed to caged bioassay mosquitos, should accompany control efforts in order to determine the impact of the spray programme on each habitat.

Aerial spraying is often used when an extensive area must be treated in a short time. Although the equipment (aircraft equipped with a spray system) may have a high initial cost, this form of application may be the most cost-effective, since very large areas can be treated during a single flight. As with vehicle-mounted operations, it is important to ensure that insecticide is reaching the actual mosquito habitats. Insecticides that can be used in aerial ULV applications are malathion, fenitrothion, naled, pirimiphos-methyl, resmethrin, cypermethrin, lambda-cyhalothrin and deltamethrin. Parameters for the aerial application of insecticides vary according to the types of aircraft, local insecticide susceptibility and the equipment used. Early morning applications are preferable, temperature should be less than 27 °C (80 °F) and wind velocity should be less than 16 km (10 miles) per hour.

Guidelines for chemical control

The indiscriminate use of insecticides for prevention and control of dengue infection should be discouraged. During periods of little or no dengue virus activity, the routine source reduction measures described above in the section *Methods for environmental management* can be integrated with 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 insecticides, using the techniques described above.

Safety precautions for chemical control

All pesticides are toxic to some degree; safety precautions should 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 can be organized along the following lines:

- Instructions on pesticide labels should be carefully followed.
- Spray operators should be provided with at least two uniforms to allow for frequent changes.
- Safety gloves and masks should be used for high-exposure activities like machine calibration.
- Changing and washing facilities with sufficient water and soap should be available.
- All work clothes should be removed at the end of each day's operations and a shower or bath taken.
- Work clothes should be washed regularly.
- Particular attention should be given to washing gloves, as wearing contaminated gloves can be dangerous.
- Spray operators should wash their hands before eating and should not smoke during work hours.
- Spray operators should not be exposed to toxic material for periods that are longer than recommended.
- Care must be taken in the disposal of used insecticide containers.
- Blood cholinesterase levels should be monitored if organophosphate insecticides are used.
- Supervision by a well trained individual is essential.

Specific guidelines on insecticides and safety procedures are included in *Safe use of pesticides. Fourteenth report of the WHO Expert Committee on Vector Biology and Control*, Geneva, World Health Organization, 1991 (WHO Technical Report Series, No. 813), and *Chemical methods for the control of vectors and pests of public health importance*, Geneva, World Health Organization, 1997 (unpublished document WHO/CTO/WHOPES/97.2, available on request from Division of Control of Tropical Diseases, World Health Organization, 1211 Geneva 27, Switzerland).

Insecticide susceptibility monitoring

During the past 40 years, chemicals have been widely used to control mosquitos and other insects 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 control operations are started and to continue monitoring susceptibility levels periodically. WHO kits are available for testing the susceptibility of adult and larval mosquitos and other arthropod vectors. These can be obtained from the Division of Control of Tropical Diseases, World Health Organization, 1211 Geneva 27, Switzerland (fax: 41 22 791 4777).

Personal protection

Personal protection measures have been extensively used in efforts to protect indigenous and rural populations against malaria. Pyrethroid-impregnated bednets or curtains appear to be effective against mosquitos that feed at night. In the case of the day-feeding *Aedes* vectors of dengue, however, these measures have less relevance. Nevertheless, they may be useful to special groups, such as the bed-ridden, infants or those who must sleep during the day. Tourists and short-term visitors to dengue endemic areas should use commercially available insect repellents. For residents and those staying longer in endemic areas, clothing can be impregnated with permethrin.

Biological control

Interventions based on the introduction of organisms that prey upon, parasitize, compete with or otherwise reduce the numbers of *Ae. aegypti* or *Ae. albopictus* remain largely experimental, and information on their efficacy is based on the results of small-scale field operations. Larvivorous fish and the biocide *Bacillus thuringiensis* H-14 (BTI) are the two organisms most frequently employed. The advantages of biological control measures include no chemical contamination of the environment, specificity against target organisms (the effect of BTI, for example, is limited to mosquitos and related Diptera) and the self-dispersion of some agents into sites that could not be easily treated by other means.

The disadvantages of biological control measures include the expense of raising the organisms, difficulty in their application and production and their limited utility in aquatic sites where temperature, pH and organic pollution may exceed the narrow requirements of the agent, as well as the fact that they are only effective against the immature stages of vector mosquitos. Moreover, a reduction in larval numbers does not necessarily result in a corresponding reduction of disease transmission, since if food is limited, lower larval densities may result in larger, healthier adults that are better able to survive.

BTI is a proven, environmentally non-intrusive mosquito larvicide that appears to be entirely safe for humans. It is commercially available under a number of trade names. The large parasporal body that forms in this agent contains a toxin that degranulates solely in the alkaline environment of the mosquito midgut. The advantage of this material is that an application destroys larval mosquitos but spares any entomophagous predators that may be present. BTI formulations tend to settle at the bottom of water containers soon after application and require frequent reapplications. In addition, the toxin is photolabile and is destroyed by sunlight. Briquette formulations that appear to have greater residual activity are commercially available and can be used with confidence in drinking-water.

Integrated control

Integrated vector control is the combination of available control methods in the most effective, economical and safe manner to maintain vector populations at acceptable levels. The 1981 *Ae. aegypti* eradication campaign in Cuba combined the reduction of larval habitats (source reduction) and modification of drinking-water storage tanks with a variety of other interventions, including legislative measures to encourage householder compliance, health education, biological control and chemical control. This effort markedly reduced the densities of this vector. Control of *Ae. aegypti* can also be combined with the control of other disease vectors, as was done in urban areas of Suva, Fiji (1978–1979), and of the United Republic of Tanzania (1972), and in Singapore (1968–1980s). The Suva programme consisted of clean-up campaigns, house inspections, ULV malathion spray, legislative measures and health education. Monthly inspections of larval habitats were a major adjunct to reducing larval indices. The Tanzanian programme combined source reduction, public education and clean-up campaigns and reduced adult populations of both *Ae. aegypti* and *Culex pipiens quinquefasciatus*.

The joint *Ae. aegypti* and *Ae. albopictus* control programme in Singapore consisted of slum clearance, resettlement of displaced persons, source reduction by uniformed health officers, drain cleaning, mosquito-proofing of water tanks, health education, and strict enforcement of control measures, including fines. The *Aedes* house index in the slums fell from 27.2% of houses infested during 1966–1968 to 5.4% in 1969 after slum clearance and resettlement, and to 1.61% city-wide in 1981. The Singapore programme cost US$1–1.50 per person per year and resulted in a reduced incidence of dengue and lower densities of both *Aedes* and *C. pipiens quinquefasciatus* mosquitos.

Environmental management of dengue virus vectors can be successfully combined with health education and public health communication, where source reduction activities are promoted by local health care workers. For example, a campaign was undertaken in Indonesia in which local clinic workers trained primary school teachers and volunteers from women's clubs to recognize cases of dengue fever and to implement a programme to reduce the number of *Ae. aegypti* breeding habitats. As a result, *Ae. aegypti* populations were significantly reduced within six months of implementing the campaign.

Disease surveillance and outbreak prevention and control

Factors increasing the risk of DHF outbreaks

The occurrence of DHF outbreaks is linked to a number of factors, including the density of mosquito vectors, particularly that of *Aedes aegypti*. The precise population density of *Ae. aegypti* that is needed to sustain dengue virus transmission epidemically or endemically has yet to be determined, but experience in Singapore in recent years suggests that house indices as low as 2% are sufficient for the epidemic transmission of dengue in areas where there is a low level of immunity in the human population. In many instances, a small number of actively biting female mosquitos has infected an entire household. Virus transmission is, of course, increased by denser human populations. Urbanization in tropical countries has resulted in both a proliferation of *Ae. aegypti* and an increase in the number of susceptible human hosts.

In cities, the movement of viraemic persons is a more important means of transporting dengue viruses than the movement of *Ae. aegypti* mosquitos. Places where people congregate during the day may be important sites of dengue virus transmission. For example, children at school bitten by infected mosquitos may take the virus home or to other parts of the city. Dengue virus may also spread in settings involving large numbers of people, such as in hospitals where visitors, patients and staff may be bitten by infected *Ae. aegypti*.

Infected persons may carry dengue virus to towns and rural areas from cities where the disease is epidemic or endemic, but the factors affecting dengue virus transmission and maintenance in towns are not well documented. Introduction of dengue virus by the air travel of infected passengers over long distances has repeatedly occurred in the Pacific and Caribbean regions during the past 30 years.

A distinct seasonal pattern in DHF outbreaks is evident in most places. In tropical regions where monsoon weather patterns prevail, DHF hospitalization rates increase during the rainy season and decline several months after the cessation of the rains. This decline may be related to a decrease in mosquito biting activity, a decrease in the longevity of female mosquitos, or both, and

possibly to a small decrease in the vector population. During these seasonal lulls, virus transmission is most likely to occur in endemic urban areas where high densities of human population ensure a constant supply of susceptible individuals, and numerous vector breeding and resting sites around human dwellings insulate vector populations from the effects of seasonal rainfall.

Surveillance of dengue

The objective of a DF and DHF surveillance programme is the early detection of outbreaks that permits the prompt implementation of control measures. In order to accomplish this, the factors favouring an outbreak should be monitored. This requires the monitoring of suspected cases of DF and DHF (using the diagnostic criteria outlined in Chapter 2), case reporting, and epidemiological and entomological investigations. Surveillance is indicated in all endemic and receptive areas, defined as locations where *Ae. aegypti* is known to be present. With modern air travel, a viraemic patient can quickly move from an endemic to a receptive area. Thus, the introduction of dengue virus into areas with *Ae. aegypti* should be expected at any time. The following activities, therefore, should be included in a basic programme of DF and DHF surveillance.

Fever surveillance

For the surveillance of fever cases, sentinel clinics at strategic locations throughout high-risk areas should report to the national public health authority on a weekly basis the number of patients seen and the number of patients with an oral or axillary temperature higher than 38 °C. In this way, abnormal increases in the incidence of febrile illnesses can be detected. If an increase is reported there should be an attempt to determine the etiology of the illness by virus culture and serology (see Chapter 4).

Recognition of dengue haemorrhagic fever cases

The standard criteria for the clinical diagnosis (see Chapter 2) and laboratory confirmation (see Chapter 4) of DHF should be followed. Most dengue virus infections in young children are mild and difficult to distinguish from other acute febrile diseases. Classical dengue fever is most commonly seen in adolescents and adults; but in areas where dengue virus is endemic, resident adults are often immune and overt disease may be limited to arriving susceptible adults such as travellers. Considerable numbers of mild dengue infection precede and accompany DHF epidemics, and the magnitude of this uncomplicated DF may be difficult to assess. It has been estimated that, during outbreaks, between 150 and 200 cases of dengue infection occur for each patient

with DSS seen in a hospital. Recognition of this is essential for the planning of prevention and control programmes.

Reporting cases to health authorities

Presumptive cases of DHF (designated as with or without shock) should be reported to the appropriate local, national and international health authorities. An agency within the national public health authority is often designated to receive and compile this information as a part of dengue fever surveillance. These data should be processed rapidly, and reports should be submitted through the national public health authorities to WHO and others as required, with copies to the individuals and institutions who contributed the data. Reports should include the number of patients with and deaths due to DHF by age, sex, location and date. Reports may be submitted in either narrative or tabular form (a sample reporting form is shown in Annex 7) and should be signed by the responsible medical officer.

Aedes *surveillance*

Country-wide surveys should be made to determine the presence, population density, and seasonal prevalence of *Aedes* vectors and their susceptibility to available insecticides (see Chapter 5). Countries may obtain assistance from WHO Headquarters or Regional Offices in organizing and conducting entomological surveys and surveillance programmes. The information from national surveys should be submitted to WHO so that the Organization can track potential problem areas.

Virological surveillance

Monitoring dengue virus infections and the surveillance of dengue serotypes in endemic areas may be instituted if facilities and trained staff are available. These efforts could consist, for instance, of obtaining reports of virus isolation from febrile patients and the systematic collection and processing of suspect mosquitos for virus isolation attempts. Original specimens (viraemic serum or infected mosquito pools), as well as viral strains that have been successfully cultured, should be preserved for future study. The isolation of virus from patients suspected to have DHF is important, and WHO or the WHO Collaborating Centres listed in Annex 6 can be contacted for advice and assistance on virus isolation procedures and the proper storage or transport of dengue virus (see also Chapter 4).

Development of epidemic contingency plans

Contingency planning should involve estimating the number of people at risk, determining the quantity of equipment (including hospital beds and intensive

care facilities), supplies and personnel required for vector control and patient management (Annex 8), and documenting the location of these resources. These efforts should be supplemented with clinical training for physicians, nurses and laboratory staff on the management of dengue patients (see Chapter 3). The fundamental therapeutic principle for the treatment of DHF is the rapid replacement of fluids by intravenous infusion. The following assumptions make it possible to estimate the quantity of supplies required during a DHF outbreak:

- In the worst situation to date (Cuba, 1981), the prevalence of seriously ill patients admitted to hospital approached 1 per 100 population.
- Hospitalized patients usually require intravenous therapy.
- About 20% of hospitalized patients require intravascular volume expanders, such as dextran 40, albumin or plasma.
- About 10% of hospitalized patients require blood transfusion.

On the basis of these estimates, the following supplies would be required per 10 000 population (100 possible cases of DHF):

- 100 cases of DHF: 200–300 litres normal saline
- 20 cases of DHF with hypovolaemia: 20 litres of a volume expander in appropriate units
- 10 cases of DHF with haemorrhage: at least 10 units of whole blood.

Most urban hospitals would be expected to have these quantities in stock. Adjustments should be made to provide for the population at risk in a given area and provisions made for timely resupply in the case of an epidemic.

Control of dengue haemorrhagic fever

To control outbreaks of DHF, two operations must be conducted simultaneously: emergency mosquito control and treatment of patients in hospital.

Emergency mosquito control

The following steps should be immediately taken when an outbreak of dengue or DHF is suspected:

- A public information campaign should be instituted, stressing the basic epidemiological characteristics of dengue and DHF and the measures the individual can take to reduce the risk of infection, e.g. personal protective measures, the use of household aerosol insecticides, source reduction efforts at home and in the neighbourhood.
- The geographical area should be defined in order to determine the extent of the insecticide spraying operation required. For this purpose, presumptive cases of dengue and DHF should be confirmed in the laboratory by serological examination of paired sera.

- An inventory of the location, quantity and availability of pesticides and the equipment for their application (see Annex 8) should be made.

Operations for emergency mosquito control are described in Chapter 5. The objective of these measures is to eliminate infected mosquitos and to break the transmission cycle by reducing mosquito populations to extremely low levels during the time necessary for viraemic subjects to recover. Control of an epidemic may not be feasible if adult populations of *Ae. aegypti* cannot be sufficiently reduced. However, a sustained reduction of vector populations will inevitably result in fewer cases.

Management of clinical care

Organizational aspects

An organizing or coordinating committee should be established and should consist of administrators, epidemiologists, clinicians, entomologists and workers from virus laboratories. The responsibility for establishing this committee is usually vested in the ministry of health. The committee should:

- Design and distribute protocols for the clinical diagnosis and treatment of DHF/DSS.
- Prepare and circulate information on DHF/DSS for health care workers, the public and the press.
- Plan and implement training programmes for health care workers and auxiliaries (e.g. hospital staff, medical students, nurses and laboratory technicians).
- Assess the need for intravenous fluids, medications, blood products, intensive care equipment, teaching materials and equipment for transporting patients.
- Supervise the use of supplies and the outcome of clinical care programmes (daily, if necessary).
- Coordinate clinical research on DHF/DSS during any outbreak.

It may be necessary for hospitals to postpone elective surgery and other non-critical care in order to provide beds for the acute care of DHF/DSS patients. Even a moderate DHF outbreak, such as that which occurred in Venezuela in 1989–1990, may significantly burden the health care system, especially if an effective contingency plan has not been implemented. It may be necessary to set up hospitals in schools or other institutions as was done in Cuba in 1981, but this should be considered only if medical personnel and a laboratory capable of performing haematocrit and platelet count determinations are also available, since these are essential for the successful treatment of DHF patients.

Triage

During epidemics, outpatient and inpatient facilities may be overwhelmed and medical staff can rapidly become exhausted. In these circumstances, only those persons genuinely requiring hospital care should be admitted (see the outpatient and hospital flow charts in Annexes 3 and 4). A fever and a positive tourniquet test, or other manifestations of bleeding, are sufficient for DHF to be suspected. When possible, haematocrit and platelet count determinations should be completed in the outpatient department. Patients with thrombocytopenia and an elevated haematocrit can be sent to a rehydration ward or, if circulatory failure is suspected, admitted to a hospital (see Chapter 3). It may also be necessary to admit less seriously ill patients who live far from a hospital and who do not have access to nearby accommodation. For those patients who can be treated as outpatients, they and their parents or other care providers should be carefully instructed to return to the hospital promptly if they experience restlessness, lethargy, abdominal pain, oliguria or circumoral cyanosis.

Paramedical workers can also perform patient triage if properly instructed. Competent laboratory assistance is desirable, but without a laboratory patients can be evaluated by physical examination—a rapid pulse, skin congestion, circumoral cyanosis or cool extremities are indications that a patient should be admitted to hospital. If possible, patients who have been admitted to hospital should stay in hospital for observation, or be warned to remain near the hospital, until two days after their fever has subsided.

Prevention of dengue haemorrhagic fever outbreaks

Prevention of DHF outbreaks is based on vector control (see Chapter 5), as a vaccine is not yet available. Currently, the only effective way to avoid dengue virus infection is to avoid being bitten by infected mosquitos.

A broad approach to the prevention of DHF involves integration of the measures described in previous chapters. Such a programme would combine two or more of the following components:

- Disease surveillance and treatment, whether centralized or based on local health care systems.
- Vector surveillance and control, with a mixture of environmental management and chemical and biological control.
- Provision of reliable potable water, sanitation and solid waste management.
- Health education, public health communication and community participation.

Dengue virus transmission is often a problem of domestic environmental management, and members of a household can frequently reduce their risk of

DF and DHF at little or no cost by controlling larval habitats and combating adult mosquitos by screening windows and doors and using household insecticide space sprays. A challenge for public health authorities is to find ways of getting a community to recognize the problem, assume a share of the responsibility for its solution and acquire the capability and motivation to prevent and control dengue fever.

Exchange of information

Exchange of information is essential for preventing and controlling outbreaks of DF and DHF. Narrative epidemiological reports, results of clinical studies, dengue virus isolations (with source and date), entomological surveys of dengue vectors, details of control measures planned or carried out, new developments in insecticides and spray equipment and other pertinent information are published in the *Dengue bulletin* of the WHO Regional Office for South-East Asia, the *Dengue surveillance summary* of the Centers for Disease Control and Prevention (in Puerto Rico), the *Epidemiological bulletin* of the WHO Regional Office for the Americas and the WHO *Weekly epidemiological record*. Addresses of editorial offices are given in Annex 8.

An increasing emphasis is being placed on the surveillance and reporting of DF and DHF in order to make a better estimate of the global burden of these diseases; exchange of this information is strongly encouraged.

Primary health care

DF and DHF are often, though not exclusively, associated with poor environmental sanitation, inferior housing and inadequate water supplies. Where such conditions prevail, communities need to be instructed in what steps they can take to prevent and control the disease. The diagnosis and management of DHF, as well as the control of outbreaks, may be a problem that can be addressed by primary health care workers. The disease tends to spread from large cities to smaller ones and to villages infested by vector mosquitos. Transmission of the disease can be reduced by community participation in vector control. In addition, the case fatality rate of DHF can be considerably decreased if appropriate fluid replacement therapy is given early in the course of the disease. Referral to a well equipped hospital is not always possible; therefore, health care workers, particularly in rural areas, should be instructed in the early diagnosis and effective management of patients suspected of having DHF.

Recognizing cases of dengue haemorrhagic fever

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

- Children are suffering from an undiagnosed febrile illness characterized by a continuous high or saddle-backed fever of 2–7 days' duration.
- Patients have petechiae, bleeding from the nose or gums, haematemesis or melaena.
- Patients remain ill despite a drop in temperature, and their clinical condition deteriorates with the development of clammy skin, cold and sweaty extremities, drowsiness or restlessness.
- Unexplained deaths due to shock, with or without haemorrhage, occur within 1 week after the onset of an acute febrile illness.

The first step towards community involvement in DHF control is for parents to learn that seeking early medical care for their children may prevent serious illness or death. To encourage parents promptly to take their children for treatment, they should be taught to recognize symptoms suggestive of DHF, in particular a high fever lasting 2–7 days that may be accompanied by anorexia, nausea, vomiting, abdominal pain and subsequent evidence of bleeding (per-

sistent red spots on the skin or in the nose, bleeding gums, "coffee-ground" vomit or dark stools). Most importantly, they should be able to recognize early signs of shock—their child remains ill despite a fall in temperature and develops cold, clammy skin, restlessness or drowsiness.

Management of dengue haemorrhagic fever patients

Chapter 3 provides detailed information on the treatment of DHF; but for the purposes of primary health care, the following principles of treatment can be applied:

- High fever should be treated by sponging and the appropriate use of paracetamol. (Acetylsalicylic acid (aspirin) and other salicylates should not be given because they can lead to bleeding and cause gastric irritation and acidosis.)
- Oral rehydration therapy should be administered in the early stages of fever.
- The patient should be referred immediately to a hospital if there is any evidence of bleeding.
- Prompt referral to a hospital or suitable health centre is necessary for the administration of intravenous fluid if the body temperature drops, the extremities become cold or the patient becomes restless. If referral is not possible, oral rehydration should be continued until the patient has a normal urine output and the skin becomes warm.

Collection of specimens for laboratory examination

Proof that an outbreak of illness is caused by dengue virus must be obtained as soon as possible after the first suspected cases. As indicated in Chapter 4, blood specimens should be collected and sent with clinical data to a laboratory that is equipped to diagnose dengue virus infections.

The recognition of cases and collection of specimens can be facilitated if a member of the community has been designated as the "health communicator" who is responsible for providing liaison between the community and the national committee responsible for supervision and management of dengue clinical care.

Vector control

Vectors of the dengue virus breed in and around houses and, in principle, can be controlled though individual and community action. A preventive approach should be adopted in extending vector control efforts to communities that do not routinely benefit from organized vector control. It may be assumed that the vector is *Ae. aegypti*, which feeds during the day, rests indoors and lays its eggs in artificial water containers. As previously discussed (see Chapter 5), local

residents may play a key role in the effective control of vector mosquitos by eliminating larval habitats, using insect repellents and indoor space-spray insecticides, placing screens on their windows and doors, and using bednets if they sleep during the day.

Countries or territories in which dengue fever or dengue haemorrhagic fever is known to occur, by WHO Region, 1975–1996

African Region
Angola
Burkina Faso
Comoros
Côte d'Ivoire
Ethiopia
Ghana
Guinea
Kenya
Madagascar[1]
Mauritius
Mozambique
Nigeria
Réunion
Senegal
Seychelles
Sierra Leone
South Africa
United Republic of Tanzania
Zaire[2]

Region of the Americas
Antigua and Barbuda
Aruba
Bahamas
Barbados
Belize
Bolivia
Bonaire

Brazil
British Virgin Islands
Colombia
Costa Rica
Cuba
Curaçao
Dominica
Dominican Republic
Ecuador
El Salvador
French Guiana
Grenada
Guadeloupe
Guatemala
Guyana
Haiti
Honduras
Jamaica
Martinique
Mexico
Montserrat
Nicaragua
Panama
Paraguay
Peru
Puerto Rico
St Kitts & Nevis
St Lucia
St Martin

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[1] Possibly imported
[2] Democratic Republic of the Congo

St Vincent & the Grenadines
Suriname
Trinidad & Tobago
Turks & Caicos Islands
United States of America
Venezuela
Virgin Islands of the United States

South-East Asia Region
Bangladesh
India
Indonesia
Maldives
Myanmar
Sri Lanka
Thailand

Eastern Mediterranean Region
Djibouti
Pakistan
Somalia
Saudi Arabia
Sudan

Western Pacific Region
Australia
Brunei Darussalam

Cambodia
China
Cook Islands
Fiji
French Polynesia
Guam
Kiribati
Lao People's Democratic Republic
Malaysia
Marshall Islands
Nauru
New Caledonia
New Zealand
Niue
Palau
Papua New Guinea
Philippines
Samoa
Singapore
Solomon Islands
Tokelau
Tonga
Tuvalu
Vanuatu
Viet Nam
Wallis & Futuna Islands

Daily dengue haemorrhagic fever record sheet

Name . . . . Age . . . . Sex . . . . Hospital No. . . . .
Weight on admission . . . .
Address . . . .
Month/Date of hospitalization . . . .

Day of illness:[a]	1	2	3	4	5	6	7	8	9	10	11	12	13
Max. temp. (°C)													
Pulse/blood pressure													
Tourniquet test													
Petechiae													
Purpura/ecchymosis													
Epistaxis													
Haematemesis/melaena													
Other bleeding													
Hepatomegaly (size)													
Shock Cold extremities Cold, clammy sweating													
Restlessness													
Lethargy													
Heart/lung signs													
Rash													
Lymph nodes													
Other signs													
Haematocrit (erythrocyte volume fraction)													
Platelets (thousands)													
White blood count													
Neutrophils													
Lymphocytes/atypical lymphocytes													
Blood for culture/serology Acute Convalescent													

[a] Circle day of illness during which the patient was hospitalized.

Day of illness	1	2	3	4	5	6	7	8	9	10	11	12	13
Treatment													
Physiological saline													
Ringer's lactate													
Plasma													
Plasma expander													
Blood													
Other													

ANNEX 3
Outpatient flow chart

FEVER >38 °C
(for <7 days)

Collect acute serum,
check platelet count
and haematocrit,
perform tourniquet test

BLEEDING OR
RESTLESSNESS
(signs of shock)

Yes → Admit to hospital

No

HIGH
HAEMATOCRIT,
PLATELET COUNT
<100 000/mm³

Yes

No

Administer:
–oral rehydration solution
–paracetamol
Follow:
–haematocrit
–tourniquet test
–platelet count

RISING
HAEMATOCRIT,
PLATELET COUNT
<100 000/mm³

Yes → Administer
intravenous therapy
(follow Fig. 3.1)

IMPROVEMENT

Yes → Continue
to follow

No

Admit to hospital

No

Continue to follow:
–haematocrit
–platelet count
–clinical condition

DETERIORATION
(shock, unstable vital
signs, haematocrit
rising)

Yes

No

Discharge, collect
convalescent serum

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Hospital flow chart

```
                    ┌─────────────────┐
                    │    ADMISSION    │
                    └─────────────────┘
                             │
                             ▼
            ┌────────────────────────────────┐
            │ Administer intravenous         │
            │ therapy (follow Fig. 3.1),     │
            │ give oral fluids ad lib.,      │
            │ collect acute serum,           │
            │ monitor:                       │
            │ −haematocrit                   │
            │ −fluid intake/output           │
            └────────────────────────────────┘
                             │
                             ▼
```

BLEEDING OR RESTLESSNESS (signs of shock) —Yes→ Administer intravenous fluid bolus (follow Fig. 3.2), sedate if necessary monitor: −serum electrolytes −blood gases —→ IMPROVEMENT (pulse pressure >20 mmHg (2.7 kPa), haematocrit normal) —Yes→ Discontinue intravenous therapy, discharge when indicated, collect convalescent serum

BLEEDING OR RESTLESSNESS —No→ Continue to administer intravenous therapy (follow Fig. 3.1)

IMPROVEMENT —No→ Continue to administer intravenous colloid therapy (follow Fig. 3.2)

DETERIORATION —Yes→ HAEMATOCRIT RISING —Yes→ Administer intravenous colloid therapy (see Fig. 3.2)

DETERIORATION —No→ Discontinue intravenous therapy when appropriate, discharge when indicated, collect convalescent serum

HAEMATOCRIT RISING —No→ Search for haemorrhage, administer blood transfusion

IMPROVEMENT —Yes→ Discontinue intravenous therapy, discharge when indicated, collect convalescent serum

IMPROVEMENT —No→ FLUID OVERLOAD? (Pulmonary oedema, filling neck veins, rales, rapidly enlarging liver, heart rate >120/min) —Yes→ Consider treating with furosemide

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ANNEX 5

Arbovirus laboratory request form for use with filter-paper discs
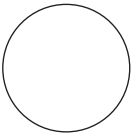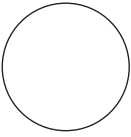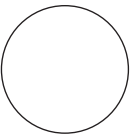
(page 1)

Name of patient _____ Hospital No. _____ Address _____ Hospital _____ Age _____

Sex _____ Physician _____ Date of admission _____ Admission complaint _____ Date of onset _____

Clinical findings: 1. Fever _____ °C (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 or kPa. Pulse _____ /min.

Restlessness/Lethargy _____ Cold extremities/body _____

Clinical laboratory findings:

Platelets (thousands) _____ /mm³ (on _____ day of illness).

Haematocrit (erythrocyte volume fraction) _____ (max) _____ (min)

Blood specimens

(Acute)
Hospital admission
Date _____

Hospital discharge
Date _____

Convalescent
Date _____

Instructions: Fill out information requested and clinical findings on both pages. Saturate filter-paper discs completely, dry them and clip them on the form. Obtain admission and discharge specimens from all patients. If patient does not return for convalescent sample, mail within 1 week of discharge.

ANNEX 5 (*continued*)

Arbovirus laboratory reporting form for use with filter-paper discs

(page 2)

To: Physician _____ Hospital _____ Address _____ Patient _____ Hospital No. _____

Date _____

Clinical findings: 1. Fever ____ °C (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 or kPa. Pulse _____ /min.
Restlessness/Lethargy _____ Cold extremities/body _____

Clinical laboratory findings:

Platelets (thousands) _____ /mm³ (on _____ day of illness).
Haematocrit (erythrocyte volume fraction) _____ (max) _____ (min)

FOR LABORATORY
USE ONLY

Serology results

Dates of specimens

_____

_____

_____

Interpretation

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WHO Collaborating Centres

WHO Collaborating Centres for Arboviruses and/or Haemorrhagic Fever Reference and Research

Argentina

Instituto Nacional de Enfermedades Virales Humanas, "Dr Julio Isidro Maiztegui", Casilla de Correo 195, 2700 Pergamino. Fax: (+54) 477 7733045.

Australia

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

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.

Central African Republic

Institut Pasteur de Bangui, P.O. Box 923, Bangui. Fax: (+236) 610109.

Cuba

Instituto de Medecina Tropical "Pedro Kouri", Apartado 61, Marianao 13, Ciudad de La Habana. Fax: (+53) 7 21957.

Finland

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

France

Centre National de Référence pour les Fièvres Hémorragiques et les Arbovirus, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15. Fax: (+33) 1 40613151.

Germany
Institut für Molekulare Genetik, Universität Heidelberg, 203 Im Neuenheimer Feld, 69120 Heidelberg. Fax: (+49) 6221 545678.

Greece
Aristotelian University of Thessaloniki, School of Medicine, Department of Microbiology, 54006 Thessaloniki. Fax: (+30) 31 999149.

India
National Institute of Virology, 20-A Dr Ambedkar Road, P.O. Box 11, 411001 Poona. Fax: (+91) 212 622669.

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Laboratory of Virology, Arbovirus Unit, Istituto Superiore de Sanitá, 299 Viale Regina Elena, 00161 Rome. Fax: (+39) 6 49902082.

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

Kenya
Kenya Medical Research Institute, Mbagathi Road, P.O. Box 54628, Nairobi. Fax: (+254) 2 725950.

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

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

Nigeria
Department of Virology, College of Medicine, University of Ibadan, Ibadan. Fax: (+234) 2 413545.

Puerto Rico
San Juan Laboratories, Centers for Disease Control and Prevention, 2 Calle Casia, 00921-3200 San Juan. Fax: (+1) 809 7666596.

Russian Federation
Ivanovsky Institute of Virology, Department of Arboviruses, 16 Gamaleya Street, 123098 Moscow. Fax: (+7) 095 1907485.

Institute of Poliomyelitis and Viral Encephalitides, Academy of Medical Sciences of Russia, 142782 Moscow. Fax: (+7) 095 1310012.

Senegal
Institut Pasteur de Dakar, 36 Avenue Pasteur, Dakar. Fax: (+221) 238772.

Slovakia
Institute of Virology, Slovak Academy of Sciences, 9 Dubravska Cesta, 84246 Bratislava. Fax: (+42) 7 374284.

Slovenia
Medical Faculty of Ljubljana, Institute of Microbiology, 4 Zaloska, 1105 Ljubljana. Fax: (+386) 61 302895.

South Africa
National Institute for Virology, Special Pathogens Unit, Private Bag X4, Sandringham 2131, Zaloska 4. Fax: (+27) 11 8820596.

Sweden
Swedish Institute for Infectious Disease Control, Lundagatan 2, Sona, 10521 Stockholm. Fax: (+46) 8 7356615.

Uganda
Uganda Virus Research Institute, P.O. Box 49, Entebbe. Fax: (+256) 42 20631.

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.

U.S. Army Medical Institute of Infectious Diseases, Fort Detrick, MD, 21702. Fax: (+1) 301 6194625.

WHO Collaborating Centres for New, Emerging and Re-emerging Diseases

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

Indonesia
U.S. Naval Medical Research Unit No. 2, NAMRU-2 Laboratory, Kotak Pos 226, Jakarta Pusat 10570. Fax: (+62) 21 4244507.

WHO Collaborating Centre for Case Management of Dengue/DHF/DSS

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

Dengue haemorrhagic fever case-reporting form

Covering period from . . . . to . . . .
Country/district/town . . . .

With shock				Without shock			
Number of presumptive cases		Number of deaths		Number of presumptive cases		Number of deaths	
<15 years M F	>15 years M F	<15 years M F	>15 years M F	<15 years M F	>15 years M F	<15 years M F	>15 years M F

Date . . . . Signature . . . .

Check-list for management of dengue haemorrhagic fever outbreaks, surveillance and reporting

Patient management

- — hospital beds and intensive care facilities
- — intravenous fluids
    - physiological saline
    - Ringer's lactate
    - 0.167 mol/litre sodium bicarbonate
- — colloidal fluids (one or more of the following)
    - plasma, fresh frozen or dried
    - plasma protein fraction, human 5%
    - dextran-40
    - other plasma substitutes
    - blood products
    - whole blood
    - platelet concentrate
- — oral rehydration solution (see page 25)
- — paracetamol
- — chloral hydrate
- — furosemide

Laboratory

- — blood-drawing equipment
- — specimen containers or filter paper
- — haematocrit centrifuge
- — platelet counting equipment
- — serum or plasma storage facilities
    - $-20\,^{\circ}$C freezer for serological specimens
    - $-70\,^{\circ}$C freezer (or liquid nitrogen) for virus isolation specimens
- — specimen shipping equipment (consult local or regional virology laboratory)

Control

- — pesticides for adult mosquitos
    - ULV formulation of residual space-spray insecticide

— spray equipment
> vehicle mounted ULV aerosol generator or thermal fogger
> mist blower, back-pack with ULV nozzle
> swing-fog machine

— mosquito screening for hospitals
— larvicides (e.g. 1% temephos sand granules)
— ovitraps (jars and paddles)
— teaching and information material
> posters, pamphlets, films, slide cassettes, video tapes,
> articles for newspapers, radio and television, etc.

Surveillance

— case-reporting forms (Annex 7)
— arbovirus laboratory diagnosis and reporting forms (Annex 5)
— names, addresses, telephone numbers
> local or national epidemiologist
> local or national public health officer
> local or national virology laboratory supervisor
> local or national vector control supervisor

— names and addresses of appropriate national and international public health authorities
— addresses for case- or outbreak-reporting

> Editor, *Weekly epidemiological record*
> World Health Organization
> 1211 Geneva 27
> Switzerland

> Editor, *Dengue bulletin*
> WHO Regional Office for South-East Asia
> World Health House
> New Delhi 110002
> India

> Editor, *Epidemiological bulletin*
> Epidemiology Coordination
> Pan American Health Organization
> 525 23rd Street, N.W.
> Washington, DC 20037
> USA

> Editor, *Dengue surveillance summary*
> San Juan Laboratories
> Centers for Disease Control and Prevention
> 2 Calle Casia
> San Juan, Puerto Rico 00921