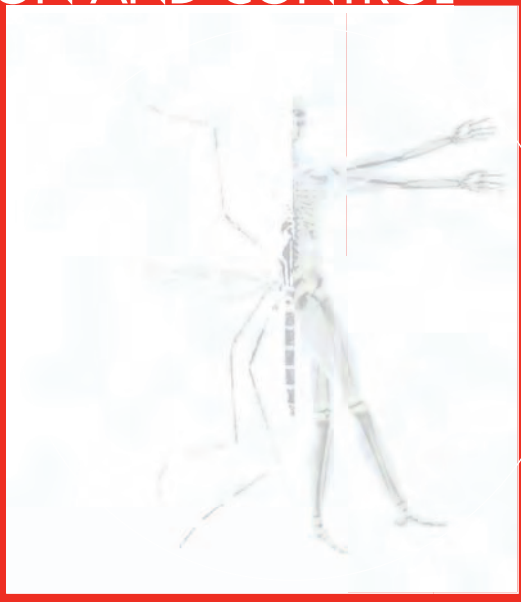
DENGUE

GUIDELINES FOR DIAGNOSIS, TREATMENT, PREVENTION AND CONTROL



New edition

2009

World Health Organization

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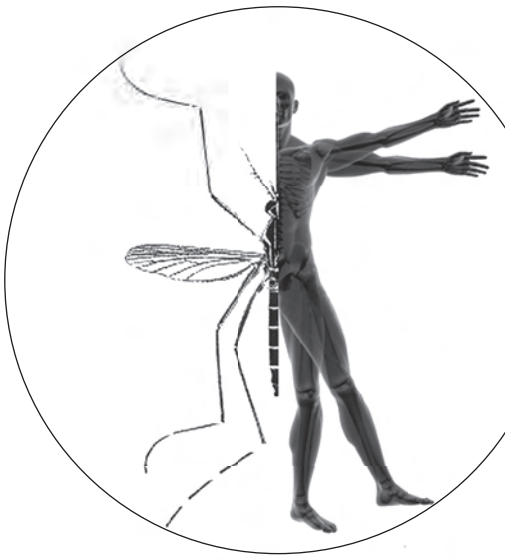

New edition

2009

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CONTENTS

PREFACE

Since the second edition of *Dengue haemorrhagic fever: diagnosis, treatment, prevention and control* was published by the World Health Organization (WHO) in 1997, the magnitude of the dengue problem has increased dramatically and has extended geographically to many previously unaffected areas. It was then, and remains today, the most important arthropod-borne viral disease of humans.

Activities undertaken by WHO regarding dengue are most recently guided at the global policy level by World Health Assembly resolution WHA55.17 (adopted by the Fifty-fifth World Health Assembly in 2002) and at the regional level by resolution CE140.R17 of the Pan American Sanitary Conference (2007), resolution WPR/RC59.R6 of the WHO Regional Committee for the Western Pacific (2008) and resolution SEA/RC61/R5 of the WHO Regional Committee for South-East Asia (2008).

This new edition has been produced to make widely available to health practitioners, laboratory personnel, those involved in vector control and other public health officials, a concise source of information of worldwide relevance on dengue. The guidelines provide updated practical information on the clinical management and delivery of clinical services; vector management and delivery of vector control services; laboratory diagnosis and diagnostic tests; and surveillance, emergency preparedness and response. Looking ahead, some indications of new and promising avenues of research are also described. Additional and more detailed specific guidance on the various specialist areas related to dengue are available from other sources in WHO and elsewhere, some of which are cited in the references.

The contributions of, and review by, many experts both within and outside WHO have facilitated the preparation of this publication through consultative and peer review processes. All contributors are gratefully acknowledged, a list of whom appears under "Acknowledgements". These guidelines are the result of collaboration between the WHO Department of Control Neglected Tropical Diseases, the WHO Department of Epidemic and Pandemic Alert and Response, and the Special Programme for Research and Training in Tropical Diseases.

This publication is intended to contribute to prevention and control of the morbidity and mortality associated with dengue and to serve as an authoritative reference source for health workers and researchers. These guidelines are not intended to replace national guidelines but to assist in the development of national or regional guidelines. They are expected to remain valid for five years (until 2014), although developments in research could change their validity, since many aspects of the prevention and control of dengue are currently being investigated in a variety of studies. The guidelines contain the most up-to-date information at the time of writing. However, the results of studies are being published regularly and should be taken into account. To address this challenge, the guide is also available on the Internet and will be updated regularly by WHO.

METHODOLOGY

These guidelines were written using the following methodology:

1. Writing team

Each chapter was allocated to a WHO coordinator and at least one non-WHO lead writer. The non-WHO lead writers received a small fee for their work. Declarations of interest were obtained from all lead writers and no conflicting interests were declared. The lead writers were chosen because of their expertise in the field and their willingness to undertake the work.

Since this guide has the broad scope of all aspects of prevention and control of dengue, the lead writers were selected for technical expertise in the areas of epidemiology, pathogenesis and transmission, clinical aspects, vector control, laboratory aspects, surveillance and response, and drug and vaccine development.

2. Peer review

All the chapters were submitted to peer review. The peer review groups were determined by the WHO coordinator and the non-WHO lead writers of each chapter. The groups consisted of five or more peer reviewers, who were not paid for their work. Declarations of interest were obtained from all peer reviewers. For those peer reviewers with potential conflicting interests, the interests are declared below.[1]

For each chapter, the process of reaching agreement on disputed issues differed. For chapters 1, 3, 4 and 6, the comments of the peer reviewers were discussed electronically within the group. Chapter 2 had a larger group whose members met for a consensus group discussion. Chapter 5 required extensive discussion, but consensus was reached without a consensus group meeting. Agreement on the chapter content was reached for all the groups.

3. Use of evidence

For each chapter, items are referenced that (1) provide new data, (2) challenge current practice, (3) describe ongoing research and (4) reflect key developments in knowledge about dengue prevention and control.

Priority was given to systematic reviews when available. Additional literature searches were conducted by the writing teams when items under 1–3 were identified, and references from personal collections of experts were added when appropriate under 4. The writing teams referred to the items under 1–4 in the text, and lists of references were added at the end of each chapter.

[1] Declared interests:

Chapter 1. Dr Anne Wilder Smith: principal investigator in dengue vaccine trial starting in 2009.

Chapter 4. Dr Mary Jane Cardosa: shareholder and director of company developing dengue diagnostic tests.

Chapter 6. Dr Robert Edelman: consultant for company involved in dengue vaccine research.

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This new edition of the dengue guidelines would not have been possible without the initiative, practical experience of many years of working in dengue, and writing contribution of Dr Michael B. Nathan, now retired from the World Health Organization (WHO).

Dr Axel Kroeger of the Special Programme for Research and Training in Tropical Diseases (WHO/TDR) equally contributed to all parts of the guidelines.

Dr John Ehrenberg, Dr Chusak Prasittisuk and Dr Jose Luis San Martin, as WHO regional advisers on dengue, contributed their unique experience to all chapters.

Dr Renu Dayal Drager (WHO) and Dr Jeremy Farrar (the Wellcome Trust) contributed technical advice to several chapters.

Dr Raman Velayudhan (WHO) coordinated the finalization and publication of the guide and advised on all the chapters.

Dr Olaf Horstick (WHO/TDR) assembled the evidence base, contributed to all chapters and contributed to the finalization of the guide.

Special thanks are due to the editorial team of Mrs Karen Ciceri and Mr Patrick Tissot at WHO.

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ABBREVIATIONS

a.i.	ad interim
ADE	antibody-dependent enhancement
ALT	alanine amino transferase
AST	aspartate amino transferase
BP	blood pressure
BSL	biosafety level
Bti	*Bacillus thuringiensis israelensis*
CD4	cluster of differentiation 4, T helper cell surface glycoprotein
CD8	cluster of differentiation 8, T cell co-receptor transmembrane glycoprotein
CFR	case-fatality rate
COMBI	communication for behavioural impact
DALY	disability-adjusted life years
DEET	diethyl-meta-toluamide
DENCO	Dengue and Control study (multi-country study)
DEN	dengue
DDT	dichlorodiphenyltrichloroethane
DF	dengue fever
DHF	dengue haemorrhagic fever
DNA	deoxyribonucleic acid
DSS	dengue shock syndrome
DT	tablet for direct application
EC	emulsifiable concentrate
ELISA	enzyme-linked immunosorbent assay
E/M	envelop/membrane antigen
FBC	full blood count
Fc-receptor	fragment, crystallisable region, a cell receptor
FRhL	fetal rhesus lung cells
GAC	E/M-specific capture IgG ELISA
GIS	Geographical Information System
GOARN	Global Outbreak Alert and Response Network
GPS	global positioning system
GR	granule
HI	haemagglutination-inhibition
HIV/AIDS	human immunodeficiency virus/acquired immunodeficiency syndrome
ICU	intensive care unit
IEC	information, education, communication
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
INF gamma	interferon gamma

IPCS	International Programme on Chemical Safety
IR3535	3-[N-acetyl-N-butyl]-aminopropionic acid ethyl ester
ITM	insecticide treated material
IV	intravenous
LAV	live attenuated vaccine
MAC-ELISA	IgM antibody-capture enzyme-linked immunosorbent assay
MIA	microsphere-based immunoassays
MoE	Ministry of Education
MoH	Ministry of Health
NAAT	nucleic acid amplification test
NASBA	nucleic acid sequence based amplification
NGO	nongovernmental organization
NS	non-structural protein
NSAID	non-steroidal anti-inflammatory drugs
OD	optical density
ORS	oral rehydration solution
PAHO	Pan American Health Organization
PCR	polymerase chain reaction
PDVI	Pediatric Dengue Vaccine Initiative
pH	measure of the acidity or basicity of a solution
prM	a region of the dengue genome
PRNT	plaque reduction and neutralization test
RNA	ribonucleic acid
RT-PCR	reverse transcriptase-polymerase chain reaction
SC	suspension concentrate
TNF alfa	tumor necrosis factor alfa
T cells	A group of lymphocytes important for cell-mediated immunity
TDR	Special Programme for Research and Training in Tropical Diseases
WBC	white blood cells
WG	Water-dispersible granule
WHO	World Health Organizaion
WP	wettable powder
YF	yellow fever

CHAPTER 1

EPIDEMIOLOGY, BURDEN OF DISEASE AND TRANSMISSION

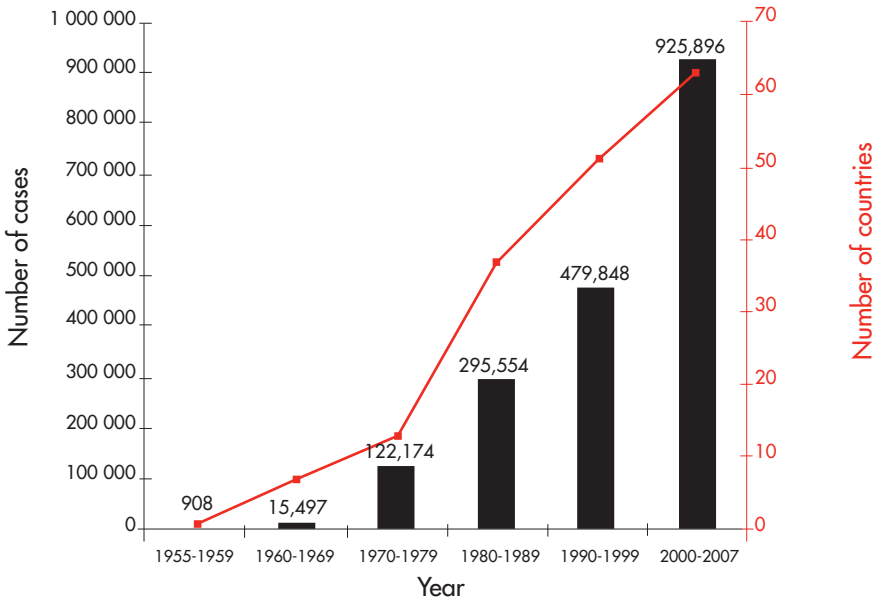
CHAPTER 1. EPIDEMIOLOGY, BURDEN OF DISEASE AND TRANSMISSION

1.1 DENGUE EPIDEMIOLOGY

Dengue is the most rapidly spreading mosquito-borne viral disease in the world. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings (Figure 1.1). An estimated 50 million dengue infections occur annually (Figure 1.2) and approximately 2.5 billion people live in dengue endemic countries (*1*). The 2002 World Health Assembly resolution WHA55.17 (*2*) urged greater commitment to dengue by WHO and its Member States. Of particular significance is the 2005 World Health Assembly resolution WHA58.3 on the revision of the International Health Regulations (IHR) (*3*), which includes dengue as an example of a disease that may constitute a public health emergency of international concern with implications for health security due to disruption and rapid epidemic spread beyond national borders.

Figure 1.1 Countries/areas at risk of dengue transmission, 2008

Figure 1.2 Average annual number of dengue fever (DF) and dengue haemorrhagic fever (DHF) cases reported to WHO, and of countries reporting dengue, 1955–2007



The following sections give an overview of the epidemiology and burden of disease in the different WHO regions. All data are from country reports from the WHO regional offices, unless referenced to a different source.

1.1.1 Dengue in Asia and the Pacific

Some 1.8 billion (more than 70%) of the population at risk for dengue worldwide live in member states of the WHO South-East Asia Region and Western Pacific Region, which bear nearly 75% of the current global disease burden due to dengue. The Asia Pacific Dengue Strategic Plan for both regions (2008–2015) has been prepared in consultation with member countries and development partners in response to the increasing threat from dengue, which is spreading to new geographical areas and causing high mortality during the early phase of outbreaks. The strategic plan aims to aid countries to reverse the rising trend of dengue by enhancing their preparedness to detect, characterize and contain outbreaks rapidly and to stop the spread to new areas.

1.1.1.1 Dengue in the WHO South-East Asia Region

Since 2000, epidemic dengue has spread to new areas and has increased in the already affected areas of the region. In 2003, eight countries – Bangladesh, India, Indonesia, Maldives, Myanmar, Sri Lanka, Thailand and Timor-Leste – reported dengue cases. In 2004, Bhutan reported the country's first dengue outbreak. In 2005, WHO's Global Outbreak Alert and Response Network (GOARN) responded to an outbreak with a high case-fatality rate (3.55%) in Timor-Leste. In November 2006, Nepal reported indigenous dengue cases for the first time. The Democratic Peoples' Republic of Korea is the only country of the South-East Region that has no reports of indigenous dengue.

The countries of the region have been divided into four distinct climatic zones with different dengue transmission potential. Epidemic dengue is a major public health problem in Indonesia, Myanmar, Sri Lanka, Thailand and Timor-Leste which are in the tropical monsoon and equatorial zone where *Aedes aegypti* is widespread in both urban and rural areas, where multiple virus serotypes are circulating, and where dengue is a leading cause of hospitalization and death in children. Cyclic epidemics are increasing in frequency and in-country geographic expansion is occurring in Bangladesh, India and Maldives – countries in the deciduous dry and wet climatic zone with multiple virus serotypes circulating. Over the past four years, epidemic dengue activity has spread to Bhutan and Nepal in the sub-Himalayan foothills.

Reported case fatality rates for the region are approximately 1%, but in India, Indonesia and Myanmar, focal outbreaks away from the urban areas have reported case-fatality rates of 3–5%.

In Indonesia, where more than 35% of the country's population lives in urban areas, 150 000 cases were reported in 2007 (the highest on record) with over 25 000 cases reported from both Jakarta and West Java. The case-fatality rate was approximately 1%.

In Myanmar in 2007 the states/divisions that reported the highest number of cases were Ayayarwaddy, Kayin, Magway, Mandalay, Mon, Rakhine, Sagaing, Tanintharyi and Yangon. From January to September 2007, Myanmar reported 9578 cases. The reported case-fatality rate in Myanmar is slightly above 1%.

In Thailand, dengue is reported from all four regions: Northern, Central, North-Eastern and Southern. In June 2007, outbreaks were reported from Trat province, Bangkok, Chiangrai, Phetchabun, Phitsanulok, Khamkaeng Phet, Nakhon Sawan and Phit Chit. A total of 58 836 cases were reported from January to November 2007. The case-fatality rate in Thailand is below 0.2%.

Dengue prevention and control will be implemented through the Bi-regional Dengue Strategy (2008–2015) of the WHO South-East Asia and Western Pacific regions. This consists of six elements: (i) dengue surveillance, (ii) case management, (iii) outbreak response, (iv) integrated vector management, (v) social mobilization and communication for dengue and (vi) dengue research (a combination of both formative and operational research). The strategy has been endorsed by resolution SEA/RC61/R5 of the WHO Regional Committee for South-East Asia in 2008 (*4*).

1.1.1.2 Dengue in the WHO Western Pacific Region

Dengue has emerged as a serious public health problem in the Western Pacific Region (*5*). Since the last major pandemic in 1998, epidemics have recurred in much of the area. Lack of reporting remains one of the most important challenges in dengue prevention and control.

Between 2001 and 2008, 1 020 333 cases were reported in Cambodia, Malaysia, Philippines, and Viet Nam – the four countries in the Western Pacific Region with the highest numbers of cases and deaths. The combined death toll for these four countries was 4798 (official country reports). Compared with other countries in the same region, the number of cases and deaths remained highest in Cambodia and the Philippines in 2008. Overall, case management has improved in the Western Pacific Region, leading to a decrease in case fatality rates.

Dengue has also spread throughout the Pacific Island countries and areas. Between 2001 and 2008, the six most affected Pacific island countries and areas were French Polynesia (35 869 cases), New Caledonia (6836 cases), Cook Islands (3735 cases), American Samoa (1816 cases), Palau (1108 cases) and the Federal States of Micronesia (664 cases). The total number of deaths for the six island countries was 34 (official country reports). Although no official reports have been submitted to WHO by Kiribati, the country did experience a dengue outbreak in 2008, reporting a total of 837 cases and causing great concern among the national authorities and among some of the other countries in the region.

Historically, dengue has been reported predominantly among urban and peri-urban populations where high population density facilitates transmission. However, evidence from recent outbreaks, as seen in Cambodia in 2007, suggests that they are now occurring in rural areas.

Implementing the Bi-regional Dengue Strategy for Asia and the Pacific (2008–2015) is a priority following endorsement by the 2008 resolution WPR/RC59.R6 of the WHO Regional Committee for the Western Pacific (*6*).

1.1.2 Dengue in the Americas

Interruption of dengue transmission in much the WHO Region of the Americas resulted from the *Ae. aegypti* eradication campaign in the Americas, mainly during the 1960s and early 1970s. However, vector surveillance and control measures were not sustained and there were subsequent reinfestations of the mosquito, followed by outbreaks in the Caribbean, and in Central and South America (*7*). Dengue fever has since spread with cyclical outbreaks occurring every 3–5 years. The biggest outbreak occurred in 2002 with more than 1 million reported cases.

From 2001 to 2007, more than 30 countries of the Americas notified a total of 4 332 731 cases of dengue (*8*). The number of cases of dengue haemorrhagic fever (DHF) in the same period was 106 037. The total number of dengue deaths from 2001 to

2007 was 1299, with a DHF case fatality rate of 1.2%. The four serotypes of the dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4) circulate in the region. In Barbados, Colombia, Dominican Republic, El Salvador, Guatemala, French Guyana, Mexico, Peru, Puerto Rico and Venezuela, all four serotypes were simultaneously identified in one year during this period.

By subregion of the Americas, dengue is characterized as described below. All data are from the Pan American Health Organization (PAHO) (*8*).

The Southern Cone countries
Argentina, Brazil, Chile, Paraguay and Uruguay are located in this subregion. In the period from 2001 to 2007, 64.6% (2 798 601) of all dengue cases in the Americas were notified in this subregion, of which 6733 were DHF with a total of 500 deaths. Some 98.5% of the cases were notified by Brazil, which also reports the highest case fatality rate in the subregion. In the subregion, DEN-1, -2 and -3 circulate.

Andean countries
This subregion includes Bolivia, Colombia, Ecuador, Peru and Venezuela, and contributed 19% (819 466) of dengue cases in the Americas from 2001 to 2007. It is the subregion with the highest number of reported DHF cases, with 58% of all cases (61 341) in the Americas, and 306 deaths. Colombia and Venezuela have most cases in the subregion (81%), and in Colombia there were most dengue deaths (225, or 73%). In Colombia, Peru and Venezuela all four dengue serotypes were identified.

Central American countries and Mexico
During 2001–2007, a total of 545 049 cases, representing 12.5% of dengue in the Americas, was reported, with 35 746 cases of DHF and 209 deaths. Nicaragua had 64 deaths (31%), followed by Honduras with 52 (25%) and Mexico with 29 (14%). Costa Rica, Honduras and Mexico reported the highest number of cases in this period. DEN-1, -2 and -3 were the serotypes most frequently reported.

Caribbean countries
In this subregion 3.9% (168 819) of the cases of dengue were notified, with 2217 DHF cases and 284 deaths. Countries with the highest number of dengue cases in the Latin Caribbean were Cuba, Puerto Rico and the Dominican Republic, whereas in the English and French Caribbean, Martinique, Trinidad and Tobago and French Guiana reported the highest numbers of cases. The Dominican Republic reported 77% of deaths (220) during the period 2001–2007. All four serotypes circulate in the Caribbean area, but predominantly DEN-1 and -2.

North American countries
The majority of the notified cases of dengue in Canada and the United States are persons who had travelled to endemic areas in Asia, the Caribbean, or Central or South America (*9*). From 2001 to 2007, 796 cases of dengue were reported in the United States, the majority imported. Nevertheless, outbreaks of dengue in Hawaii have been reported, and there were outbreaks sporadically with local transmission in Texas at the border with Mexico (*10,11*).

The Regional Dengue Programme of PAHO focuses public policies towards a multisectoral and interdisciplinary integration. This allows the formulation, implementation, monitoring and evaluation of national programmes through the Integrated Management Strategy for Prevention and Control of Dengue (EGI-dengue, from its acronym in Spanish). This has six key components: (i) social communication (using Communication for Behavioural Impact (COMBI)), (ii) entomology, (iii) epidemiology, (iv) laboratory diagnosis, (v) case management and (vi) environment. This strategy has been endorsed by PAHO resolutions (*12–15*). Sixteen countries and three subregions (Central America, Mercosur and the Andean subregion) agreed to use EGI-dengue as a strategy and are in the process of implementation.

1.1.3 Dengue in the WHO African Region

Although dengue exists in the WHO African Region, surveillance data are poor. Outbreak reports exist, although they are not complete, and there is evidence that dengue outbreaks are increasing in size and frequency (*16*). Dengue is not officially reported to WHO by countries in the region. Dengue-like illness has been recorded in Africa though usually without laboratory confirmation and could be due to infection with dengue virus or with viruses such as chikungunya that produce similar clinical symptoms.

Dengue has mostly been documented in Africa from published reports of serosurveys or from diagnosis in travellers returning from Africa, and dengue cases from countries in Sub-Saharan Africa. A serosurvey (*17*) suggests that dengue existed in Africa as far back as 1926–1927, when the disease caused an epidemic in Durban, South Africa. Cases of dengue imported from India were detected in the 1980s (*18*).

For eastern Africa, the available evidence so far indicates that DEN-1, -2 and -3 appear to be common causes of acute fever. Examples of this are outbreaks in the Comoros in various years (1948, 1984 and 1993, DEN-1 and -2) (*19*) and Mozambique (1984–1985, DEN-3) (*20*).

In western Africa in the 1960s, DEN-1, -2 and -3 were isolated for the first time from samples taken from humans in Nigeria (*21*). Subsequent dengue outbreaks have been reported from different countries, as for example from Burkina Faso (1982, DEN-2) (*22*) and Senegal (1999, DEN-2) (*23*). Also DEN-2 and DEN-3 cases were confirmed in Côte d'Ivoire in 2006 and 2008.

Despite poor surveillance for dengue in Africa, it is clear that epidemic dengue fever caused by all four dengue serotypes has increased dramatically since 1980, with most epidemics occurring in eastern Africa, and to a smaller extent in western Africa, though this situation may be changing in 2008.

While dengue may not appear to be a major public health problem in Africa compared to the widespread incidence of malaria and HIV/AIDS, the increasing frequency and severity of dengue epidemics worldwide calls for a better understanding of the epidemiology of dengue infections with regard to the susceptibility of African populations to dengue and the interference between dengue and the other major communicable diseases of the continent.

1.1.4 Dengue in the WHO Eastern Mediterranean Region (Figure 1.3)

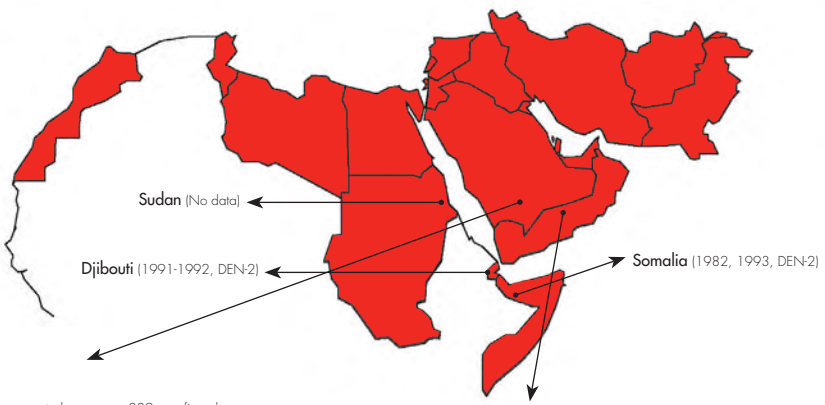
Outbreaks of dengue have been documented in the Eastern Mediterranean Region possibly as early as 1799 in Egypt (*24*). The frequency of reported outbreaks continue to increase, with outbreaks for example in Sudan (1985, DEN-1 and -2) (*25*) and in Djibouti (1991, DEN-2) (*26*).

Recent outbreaks of suspected dengue have been recorded in Pakistan, Saudi Arabia, Sudan and Yemen, 2005–2006 (*24*). In Pakistan, the first confirmed outbreak of DHF occurred in 1994. A DEN-3 epidemic with DHF was first reported in 2005 (*27*). Since then, the expansion of dengue infections with increasing frequency and severity has been reported from large cities in Pakistan as far north as the North-West Frontier Province in 2008. Dengue is now a reportable disease in Pakistan. A pertinent issue for this region is the need to better understand the epidemiological situation of dengue in areas that are endemic for Crimean-Congo haemorrhagic fever and co-infections of these pathogens.

Yemen is also affected by the increasing frequency and geographic spread of epidemic dengue, and the number of cases has risen since the major DEN-3 epidemic that occurred in the western al-Hudeidah governorate in 2005. In 2008 dengue affected the southern province of Shabwa.

Since the first case of DHF died in Jeddah in 1993, Saudi Arabia has reported three major epidemics: a DEN-2 epidemic in 1994 with 469 cases of dengue, 23 cases of DHF, two cases of dengue shock syndrome (DSS) and two deaths; a DEN-1 epidemic in 2006 with 1269 cases of dengue, 27 cases of DHF, 12 cases of DSS and six

Figure 1.3 Outbreaks of dengue fever in the WHO Eastern Mediterranean Region, 1994–2005



DEN-2:

1994:	673	suspected cases,	289	confirmed cases
1995:	136	suspected cases,	6	confirmed cases
1996:	57	suspected cases,	2	confirmed cases
1997:	62	suspected cases,	15	confirmed cases
1998:	31	suspected cases,	0	confirmed cases
1999:	26	suspected cases,	3	confirmed cases
2000:	17	suspected cases,	0	confirmed cases
2001:	7	suspected cases,	0	confirmed cases
2005:	32	suspected (confirmed)		

Al-Hudaydah, Mukkala, Shaabwa
(1994, DEN-3, no data);
Al-Hudaydah, Yemen
(September 2000, DEN-2, 653 suspected cases, 80 deaths (CFR = 12%));
Al-Hudaydah, Yemen
(March 2004, 45 suspected cases, 2 deaths);
Al-Hudaydah, Mukkala
(March 2005, 403 suspected cases, 2 deaths);

deaths; and a DEN-3 epidemic in 2008 with 775 cases of dengue, nine cases of DHF, four cases of DSS and four deaths. A pertinent issue for the IHR is that Jeddah is a Haj entry point – as well as being the largest commercial port in the country, and the largest city with the busiest airport in the western region – with large numbers of people coming from high-burden dengue countries such as Indonesia, Malaysia and Thailand, in addition to the dengue-affected countries of the region.

1.1.5 Dengue in other regions

As described above, dengue is now endemic in all WHO regions except the WHO European Region. Data available for the European region (http://data.euro.who.int/cisid/) indicate that most cases in the region have been reported by European Union member states, either as incidents in overseas territories or importations from endemic countries. [See also a report from the European Centre for Disease Prevention and Control (*28*)]. However, in the past, dengue has been endemic in some Balkan and Mediterranean countries of the region, and imported cases in the presence of known mosquito vectors (e.g. *Aedes albopictus*) cannot exclude future disease spread.

Globally, reporting on dengue cases shows cyclical variation with high epidemic years and non-epidemic years. Dengue often presents in the form of large outbreaks. There is, however, also a seasonality of dengue, with outbreaks occurring in different periods of the year. This seasonality is determined by peak transmission of the disease, influenced by characteristics of the host, the vector and the agent.

1.1.6 Dengue case classification

Dengue has a wide spectrum of clinical presentations, often with unpredictable clinical evolution and outcome. While most patients recover following a self-limiting non-severe clinical course, a small proportion progress to severe disease, mostly characterized by plasma leakage with or without haemorrhage. Intravenous rehydration is the therapy of choice; this intervention can reduce the case fatality rate to less than 1% of severe cases. The group progressing from non-severe to severe disease is difficult to define, but this is an important concern since appropriate treatment may prevent these patients from developing more severe clinical conditions.

Triage, appropriate treatment, and the decision as to where this treatment should be given (in a health care facility or at home) are influenced by the case classification for dengue. This is even more the case during the frequent dengue outbreaks worldwide, where health services need to be adapted to cope with the sudden surge in demand.

Changes in the epidemiology of dengue, as described in the previous sections, lead to problems with the use of the existing WHO classification. Symptomatic dengue virus infections were grouped into three categories: undifferentiated fever, dengue fever (DF) and dengue haemorrhagic fever (DHF). DHF was further classified into four severity grades, with grades III and IV being defined as dengue shock syndrome (DSS) (*29*). There have been many reports of difficulties in the use of this classification (*30–32*), which were summarized in a systematic literature review (*33*). Difficulties in applying the criteria for DHF in the clinical situation, together with the increase in clinically

severe dengue cases which did not fulfil the strict criteria of DHF, led to the request for the classification to be reconsidered. Currently the classification into DF/DHF/DSS continues to be widely used. (*29*)

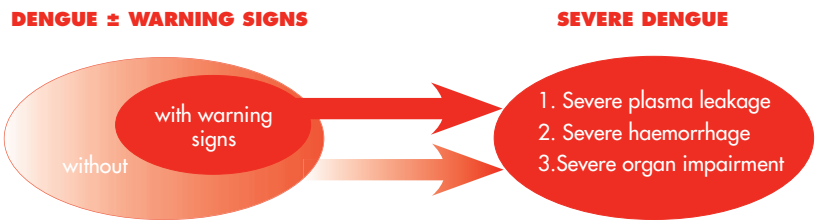
A WHO/TDR-supported prospective clinical multicentre study across dengue-endemic regions was set up to collect evidence about criteria for classifying dengue into levels of severity. The study findings confirmed that, by using a set of clinical and/or laboratory parameters, one sees a clear-cut difference between patients with severe dengue and those with non-severe dengue. However, for practical reasons it was desirable to split the large group of patients with non-severe dengue into two subgroups – patients with warning signs and those without them. Criteria for diagnosing dengue (with or without warning signs) and severe dengue are presented in Figure 1.4. It must be kept in mind that even dengue patients without warning signs may develop severe dengue.

Expert consensus groups in Latin America (Havana, Cuba, 2007), South-East Asia (Kuala Lumpur, Malaysia, 2007), and at WHO headquarters in Geneva, Switzerland in 2008 agreed that:

"dengue is one disease entity with different clinical presentations and often with unpredictable clinical evolution and outcome";

the classification into levels of severity has a high potential for being of practical use in the clinicians' decision as to where and how intensively the patient should be observed and treated (i.e. triage, which is particularly useful in outbreaks), in more consistent reporting in the national and international surveillance system, and as an end-point measure in dengue vaccine and drug trials.

Figure 1.4 Suggested dengue case classification and levels of severity



CRITERIA FOR DENGUE ± WARNING SIGNS

Probable dengue

live in /travel to dengue endemic area.
Fever and 2 of the following criteria:

- Nausea, vomiting
- Rash
- Aches and pains
- Tourniquet test positive
- Leukopenia
- Any warning sign

Laboratory-confirmed dengue

(important when no sign of plasma leakage)

Warning signs*

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargment >2 cm
- Laboratory: increase in HCT concurrent with rapid decrease in platelet count

*(requiring strict observation and medical intervention)

CRITERIA FOR SEVERE DENGUE

Severe plasma leakage

leading to:

- Shock (DSS)
- Fluid accumulation with respiratory distress

Severe bleeding

as evaluated by clinician

Severe organ involvement

- Liver: AST or ALT >=1000
- CNS: Impaired consciousness
- Heart and other organs

This model for classifying dengue has been suggested by an expert group (Geneva, Switzerland, 2008) and is currently being tested in 18 countries by comparing its performance in practical settings to the existing WHO case classification. The process will be finalized in 2010. For practical reasons this guide adapts the distinction between dengue and severe dengue.

Additionally the guide uses three categories for case management (A, B, C) (Chapter 2).

1.2 BURDEN OF DISEASE

Dengue inflicts a significant health, economic and social burden on the populations of endemic areas. Globally the estimated number of disability-adjusted life years (DALYs) lost to dengue in 2001 was 528 (*34*). In Puerto Rico, an estimated yearly mean of 580 DALYs per million population were lost to dengue between 1984 and 1994 – similar to the cumulative total of DALYs lost to malaria, tuberculosis, intestinal helminths and the childhood disease cluster in all of Latin America and the Caribbean (*35*).

The number of cases reported annually to WHO ranged from 0.4 to 1.3 million in the decade 1996 – 2005. As an infectious disease, the number of cases varies substantially from year to year. Underreporting and misdiagnoses are major obstacles to understanding the full burden of dengue (*36*).

Available data from South-East Asia is largely derived from hospitalized cases among children but the burden due to uncomplicated dengue fever is also considerable. In a prospective study of schoolchildren in northern Thailand the mean annual burden of dengue over a five-year period was 465.3 DALYs per million, with non-hospitalized patients with dengue illness contributing 44 – 73% of the total (*37*).

Studies on the cost of dengue were conducted in eight countries in 2005-2006: five in the Americas (Brazil, El Salvador, Guatemala, Panama, Venezuela) and three in Asia (Cambodia, Malaysia, Thailand) (*38*). As dengue also affected other household members who helped care for the dengue patient, an average episode represented 14.8 lost days for ambulatory patients and 18.9 days for hospitalized patients. The overall cost of a non-fatal ambulatory case averaged US$ 514, while the cost of a non-fatal hospitalized case averaged US$ 1491. On average, a hospitalized case of dengue cost three times what an ambulatory case costs. Combining the ambulatory and hospitalized patients and factoring in the risk of death, the overall cost of a dengue case is US$ 828. Merging this number with the average annual number of officially reported dengue cases from the eight countries studied in the period 2001 – 2005 (532 000 cases) gives a cost of officially reported dengue of US$ 440 million. This very conservative estimate ignores not only the underreporting of cases but also the substantial costs associated with dengue surveillance and vector control programmes. This study showed that a treated dengue episode imposes substantial costs on both the health sector and the overall economy. If a vaccine were able to prevent much of this burden, the economic gains would be substantial.

Children are at a higher risk of severe dengue (*39*). Intensive care is required for severely ill patients, including intravenous fluids, blood or plasma transfusion and medicines.

Dengue afflicts all levels of society but the burden may be higher among the poorest who grow up in communities with inadequate water supply and solid waste infrastructure, and where conditions are most favourable for multiplication of the main vector, *Ae. aegypti*.

1.3 DENGUE IN INTERNATIONAL TRAVEL

Travellers play an essential role in the global epidemiology of dengue infections, as viraemic travellers carry various dengue serotypes and strains into areas with mosquitoes that can transmit infection (*40*). Furthermore, travellers perform another essential service in providing early alerts to events in other parts of the world. Travellers often transport the dengue virus from areas in tropical developing countries, where limited laboratory facilities exist, to developed countries with laboratories that can identify virus serotypes (*41*). Access to research facilities makes it possible to obtain more detailed information about a virus, including serotype and even sequencing, when that information would be valuable. Systematic collection of clinical specimens and banking of serum or isolates may have future benefits as new technologies become available.

From the data collected longitudinally over a decade by the GeoSentinel Surveillance Network (www.geosentinel.org) it was possible, for example, to examine month-by-month morbidity from a sample of 522 cases of dengue as a proportion of all diagnoses in 24 920 ill returned travellers seen at 33 surveillance sites. Travel-related dengue demonstrated a defined seasonality for multiple regions (South-East Asia, South Central Asia, Caribbean, South America) (*42*).

Information about dengue in travellers, using sentinel surveillance, can be shared rapidly to alert the international community to the onset of epidemics in endemic areas where there is no surveillance and reporting of dengue, as well as the geographic spread of virus serotypes and genotypes to new areas which increases the risk of severe dengue. The information can also assist clinicians in temperate regions – most of whom are not trained in clinical tropical diseases – to be alert for cases of dengue fever in ill returned travellers. The clinical manifestations and complications of dengue can also be studied in travellers (most of them adult and non-immune) as dengue may present differently compared with the endemic population (most of them in the paediatric age group and with pre-existing immunity). The disadvantage of such sentinel surveillance, however, is the lack of a denominator: true risk incidence cannot be determined. An increase in cases in travellers could be due to increased travel activity to dengue endemic areas, for instance.

1.4 TRANSMISSION

1.4.1 The virus

Dengue virus (DEN) is a small single-stranded RNA virus comprising four distinct serotypes (DEN-1 to -4). These closely related serotypes of the dengue virus belong to the genus Flavivirus, family Flaviviridae.

The mature particle of the dengue virus is spherical with a diameter of 50nm containing multiple copies of the three structural proteins, a host-derived membrane bilayer and a single copy of a positive-sense, single-stranded RNA genome. The genome is cleaved by host and viral proteases in three structural proteins (capsid, C, prM, the precursor of membrane, M, protein and envelope, E) and seven nonstructural proteins (NS).

Distinct genotypes or lineages (viruses highly related in nucleotide sequence) have been identified within each serotype, highlighting the extensive genetic variability of the dengue serotypes. Purifying selection appears to be a dominant theme in dengue viral evolution, however, such that only viruses that are "fit" for both human and vector are maintained. Among them, "Asian" genotypes of DEN-2 and DEN-3 are frequently associated with severe disease accompanying secondary dengue infections (*43–45*). Intra-host viral diversity (quasispecies) has also been described in human hosts.

1.4.2 The vectors

The various serotypes of the dengue virus are transmitted to humans through the bites of infected *Aedes* mosquitoes, principally *Ae. aegypti*. This mosquito is a tropical and subtropical species widely distributed around the world, mostly between latitudes 35 ^{0}N and 35 ^{0}S. These geographical limits correspond approximately to a winter isotherm of 10 ^{0}C. *Ae. aegypti* has been found as far north as 45 ^{0}N, but such invasions have occurred during warmer months and the mosquitoes have not survived the winters. Also, because of lower temperatures, *Ae. aegypti* is relatively uncommon above 1000 metres. The immature stages are found in water-filled habitats, mostly in artificial containers closely associated with human dwellings and often indoors. Studies suggest that most female *Ae. aegypti* may spend their lifetime in or around the houses where they emerge as adults. This means that people, rather than mosquitoes, rapidly move the virus within and between communities. Dengue outbreaks have also been attributed to *Aedes albopictus*, *Aedes polynesiensis* and several species of the *Aedes scutellaris* complex. Each of these species has a particular ecology, behaviour and geographical distribution. In recent decades *Aedes albopictus* has spread from Asia to Africa, the Americas and Europe, notably aided by the international trade in used tyres in which eggs are deposited when they contain rainwater. The eggs can remain viable for many months in the absence of water (Chapter 3).

1.4.2 The host

After an incubation period of 4–10 days, infection by any of the four virus serotypes can produce a wide spectrum of illness, although most infections are asymptomatic or subclinical (Chapter 2). Primary infection is thought to induce lifelong protective immunity to the infecting serotype (*46*). Individuals suffering an infection are protected from clinical illness with a different serotype within 2–3 months of the primary infection but with no long-term cross-protective immunity.

Individual risk factors determine the severity of disease and include secondary infection, age, ethnicity and possibly chronic diseases (bronchial asthma, sickle cell anaemia and diabetes mellitus). Young children in particular may be less able than adults to compensate for capillary leakage and are consequently at greater risk of dengue shock.

Seroepidemiological studies in Cuba and Thailand consistently support the role of secondary heterotypic infection as a risk factor for severe dengue, although there are a few reports of severe cases associated with primary infection (*47–50*). The time interval between infections and the particular viral sequence of infections may also be of importance. For instance, a higher case fatality rate was observed in Cuba when DEN-2 infection followed a DEN-1 infection after an interval of 20 years compared to an interval of four years. Severe dengue is also regularly observed during primary infection of infants born to dengue-immune mothers. Antibody-dependent enhancement (ADE) of infection has been hypothesized (*51,52*) as a mechanism to explain severe dengue in the course of a secondary infection and in infants with primary infections. In this model, non-neutralizing, cross-reactive antibodies raised during a primary infection, or acquired passively at birth, bind to epitopes on the surface of a heterologous infecting virus and facilitate virus entry into Fc-receptor-bearing cells. The increased number of infected cells is predicted to result in a higher viral burden and induction of a robust host immune response that includes inflammatory cytokines and mediators, some of which may contribute to capillary leakage. During a secondary infection, cross-reactive memory T cells are also rapidly activated, proliferate, express cytokines and die by apoptosis in a manner that generally correlates with overall disease severity. Host genetic determinants might influence the clinical outcome of infection (*53,54*), though most studies have been unable to adequately address this issue. Studies in the American region show the rates of severe dengue to be lower in individuals of African ancestry than those in other ethnic groups. *(54)*

The dengue virus enters via the skin while an infected mosquito is taking a bloodmeal. During the acute phase of illness the virus is present in the blood and its clearance from this compartment generally coincides with defervescence. Humoral and cellular immune responses are considered to contribute to virus clearance via the generation of neutralizing antibodies and the activation of $CD4^+$ and $CD8^+$ T lymphocytes. In addition, innate host defence may limit infection by the virus. After infection, serotype-specific and cross-reactive antibodies and $CD4^+$ and $CD8^+$ T cells remain measurable for years.

Plasma leakage, haemoconcentration and abnormalities in homeostasis characterize severe dengue. The mechanisms leading to severe illness are not well defined but the immune response, the genetic background of the individual and the virus characteristics may all contribute to severe dengue.

Recent data suggest that endothelial cell activation could mediate plasma leakage (55,56). Plasma leakage is thought to be associated with functional rather than destructive effects on endothelial cells. Activation of infected monocytes and T cells, the complement system and the production of mediators, monokines, cytokines and soluble receptors may also be involved in endothelial cell dysfunction.

Thrombocytopenia may be associated with alterations in megakaryocytopoieses by the infection of human haematopoietic cells and impaired progenitor cell growth, resulting in platelet dysfunction (platelet activation and aggregation), increased destruction or consumption (peripheral sequestration and consumption). Haemorrhage may be a consequence of the thrombocytopenia and associated platelet dysfunction or disseminated intravascular coagulation. In summary, a transient and reversible imbalance of inflammatory mediators, cytokines and chemokines occurs during severe dengue, probably driven by a high early viral burden, and leading to dysfunction of vascular endothelial cells, derangement of the haemocoagulation system then to plasma leakage, shock and bleeding.

1.4.4 Transmission of the dengue virus

Humans are the main amplifying host of the virus. Dengue virus circulating in the blood of viraemic humans is ingested by female mosquitoes during feeding. The virus then infects the mosquito mid-gut and subsequently spreads systemically over a period of 8–12 days. After this extrinsic incubation period, the virus can be transmitted to other humans during subsequent probing or feeding. The extrinsic incubation period is influenced in part by environmental conditions, especially ambient temperature. Thereafter the mosquito remains infective for the rest of its life. *Ae. aegypti* is one of the most efficient vectors for arboviruses because it is highly anthropophilic, frequently bites several times before completing oogenesis, and thrives in close proximity to humans. Vertical transmission (transovarial transmission) of dengue virus has been demonstrated in the laboratory but rarely in the field. The significance of vertical transmission for maintenance of the virus is not well understood. Sylvatic dengue strains in some parts of Africa and Asia may also lead to human infection, causing mild illness. Several factors can influence the dynamics of virus transmission – including environmental and climate factors, host-pathogen interactions and population immunological factors. Climate directly influences the biology of the vectors and thereby their abundance and distribution; it is consequently an important determinant of vector-borne disease epidemics.

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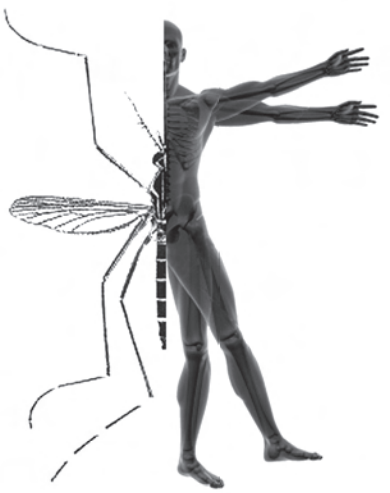


CHAPTER 2

CLINICAL MANAGEMENT AND DELIVERY OF CLINICAL SERVICES

CHAPTER 2. CLINICAL MANAGEMENT AND DELIVERY OF CLINICAL SERVICES

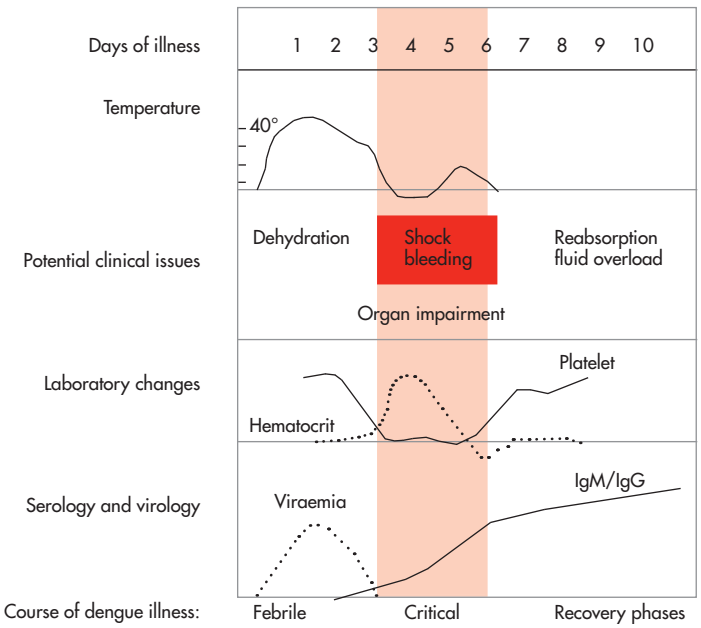
2.1 OVERVIEW

Dengue infection is a systemic and dynamic disease. It has a wide clinical spectrum that includes both severe and non-severe clinical manifestations (*1*). After the incubation period, the illness begins abruptly and is followed by the three phases – febrile, critical and recovery (Figure 2.1).

For a disease that is complex in its manifestations, management is relatively simple, inexpensive and very effective in saving lives so long as correct and timely interventions are instituted. The key is early recognition and understanding of the clinical problems during the different phases of the disease, leading to a rational approach to case management and a good clinical outcome. An overview of good and bad clinical practices is given in Textbox A.

Activities (triage and management decisions) at the primary and secondary care levels (where patients are first seen and evaluated) are critical in determining the clinical outcome of dengue. A well-managed front-line response not only reduces the number of unnecessary hospital admissions but also saves the lives of dengue patients. Early notification of dengue cases seen in primary and secondary care is crucial for identifying outbreaks and initiating an early response (Chapter 5). Differential diagnosis needs to be considered (Textbox B).

Figure 2.1 The course of dengue illness*



* Source: adapted from Yip (2) by chapter authors.

2.1.1 Febrile phase

Patients typically develop high-grade fever suddenly. This acute febrile phase usually lasts 2–7 days and is often accompanied by facial flushing, skin erythema, generalized body ache, myalgia, arthralgia and headache (1). Some patients may have sore throat, injected pharynx and conjunctival injection. Anorexia, nausea and vomiting are common. It can be difficult to distinguish dengue clinically from non-dengue febrile diseases in the early febrile phase. A positive tourniquet test in this phase increases the probability of dengue (3,4). In addition, these clinical features are indistinguishable between severe and non-severe dengue cases. Therefore monitoring for warning signs and other clinical parameters (Textbox C) is crucial to recognizing progression to the critical phase.

Mild haemorrhagic manifestations like petechiae and mucosal membrane bleeding (e.g. nose and gums) may be seen (3,5). Massive vaginal bleeding (in women of childbearing age) and gastrointestinal bleeding may occur during this phase but is not common (5). The liver is often enlarged and tender after a few days of fever (3). The earliest abnormality in the full blood count is a progressive decrease in total white cell count, which should alert the physician to a high probability of dengue.

2.1.2 Critical phase

Around the time of defervescence, when the temperature drops to 37.5–38°C or less and remains below this level, usually on days 3–7 of illness, an increase in capillary permeability in parallel with increasing haematocrit levels may occur (6,7). This marks the beginning of the critical phase. The period of clinically significant plasma leakage usually lasts 24–48 hours.

Progressive leukopenia (3) followed by a rapid decrease in platelet count usually precedes plasma leakage. At this point patients without an increase in capillary permeability will improve, while those with increased capillary permeability may become worse as a result of lost plasma volume. The degree of plasma leakage varies. Pleural effusion and ascites may be clinically detectable depending on the degree of plasma leakage and the volume of fluid therapy. Hence chest x-ray and abdominal ultrasound can be useful tools for diagnosis. The degree of increase above the baseline haematocrit often reflects the severity of plasma leakage.

Shock occurs when a critical volume of plasma is lost through leakage. It is often preceded by warning signs. The body temperature may be subnormal when shock occurs. With prolonged shock, the consequent organ hypoperfusion results in progressive organ impairment, metabolic acidosis and disseminated intravascular coagulation. This in turn leads to severe haemorrhage causing the haematocrit to decrease in severe shock. Instead of the leukopenia usually seen during this phase of dengue, the total white cell count may increase in patients with severe bleeding. In addition, severe organ impairment such as severe hepatitis, encephalitis or myocarditis and/or severe bleeding may also develop without obvious plasma leakage or shock (8).

Those who improve after defervescence are said to have non-severe dengue. Some patients progress to the critical phase of plasma leakage without defervescence and, in

these patients, changes in the full blood count should be used to guide the onset of the critical phase and plasma leakage.

Those who deteriorate will manifest with warning signs. This is called dengue with warning signs (Textbox C). Cases of dengue with warning signs will probably recover with early intravenous rehydration. Some cases will deteriorate to severe dengue (see below).

2.1.3 Recovery phase

If the patient survives the 24–48 hour critical phase, a gradual reabsorption of extravascular compartment fluid takes place in the following 48–72 hours. General well-being improves, appetite returns, gastrointestinal symptoms abate, haemodynamic status stabilizes and diuresis ensues. Some patients may have a rash of "isles of white in the sea of red" (9). Some may experience generalized pruritus. Bradycardia and electrocardiographic changes are common during this stage.

The haematocrit stabilizes or may be lower due to the dilutional effect of reabsorbed fluid. White blood cell count usually starts to rise soon after defervescence but the recovery of platelet count is typically later than that of white blood cell count.

Respiratory distress from massive pleural effusion and ascites will occur at any time if excessive intravenous fluids have been administered. During the critical and/or recovery phases, excessive fluid therapy is associated with pulmonary oedema or congestive heart failure.

The various clinical problems during the different phases of dengue can be summarized as in Table 2.1.

Table 2.1 Febrile, critical and recovery phases in dengue

1	Febrile phase	Dehydration; high fever may cause neurological disturbances and febrile seizures in young children
2	Critical phase	Shock from plasma leakage; severe haemorrhage; organ impairment
3	Recovery phase	Hypervolaemia (only if intravenous fluid therapy has been excessive and/or has extended into this period)

2.1.4 Severe dengue

Severe dengue is defined by one or more of the following: (i) plasma leakage that may lead to shock (dengue shock) and/or fluid accumulation, with or without respiratory distress, and/or (ii) severe bleeding, and/or (iii) severe organ impairment.

As dengue vascular permeability progresses, hypovolaemia worsens and results in shock. It usually takes place around defervescence, usually on day 4 or 5 (range days 3–7) of illness, preceded by the warning signs. During the initial stage of shock, the compensatory mechanism which maintains a normal systolic blood pressure also produces tachycardia and peripheral vasoconstriction with reduced skin perfusion,

resulting in cold extremities and delayed capillary refill time. Uniquely, the diastolic pressure rises towards the systolic pressure and the pulse pressure narrows as the peripheral vascular resistance increases. Patients in dengue shock often remain conscious and lucid. The inexperienced physician may measure a normal systolic pressure and misjudge the critical state of the patient. Finally, there is decompensation and both pressures disappear abruptly. Prolonged hypotensive shock and hypoxia may lead to multi-organ failure and an extremely difficult clinical course (Textbox D).

The patient is considered to have shock if the pulse pressure (i.e. the difference between the systolic and diastolic pressures) is ≤ 20 mm Hg in children or he/she has signs of poor capillary perfusion (cold extremities, delayed capillary refill, or rapid pulse rate). In adults, the pulse pressure of ≤ 20 mm Hg may indicate a more severe shock. Hypotension is usually associated with prolonged shock which is often complicated by major bleeding.

Patients with severe dengue may have coagulation abnormalities, but these are usually not sufficient to cause major bleeding. When major bleeding does occur, it is almost always associated with profound shock since this, in combination with thrombocytopaenia, hypoxia and acidosis, can lead to multiple organ failure and advanced disseminated intravascular coagulation. Massive bleeding may occur without prolonged shock in instances when acetylsalicylic acid (aspirin), ibuprofen or corticosteroids have been taken.

Unusual manifestations, including acute liver failure and encephalopathy, may be present, even in the absence of severe plasma leakage or shock. Cardiomyopathy and encephalitis are also reported in a few dengue cases. However, most deaths from dengue occur in patients with profound shock, particularly if the situation is complicated by fluid overload.

Severe dengue should be considered if the patient is from an area of dengue risk presenting with fever of 2–7 days plus any of the following features:

- There is evidence of plasma leakage, such as:
  - high or progressively rising haematocrit;
  - pleural effusions or ascites;
  - circulatory compromise or shock (tachycardia, cold and clammy extremities, capillary refill time greater than three seconds, weak or undetectable pulse, narrow pulse pressure or, in late shock, unrecordable blood pressure).
- There is significant bleeding.
- There is an altered level of consciousness (lethargy or restlessness, coma, convulsions).
- There is severe gastrointestinal involvement (persistent vomiting, increasing or intense abdominal pain, jaundice).
- There is severe organ impairment (acute liver failure, acute renal failure, encephalopathy or encephalitis, or other unusual manifestations, cardiomyopathy) or other unusual manifestations.

2.2 DELIVERY OF CLINICAL SERVICES AND CASE MANAGEMENT

2.2.1 Introduction

Reducing dengue mortality requires an organized process that guarantees early recognition of the disease, and its management and referral when necessary. The key component of the process is the delivery of good clinical services at all levels of health care, from primary to tertiary levels. Most dengue patients recover without requiring hospital admission while some may progress to severe disease. Simple but effective triage principles and management decisions applied at the primary and secondary care levels, where patients are first seen and evaluated, can help in identifying those at risk of developing severe disease and needing hospital care. This should be complemented by prompt and appropriate management of severe dengue in referral centres.

Activities at the first level of care should focus on:

- recognizing that the febrile patient could have dengue;
- notifying early to the public health authorities that the patient is a suspected case of dengue;
- managing patients in the early febrile phase of dengue;
- recognizing the early stage of plasma leakage or critical phase and initiating fluid therapy;
- recognizing patients with warning signs who need to be referred for admission and/or intravenous fluid therapy to a secondary health care facility;
- recognizing and managing severe plasma leakage and shock, severe bleeding and severe organ impairment promptly and adequately.

2.2.2. Primary and secondary health care centres

At primary and secondary levels, health care facilities are responsible for emergency/ambulatory triage assessment and treatment.

Triage is the process of rapidly screening patients soon after their arrival in the hospital or health facility in order to identify those with severe dengue (who require immediate emergency treatment to avert death), those with warning signs (who should be given priority while waiting in the queue so that they can be assessed and treated without delay), and non-urgent cases (who have neither severe dengue nor warning signs).

During the early febrile phase, it is often not possible to predict clinically whether a patient with dengue will progress to severe disease. Various forms of severe manifestations may unfold only as the disease progresses through the critical phase, but the warning signs are good indicators of a higher risk of developing severe dengue. Therefore, the patient should have daily outpatient health care assessments for disease progression with careful checking for manifestations of severe dengue and warning signs.

Health care workers at the first levels of care should apply a stepwise approach, as suggested in Table 2.2.

Table 2.2 A stepwise approach to the management of dengue

Step I. Overall assessment
I.1 History, including information on symptoms, past medical and family history
I.2 Physical examination, including full physical and mental assessment
I.3 Investigation, including routine laboratory and dengue-specific laboratory
Step II. Diagnosis, assessment of disease phase and severity
Step III. Management
III.1 Disease notification
III.2 Management decisions. Depending on the clinical manifestations and other circumstances, patients may:
– be sent home (Group A);
– be referred for in-hospital management (Group B);
– require emergency treatment and urgent referral (Group C).

Section 2.3 gives treatment recommendations for the groups A–C.

2.2.3 Referral centres

Referral centres receiving severely ill dengue patients must be able to give prompt attention to referred cases. Beds should be made available to those patients who meet the admission criteria, even if elective cases have to be deferred. If possible, there should be a designated area to cohort dengue patients, and a high-dependency unit for closer monitoring of those with shock. These units should be staffed by doctors and nurses who are trained to recognize high-risk patients and to institute appropriate treatment and monitoring.

A number of criteria may be used to decide when to transfer a patient to a high-dependency unit. These include:

- early presentation with shock (on days 2 or 3 of illness);
- severe plasma leakage and/or shock;
- undetectable pulse and blood pressure;
- severe bleeding;
- fluid overload;
- organ impairment (such as hepatic damage, cardiomyopathy, encephalopathy, encephalitis and other unusual complications).

2.2.4 Resources needed

In the detection and management of dengue, a range of resources is needed to deliver good clinical services at all levels. Resources include (*10*):

- *Human resources*: The most important resource is trained doctors and nurses. Adequate health personnel should be allocated to the first level of care to help in triage and emergency management. If possible, dengue units staffed by

experienced personnel could be set up at referral centres to receive referred cases, particularly during dengue outbreaks, when the number of personnel main need to be increased.

- *Special area*: A well equipped and well staffed area should be designated for giving immediate and transitory medical care to patients who require intravenous fluid therapy until they can be transferred to a ward or referral health facility.
- *Laboratory resources*: The most important laboratory investigation is that of serial haematocrit levels and full blood counts. These investigations should be easily accessible from the health centre. Results should be available within two hours in severe cases of dengue. If no proper laboratory services are available, the minimum standard is the point-of-care testing of haematocrit by capillary (finger prick) blood sample with the use of a microcentrifuge.
- *Consumables*: Intravenous fluids such as crystalloids, colloids and intravenous giving sets should be available.
- *Drugs*: There should be adequate stocks of antipyretics and oral rehydration salts. In severe cases, additional drugs are necessary (vitamin K1, Ca gluconate, $NaHCO_3$, glucose, furosemide, KCl solution, vasopressor, and inotropes).
- *Communication*: Facilities should be provided for easy communication, especially between secondary and tertiary levels of care and laboratories, including consultation by telephone.
- *Blood bank*: Blood and blood products will be required by only a small percentage of patients but should be made readily available to those who need them.

2.2.5 Education and training

To ensure the presence of adequate staffing at all levels, the education and training of doctors, nurses, auxiliary health care workers and laboratory staff are priorities. Educational programmes that are customized for different levels of health care and that reflect local capacity should be supported and implemented widely. The educational programmes should develop capacities for effective triage and should improve recognition, clinical management and laboratory diagnosis of dengue.

National committees should monitor and evaluate clinical management and outcomes. Review committees at different levels (e.g. national, state, district, hospital) should review all dengue deaths, and, if possible, all cases of severe dengue, evaluate the health care delivery system, and provide feedback to doctors on how to improve care.

In dengue-endemic countries, the knowledge of dengue, the vectors and transmission of disease should be incorporated into the school curriculum. The population should also be educated about dengue in order to empower patients and their families in their own care – so that they are prepared to seek medical care at the right time, avoid self-medication, identify skin bleedings, consider the day of defervescence (and during 48 hours) as the time when complications usually occur, and look for warning signs such as intense and continuous abdominal pain and frequent vomiting.

The mass media can give an important contribution if they are correctly briefed. Workshops and other meetings with journalists, editors, artists and executives can

contribute to drawing up the best strategy for health education and communication without alarming the public.

During dengue epidemics, nursing and medical students together with community activists can visit homes with the double purpose of providing health education and actively tracing dengue cases. This has been shown to be feasible, inexpensive and effective (*11*) and must be coordinated with the primary health care units. It is useful to have printed information about dengue illness and the warning signs for distribution to members of the community. Medical care providers must include health education activities such as disease prevention in their daily work.

2.3 RECOMMENDATIONS FOR TREATMENT

2.3.1 A stepwise approach to the management of dengue (see Table 2.2)

2.3.1.1 Step I—Overall assessment

History
The history should include:

- date of onset of fever/illness;
- quantity of oral intake;
- assessment for warning signs (Textbox C);
- diarrhoea;
- change in mental state/seizure/dizziness;
- urine output (frequency, volume and time of last voiding);
- other important relevant histories, such as family or neighbourhood dengue, travel to dengue endemic areas, co-existing conditions (e.g. infancy, pregnancy, obesity, diabetes mellitus, hypertension), jungle trekking and swimming in waterfall (consider leptospirosis, typhus, malaria), recent unprotected sex or drug abuse (consider acute HIV seroconversion illness).

Physical examination
The physical examination should include:

- assessment of mental state;
- assessment of hydration status;
- assessment of haemodynamic status (Textbox D);
- checking for tachypnoea/acidotic breathing/pleural effusion;
- checking for abdominal tenderness/hepatomegaly/ascites;
- examination for rash and bleeding manifestations;
- tourniquet test (repeat if previously negative or if there is no bleeding manifestation).

Investigation
A full blood count should be done at the first visit. A haematocrit test in the early febrile phase establishes the patient's own baseline haematocrit. A decreasing white blood cell count makes dengue very likely. A rapid decrease in platelet count in parallel with a rising haematocrit compared to the baseline is suggestive of progress to the plasma

leakage/critical phase of the disease. In the absence of the patient's baseline, age-specific population haematocrit levels could be used as a surrogate during the critical phase.

Laboratory tests should be performed to confirm the diagnosis. However, it is not necessary for the acute management of patients, except in cases with unusual manifestations (Chapter 4).

Additional tests should be considered as indicated (and if available). These should include tests of liver function, glucose, serum electrolytes, urea and creatinine, bicarbonate or lactate, cardiac enzymes, ECG and urine specific gravity.

2.3.1.2 Step II—Diagnosis, assessment of disease phase and severity

On the basis of evaluations of the history, physical examination and/or full blood count and haematocrit, clinicians should be able to determine whether the disease is dengue, which phase it is in (febrile, critical or recovery), whether there are warning signs, the hydration and haemodynamic status of the patient, and whether the patient requires admission (Textboxes E and F).

2.3.1.3 Step III—Management

Disease notification
In dengue-endemic countries, cases of suspected, probable and confirmed dengue should be notified as soon as possible so that appropriate public health measures can be initiated (Chapter 5). Laboratory confirmation is not necessary before notification, but should be obtained. In non-endemic countries, usually only confirmed cases will be notified.

Suggested criteria for early notification of suspected cases are that the patient lives in or has travelled to a dengue-endemic area, has fever for three days or more, has low or decreasing white cell counts, and/or has thrombocytopaenia ± positive tourniquet test.

In dengue-endemic countries, the later the notification, the more difficult it is to prevent dengue transmission.

Management decisions
Depending on the clinical manifestations and other circumstances, patients may (*12*) be sent home (Group A), be referred for in-hospital management (Group B), or require emergency treatment and urgent referral (Group C).

2.3.2 Treatment according to groups A–C

2.3.2.1 Group A – patients who may be sent home (see the home care card for dengue in Textbox G)

These are patients who are able to tolerate adequate volumes of oral fluids and pass urine at least once every six hours, and do not have any of the warning signs, particularly when fever subsides.

Ambulatory patients should be reviewed daily for disease progression (decreasing white blood cell count, defervescence and warning signs) until they are out of the critical period. Those with stable haematocrit can be sent home after being advised to return to the hospital immediately if they develop any of the warning signs and to adhere to the following action plan:

- Encourage oral intake of oral rehydration solution (ORS), fruit juice and other fluids containing electrolytes and sugar to replace losses from fever and vomiting. Adequate oral fluid intake may be able to reduce the number of hospitalizations (*13*). [Caution: fluids containing sugar/glucose may exacerbate hyperglycaemia of physiological stress from dengue and diabetes mellitus.]
- Give paracetamol for high fever if the patient is uncomfortable. The interval of paracetamol dosing should not be less than six hours. Tepid sponge if the patient still has high fever. Do not give acetylsalicylic acid (aspirin), ibuprofen or other non-steroidal anti-inflammatory agents (NSAIDs) as these drugs may aggravate gastritis or bleeding. Acetylsalicylic acid (aspirin) may be associated with Reye's Syndrome.
- Instruct the care-givers that the patient should be brought to hospital immediately if any of the following occur: no clinical improvement, deterioration around the time of defervescence, severe abdominal pain, persistent vomiting, cold and clammy extremities, lethargy or irritability/restlessness, bleeding (e.g. black stools or coffee-ground vomiting), not passing urine for more than 4–6 hours.

Patients who are sent home should be monitored daily by health care providers for temperature pattern, volume of fluid intake and losses, urine output (volume and frequency), warning signs, signs of plasma leakage and bleeding, haematocrit, and white blood cell and platelet counts (see group B).

2.3.2.2 Group B – patients who should be referred for in-hospital management

Patients may need to be admitted to a secondary health care centre for close observation, particularly as they approach the critical phase. These include patients with warning signs, those with co-existing conditions that may make dengue or its management more complicated (such as pregnancy, infancy, old age, obesity, diabetes mellitus, renal failure, chronic haemolytic diseases), and those with certain social circumstances (such as living alone, or living far from a health facility without reliable means of transport).

If the patient has dengue with warning signs, the action plan should be as follows:

- Obtain a reference haematocrit before fluid therapy. Give only isotonic solutions such as 0.9% saline, Ringer's lactate, or Hartmann's solution. Start with 5–7 ml/kg/hour for 1–2 hours, then reduce to 3–5 ml/kg/hr for 2–4 hours, and then reduce to 2–3 ml/kg/hr or less according to the clinical response (Textboxes H, J and K).
- Reassess the clinical status and repeat the haematocrit. If the haematocrit remains the same or rises only minimally, continue with the same rate (2–3 ml/kg/hr) for another 2–4 hours. If the vital signs are worsening and haematocrit is rising rapidly, increase the rate to 5–10 ml/kg/hour for 1–2 hours. Reassess the clinical status, repeat the haematocrit and review fluid infusion rates accordingly.

- Give the minimum intravenous fluid volume required to maintain good perfusion and urine output of about 0.5 ml/kg/hr. Intravenous fluids are usually needed for only 24–48 hours. Reduce intravenous fluids gradually when the rate of plasma leakage decreases towards the end of the critical phase. This is indicated by urine output and/or oral fluid intake that is/are adequate, or haematocrit decreasing below the baseline value in a stable patient.
- Patients with warning signs should be monitored by health care providers until the period of risk is over. A detailed fluid balance should be maintained. Parameters that should be monitored include vital signs and peripheral perfusion (1–4 hourly until the patient is out of the critical phase), urine output (4–6 hourly), haematocrit (before and after fluid replacement, then 6–12 hourly), blood glucose, and other organ functions (such as renal profile, liver profile, coagulation profile, as indicated).

If the patient has dengue without warning signs, the action plan should be as follows:

- Encourage oral fluids. If not tolerated, start intravenous fluid therapy of 0.9% saline or Ringer's lactate with or without dextrose at maintenance rate (Textbox H). For obese and overweight patients, use the ideal body weight for calculation of fluid infusion (Textboxes J and K). Patients may be able to take oral fluids after a few hours of intravenous fluid therapy. Thus, it is necessary to revise the fluid infusion frequently. Give the minimum volume required to maintain good perfusion and urine output. Intravenous fluids are usually needed only for 24–48 hours.
- Patients should be monitored by health care providers for temperature pattern, volume of fluid intake and losses, urine output (volume and frequency), warning signs, haematocrit, and white blood cell and platelet counts (Textbox L). Other laboratory tests (such as liver and renal functions tests) can be done, depending on the clinical picture and the facilities of the hospital or health centre.

2.3.2.3 Group C – patients who require emergency treatment and urgent referral when they have severe dengue

Patients require emergency treatment and urgent referral when they are in the critical phase of disease, i.e. when they have:

- severe plasma leakage leading to dengue shock and/or fluid accumulation with respiratory distress;
- severe haemorrhages;
- severe organ impairment (hepatic damage, renal impairment, cardiomyopathy, encephalopathy or encephalitis).

All patients with severe dengue should be admitted to a hospital with access to intensive care facilities and blood transfusion. Judicious intravenous fluid resuscitation is the essential and usually sole intervention required. The crystalloid solution should be isotonic and the volume just sufficient to maintain an effective circulation during the period of plasma leakage. Plasma losses should be replaced immediately and rapidly with isotonic crystalloid solution or, in the case of hypotensive shock, colloid solutions (Textbox M). If possible, obtain haematocrit levels before and after fluid resuscitation.

There should be continued replacement of further plasma losses to maintain effective circulation for 24–48 hours. For overweight or obese patients, the ideal body weight should be used for calculating fluid infusion rates (textboxes J and K). A group and cross-match should be done for all shock patients. Blood transfusion should be given only in cases with suspected/severe bleeding.

Fluid resuscitation must be clearly separated from simple fluid administration. This is a strategy in which larger volumes of fluids (e.g. 10–20 ml boluses) are administered for a limited period of time under close monitoring to evaluate the patient's response and to avoid the development of pulmonary oedema. The degree of intravascular volume deficit in dengue shock varies. Input is typically much greater than output, and the input/output ratio is of no utility for judging fluid resuscitation needs during this period.

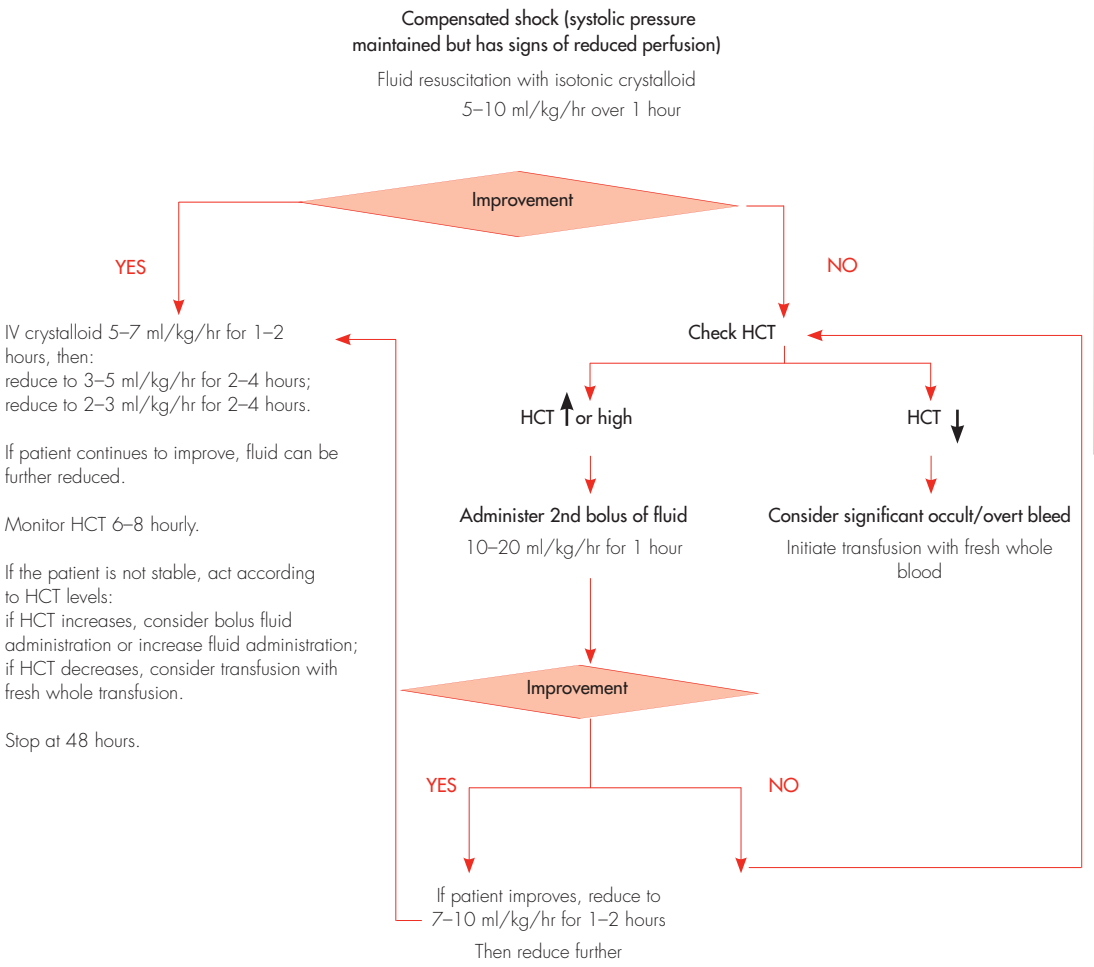
The goals of fluid resuscitation include improving central and peripheral circulation (decreasing tachycardia, improving blood pressure, pulse volume, warm and pink extremities, and capillary refill time <2 seconds) and improving end-organ perfusion – i.e. stable conscious level (more alert or less restless), urine output ≥ 0.5 ml/kg/hour, decreasing metabolic acidosis.

Treatment of shock

The action plan for treating patients with compensated shock is as follows (Textboxes D and N, Figure 2.2):

- Start intravenous fluid resuscitation with isotonic crystalloid solutions at 5–10 ml/kg/hour over one hour. Then reassess the patient's condition (vital signs, capillary refill time, haematocrit, urine output). The next steps depend on the situation.
- If the patient's condition improves, intravenous fluids should be gradually reduced to 5–7 ml/kg/hr for 1–2 hours, then to 3–5 ml/kg/hr for 2–4 hours, then to 2–3 ml/kg/hr, and then further depending on haemodynamic status, which can be maintained for up to 24–48 hours. (See textboxes H and J for a more appropriate estimate of the normal maintenance requirement based on ideal body weight).
- If vital signs are still unstable (i.e. shock persists), check the haematocrit after the first bolus. If the haematocrit increases or is still high (>50%), repeat a second bolus of crystalloid solution at 10–20 ml/kg/hr for one hour. After this second bolus, if there is improvement, reduce the rate to 7–10 ml/kg/hr for 1–2 hours, and then continue to reduce as above. If haematocrit decreases compared to the initial reference haematocrit (<40% in children and adult females, <45% in adult males), this indicates bleeding and the need to cross-match and transfuse blood as soon as possible (see treatment for haemorrhagic complications).
- Further boluses of crystalloid or colloidal solutions may need to be given during the next 24–48 hours.

Figure 2.2 Algorithm for fluid management in compensated shock



HCT = haematocrit

Patients with hypotensive shock should be managed more vigorously. The action plan for treating patients with hypotensive shock is as follows (Textboxes D and N, Figure 2.3):

- Initiate intravenous fluid resuscitation with crystalloid or colloid solution (if available) at 20 ml/kg as a bolus given over 15 minutes to bring the patient out of shock as quickly as possible.
- If the patient's condition improves, give a crystalloid/colloid infusion of 10 ml/kg/hr for one hour. Then continue with crystalloid infusion and gradually reduce to 5–7 ml/kg/hr for 1–2 hours, then to 3–5 ml/kg/hr for 2–4 hours, and then

to 2–3 ml/kg/hr or less, which can be maintained for up to 24–48 hours (textbox H).

- If vital signs are still unstable (i.e. shock persists), review the haematocrit obtained before the first bolus. If the haematocrit was low (<40% in children and adult females, <45% in adult males), this indicates bleeding and the need to cross-match and transfuse blood as soon as possible (see treatment for haemorrhagic complications).
- If the haematocrit was high compared to the baseline value (if not available, use population baseline), change intravenous fluids to colloid solutions at 10–20 ml/kg as a second bolus over 30 minutes to one hour. After the second bolus, reassess the patient. If the condition improves, reduce the rate to 7–10 ml/kg/hr for 1–2 hours, then change back to crystalloid solution and reduce the rate of infusion as mentioned above. If the condition is still unstable, repeat the haematocrit after the second bolus.
- If the haematocrit decreases compared to the previous value (<40% in children and adult females, <45% in adult males), this indicates bleeding and the need to cross-match and transfuse blood as soon as possible (see treatment for haemorrhagic complications). If the haematocrit increases compared to the previous value or remains very high (>50%), continue colloid solutions at 10–20 ml/kg as a third bolus over one hour. After this dose, reduce the rate to 7–10 ml/kg/hr for 1–2 hours, then change back to crystalloid solution and reduce the rate of infusion as mentioned above when the patient's condition improves.
- Further boluses of fluids may need to be given during the next 24 hours. The rate and volume of each bolus infusion should be titrated to the clinical response. Patients with severe dengue should be admitted to the high-dependency or intensive care area.

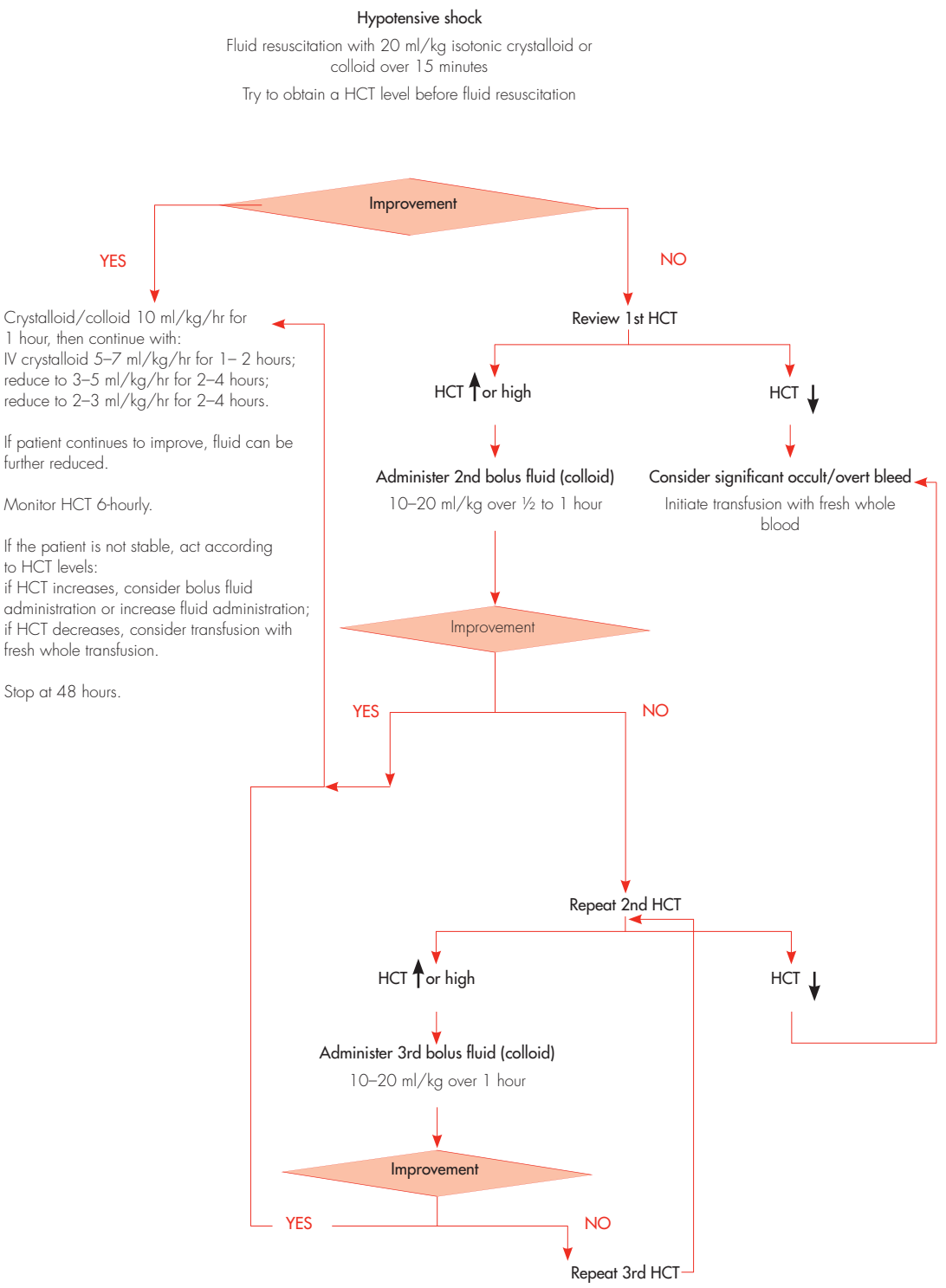
Patients with dengue shock should be frequently monitored until the danger period is over. A detailed fluid balance of all input and output should be maintained.

Parameters that should be monitored include vital signs and peripheral perfusion (every 15–30 minutes until the patient is out of shock, then 1–2 hourly). In general, the higher the fluid infusion rate, the more frequently the patient should be monitored and reviewed in order to avoid fluid overload while ensuring adequate volume replacement.

If resources are available, a patient with severe dengue should have an arterial line placed as soon as practical. The reason for this is that in shock states, estimation of blood pressure using a cuff is commonly inaccurate. The use of an indwelling arterial catheter allows for continuous and reproducible blood pressure measurements and frequent blood sampling on which decisions regarding therapy can be based. Monitoring of ECG and pulse oximetry should be available in the intensive care unit.

Urine output should be checked regularly (hourly till the patient is out of shock, then 1–2 hourly). A continuous bladder catheter enables close monitoring of urine output. An acceptable urine output would be about 0.5 ml/kg/hour. Haematocrit should be monitored (before and after fluid boluses until stable, then 4–6 hourly). In addition, there should be monitoring of arterial or venous blood gases, lactate, total carbon dioxide/bicarbonate (every 30 minutes to one hour until stable, then as indicated), blood glucose (before fluid resuscitation and repeat as indicated), and other organ

Figure 2.3 Algorithm for fluid management in hypotensive shock



functions (such as renal profile, liver profile, coagulation profile, before resuscitation and as indicated).

Changes in the haematocrit are a useful guide to treatment. However, changes must be interpreted in parallel with the haemodynamic status, the clinical response to fluid therapy and the acid-base balance. For instance, a rising or persistently high haematocrit together with unstable vital signs (particularly narrowing of the pulse pressure) indicates active plasma leakage and the need for a further bolus of fluid replacement. However, a rising or persistently high haematocrit together with stable haemodynamic status and adequate urine output does not require extra intravenous fluid. In the latter case, continue to monitor closely and it is likely that the haematocrit will start to fall within the next 24 hours as the plasma leakage stops.

A decrease in haematocrit together with unstable vital signs (particularly narrowing of the pulse pressure, tachycardia, metabolic acidosis, poor urine output) indicates major haemorrhage and the need for urgent blood transfusion. Yet a decrease in haematocrit together with stable haemodynamic status and adequate urine output indicates haemodilution and/or reabsorption of extravasated fluids, so in this case intravenous fluids must be discontinued immediately to avoid pulmonary oedema.

Treatment of haemorrhagic complications

Mucosal bleeding may occur in any patient with dengue but, if the patient remains stable with fluid resuscitation/replacement, it should be considered as minor. The bleeding usually improves rapidly during the recovery phase. In patients with profound thrombocytopaenia, ensure strict bed rest and protect from trauma to reduce the risk of bleeding. Do not give intramuscular injections to avoid haematoma. It should be noted that prophylactic platelet transfusions for severe thrombocytopaenia in otherwise haemodynamically stable patients have not been shown to be effective and are not necessary (*14*).

If major bleeding occurs it is usually from the gastrointestinal tract, and/or vagina in adult females. Internal bleeding may not become apparent for many hours until the first black stool is passed.

Patients at risk of major bleeding are those who:

- have prolonged/refractory shock;
- have hypotensive shock and renal or liver failure and/or severe and persistent metabolic acidosis;
- are given non-steroidal anti-inflammatory agents;
- have pre-existing peptic ulcer disease;
- are on anticoagulant therapy;
- have any form of trauma, including intramuscular injection.

Patients with haemolytic conditions are at risk of acute haemolysis with haemoglobinuria and will require blood transfusion.

Severe bleeding can be recognized by:

- persistent and/or severe overt bleeding in the presence of unstable haemodynamic status, regardless of the haematocrit level;
- a decrease in haematocrit after fluid resuscitation together with unstable haemodynamic status;
- refractory shock that fails to respond to consecutive fluid resuscitation of 40-60 ml/kg;
- hypotensive shock with low/normal haematocrit before fluid resuscitation;
- persistent or worsening metabolic acidosis ± a well-maintained systolic blood pressure, especially in those with severe abdominal tenderness and distension.

Blood transfusion is life-saving and should be given as soon as severe bleeding is suspected or recognized. However, blood transfusion must be given with care because of the risk of fluid overload. Do not wait for the haematocrit to drop too low before deciding on blood transfusion. Note that haematocrit of <30% as a trigger for blood transfusion, as recommended in the Surviving Sepsis Campaign Guideline (*15*), is not applicable to severe dengue. The reason for this is that, in dengue, bleeding usually occurs after a period of prolonged shock that is preceded by plasma leakage. During the plasma leakage the haematocrit increases to relatively high values before the onset of severe bleeding. When bleeding occurs, haematocrit will then drop from this high level. As a result, haematocrit levels may not be as low as in the absence of plasma leakage.

The action plan for the treatment of haemorrhagic complications is as follows:

- Give 5–10ml/kg of fresh-packed red cells or 10–20 ml/kg of fresh whole blood at an appropriate rate and observe the clinical response. It is important that fresh whole blood or fresh red cells are given. Oxygen delivery at tissue level is optimal with high levels of 2,3 di-phosphoglycerate (2,3 DPG). Stored blood loses 2,3 DPG, low levels of which impede the oxygen-releasing capacity of haemoglobin, resulting in functional tissue hypoxia. A good clinical response includes improving haemodynamic status and acid-base balance.
- Consider repeating the blood transfusion if there is further blood loss or no appropriate rise in haematocrit after blood transfusion. There is little evidence to support the practice of transfusing platelet concentrates and/or fresh-frozen plasma for severe bleeding. It is being practised when massive bleeding can not be managed with just fresh whole blood/fresh-packed cells, but it may exacerbate the fluid overload.
- Great care should be taken when inserting a naso-gastric tube which may cause severe haemorrhage and may block the airway. A lubricated oro-gastric tube may minimize the trauma during insertion. Insertion of central venous catheters should be done with ultra-sound guidance or by a very experienced person.

2.3.3 Treatment of complications and other areas of treatment

2.3.3.1 Fluid overload

Fluid overload with large pleural effusions and ascites is a common cause of acute respiratory distress and failure in severe dengue. Other causes of respiratory distress include acute pulmonary oedema, severe metabolic acidosis from severe shock, and Acute Respiratory Distress Syndrome (ARDS) (refer to standard textbook of clinical care for further guidance on management).

Causes of fluid overload are:

- excessive and/or too rapid intravenous fluids;
- incorrect use of hypotonic rather than isotonic crystalloid solutions;
- inappropriate use of large volumes of intravenous fluids in patients with unrecognized severe bleeding;
- inappropriate transfusion of fresh-frozen plasma, platelet concentrates and cryoprecipitates;
- continuation of intravenous fluids after plasma leakage has resolved (24–48 hours from defervescence);
- co-morbid conditions such as congenital or ischaemic heart disease, chronic lung and renal diseases.

Early clinical features of fluid overload are:

- respiratory distress, difficulty in breathing;
- rapid breathing;
- chest wall in-drawing;
- wheezing (rather than crepitations);
- large pleural effusions;
- tense ascites;
- increased jugular venous pressure (JVP).

Late clinical features are:

- pulmonary oedema (cough with pink or frothy sputum ± crepitations, cyanosis);
- irreversible shock (heart failure, often in combination with ongoing hypovolaemia).

Additional investigations are:

- the chest x-ray which shows cardiomegaly, pleural effusion, upward displacement of the diaphragm by the ascites and varying degrees of "bat's wings" appearance ± Kerley B lines suggestive of fluid overload and pulmonary oedema;
- ECG to exclude ischaemic changes and arrhythmia;
- arterial blood gases;
- echocardiogram for assessment of left ventricular function, dimensions and regional wall dyskinesia that may suggest underlying ischaemic heart disease;
- cardiac enzymes.

The action plan for the treatment of fluid overload is as follows:

- Oxygen therapy should be given immediately.
- Stopping intravenous fluid therapy during the recovery phase will allow fluid in the pleural and peritoneal cavities to return to the intravascular compartment. This results in diuresis and resolution of pleural effusion and ascites. Recognizing when to decrease or stop intravenous fluids is key to preventing fluid overload. When the following signs are present, intravenous fluids should be discontinued or reduced to the minimum rate necessary to maintain euglycaemia:
  - signs of cessation of plasma leakage;
  - stable blood pressure, pulse and peripheral perfusion;
  - haematocrit decreases in the presence of a good pulse volume;
  - afebrile for more than 24–48 days (without the use of antipyretics);
  - resolving bowel/abdominal symptoms;
  - improving urine output.
- The management of fluid overload varies according to the phase of the disease and the patient's haemodynamic status. If the patient has stable haemodynamic status and is out of the critical phase (more than 24–48 hours of defervescence), stop intravenous fluids but continue close monitoring. If necessary, give oral or intravenous furosemide 0.1–0.5 mg/kg/dose once or twice daily, or a continuous infusion of furosemide 0.1 mg/kg/hour. Monitor serum potassium and correct the ensuing hypokalaemia.
- If the patient has stable haemodynamic status but is still within the critical phase, reduce the intravenous fluid accordingly. Avoid diuretics during the plasma leakage phase because they may lead to intravascular volume depletion.
- Patients who remain in shock with low or normal haematocrit levels but show signs of fluid overload may have occult haemorrhage. Further infusion of large volumes of intravenous fluids will lead only to a poor outcome. Careful fresh whole blood transfusion should be initiated as soon as possible. If the patient remains in shock and the haematocrit is elevated, repeated small boluses of a colloid solution may help.

2.3.3.2 Other complications of dengue

Both hyperglycaemia and hypoglycaemia may occur, even in the absence of diabetes mellitus and/or hypoglycaemic agents. Electrolyte and acid-base imbalances are also common observations in severe dengue and are probably related to gastrointestinal losses through vomiting and diarrhoea or to the use of hypotonic solutions for resuscitation and correction of dehydration. Hyponatraemia, hypokalaemia, hyperkalaemia, serum calcium imbalances and metabolic acidosis (sodium bicarbonate for metabolic acidosis is not recommended for $pH \geq 7.15$) can occur. One should also be alert for co-infections and nosocomial infections.

2.3.3.3 Supportive care and adjuvant therapy

Supportive care and adjuvant therapy may be necessary in severe dengue. This may include:

- renal replacement therapy, with a preference to continuous veno-venous haemodialysis (CVVH), since peritoneal dialysis has a risk of bleeding;
- vasopressor and inotropic therapies as temporary measures to prevent life-threatening hypotension in dengue shock and during induction for intubation, while correction of intravascular volume is being vigorously carried out;
- further treatment of organ impairment, such as severe hepatic involvement or encephalopathy or encephalitis;
- further treatment of cardiac abnormalities, such as conduction abnormalities, may occur (the latter usually not requiring interventions).

In this context there is little or no evidence in favour of the use of steroids and intravenous immunoglobulins, or of recombinant Activated Factor VII.

Refer to standard textbooks of clinical care for more detailed information regarding the treatment of complications and other areas of treatment.

ANNEX

Textbox A. Good clinical practice and bad clinical practice

	Good practice	Bad practice
1	Assessment and follow-up of patients with non-severe dengue and careful instruction of warning signs to watch out for	Sending patients with non-severe dengue home with no follow-up and inadequate instructions
2	Administration of paracetamol for high fever if the patient is uncomfortable	Administration of acetylsalicylic acid (aspirin) or ibuprofen
3	Obtaining a haematocrit level before and after fluid boluses	Not knowing when haematocrit levels are taken with respect to fluid therapy
4	Clinical assessment of the haemodynamic status before and after each fluid bolus	No clinical assessment of patient with respect to fluid therapy
5	Interpretation of haematocrit levels in the context of fluid administered and haemodynamic assessment	Interpretation of haematocrit levels independent of clinical status
6	Administration of intravenous fluids for repeated vomiting or a high or rapidly rising haematocrit	Administration of intravenous fluids to any patient with non-severe dengue
7	Use of isotonic intravenous fluids for severe dengue	Use of hypotonic intravenous fluids for severe dengue
8	Giving intravenous fluid volume just sufficient to maintain effective circulation during the period of plasma leakage for severe dengue	Excessive or prolonged intravenous fluid administration for severe dengue
9	Avoiding intramuscular injections in dengue patients	Giving intramuscular injections to dengue patients
10	Intravenous fluid rate and frequency of monitoring and haematocrit measurement adjusted according to the patient's condition	Fixed intravenous fluid rate and unchanged frequency of monitoring and haematocrit measurement during entire hospitalization for severe dengue
11	Close monitoring of blood glucose, i.e. tight glycaemic control	Not monitoring blood glucose, unaware of the hyperglycaemic effect on osmotic diuresis and confounding hypovolaemia
12	Discontinuation or reducing fluid therapy once haemodynamic status stabilizes	Continuation and no review of intravenous fluid therapy once haemodynamic status stabilizes

Textbox B. Differential diagnosis of dengue fever

Conditions that mimic the febrile phase of dengue infection	
Flu-like syndromes	Influenza, measles, Chikungunya, infectious mononucleosis , HIV seroconversion illness
Illnesses with a rash	Rubella, measles, scarlet fever, meningococcal infection, Chikungunya, drug reactions
Diarrhoeal diseases	Rotavirus, other enteric infections
Illnesses with neurological manifestations	Meningo/encephalitis Febrile seizures
Conditions that mimic the critical phase of dengue infection	
Infectious	Acute gastroenteritis, malaria, leptospirosis, typhoid, typhus, viral hepatitis, acute HIV seroconversion illness, bacterial sepsis, septic shock
Malignancies	Acute leukaemia and other malignancies
Other clinical pictures	Acute abdomen – acute appendicitis – acute cholecystitis – perforated viscus Diabetic ketoacidosis Lactic acidosis Leukopenia and thrombocytopaenia ± bleeding Platelet disorders Renal failure Respiratory distress (Kussmaul's breathing) Systemic Lupus Erythematosus

Textbox C. Warning signs

Clinical	Abdominal pain or tenderness Persistent vomiting Clinical fluid accumulation Mucosal bleed Lethargy, restlessness Liver enlargement >2 cm
Laboratory	Increase in HCT concurrent with rapid decrease in platelet count

Textbox D. Haemodynamic assessment: continuum of haemodynamic changes

Parameters	Stable circulation	Compensated shock	Hypotensive shock
Hypotensive shock	Clear and lucid	Clear and lucid (shock can be missed if you do not touch the patient)	Change of mental state (restless, combative)
Capillary refill time	Brisk (<2 sec)	Prolonged (>2 sec)	Very prolonged, mottled skin
Extremities	Warm and pink extremities	Cool peripheries	Cold, clammy extremities
Peripheral pulse volume	Good volume	Weak and thready	Feeble or absent
Heart rate	Normal for age	Tachycardia	Severe tachycardia with bradycardia in late shock
Blood pressure	Normal for age Normal pulse pressure for age	Normal systolic pressure but rising diastolic pressure Narrowing pulse pressure Postural hypotension	Narrowed pulse pressure (<20 mmHg) Hypotension (see definition below) Unrecordable blood pressure
Respiratory rate	Normal for age	Tachypnoea	Metabolic acidosis hyperpnoea/ Kussmaul's breathing

Definition of hypotension:
Systolic blood pressure of <90 mm Hg or mean arterial pressure <70 mm Hg in adults or a systolic blood pressure decrease of >40 mm Hg or <2 SD below normal for age.
In children up to 10 years of age, the 5th centile for systolic blood pressure can be determined by the formula: 70 + (age in years x 2) mm Hg.

Textbox E. Admission criteria

Warning signs	Any of the warning signs (Textbox C)
Signs and symptoms related to hypotension (possible plasma leakage)	Dehydrated patient, unable to tolerate oral fluids Giddiness or postural hypotension Profuse perspiration, fainting, prostration during defervescence Hypotension or cold extremities
Bleeding	Spontaneous bleeding, independent of the platelet count
Organ impairment	Renal, hepatic, neurological or cardiac – enlarged, tender liver, although not yet in shock – chest pain or respiratory distress, cyanosis
Findings through further investigations	Rising haematocrit Pleural effusion, ascites or asymptomatic gall-bladder thickening
Co-existing conditions	Pregnancy Co-morbid conditions, such as diabetes mellitus, hypertension, peptic ulcer, haemolitic anemias and others Overweight or obese (rapid venous access difficult in emergency) Infancy or old age
Social circumstances	Living alone Living far from health facility Without reliable means of transport

Textbox F. Discharge criteria (all of the following conditions must be present)

Clinical	No fever for 48 hours. Improvement in clinical status (general well-being, appetite, haemodynamic status, urine output, no respiratory distress).
Laboratory	Increasing trend of platelet count. Stable haematocrit without intravenous fluids.

Textbox G. Home care card for dengue

Home care card for dengue (please take this card to your health facility for each visit)

What should be done?

- Adequate bed rest
- Adequate fluid intake (>5 glasses for average-sized adults or accordingly in children)
  - Milk, fruit juice (caution with diabetes patient) and isotonic electrolyte solution (ORS) and barley/rice water.
  - Plain water alone may cause electrolyte imbalance.
- Take paracetamol (not more than 4 grams per day for adults and accordingly in children)
- Tepid sponging
- Look for mosquito breeding places in and around the home and eliminate them

What should be avoided?

- Do not take acetylsalicylic acid (aspirin), mefenemic acid (ponstan), ibuprofen or other non-steroidal anti-inflammatory agents (NSAIDs), or steroids. If you are already taking these medications please consult your doctor.
- Antibiotics are not necessary.

If any of following is observed, take the patient immediately to the nearest hospital. These are warning signs for danger:

- Bleeding:
  - red spots or patches on the skin
  - bleeding from nose or gums
  - vomiting blood
  - black-coloured stools
  - heavy menstruation/vaginal bleeding
- Frequent vomiting
- Severe abdominal pain
- Drowsiness, mental confusion or seizures
- Pale, cold or clammy hands and feet
- Difficulty in breathing

Laboratory results monitoring

	1st Visit					
Date						
Haematocrit						
White cell count						
Platelet count						

Textbox H. Calculations for normal maintenance of intravenous fluid infusion

Normal maintenance fluid per hour can be calculated on the basis of the following formula* (equivalent to Holliday-Segar formula):

4 mL/kg/h for first 10 kg body weight
+ 2 mL/kg/h for next 10 kg body weight
+ 1 mL/kg/h for subsequent kg body weight

*For overweight/obese patients calculate normal maintenance fluid based on ideal body weight (IBW)
(Adapted from reference *16*)

IBW for overweight/obese adults can be estimated on the basis of the following formula

Female: 45.5 kg + 0.91(height -152.4) cm
Male: 50.0 kg + 0.91(height -152.4) cm
(*17*)

Textbox J. Hourly maintenance fluid regime for overweight or obese patients

Estimated ideal body weight, or IBW (kg)	Normal maintenance fluid (ml/hour) based on Holliday-Segar formula	Fluid regime based on 2–3 ml/kg /hour (ml/hour)	Fluid regime based on 1.5 –2 ml/kg/hour (ml/hour)
5	10	10–15	
10	20	20–30	
15	30	30–45	
20	60	40–60	
25	65	50–75	
30	70	60–90	
35	75	70–105	
40	80	80–120	
50	90	100–150	
60	100		90–120
70	110		105–140
80	120		120–150

Notes:
For adults with IBW >50 kg, 1.5–2 ml/kg can be used for quick calculation of hourly maintenance fluid regime.
For adults with IBW≤50 kg, 2–3 ml/kg can be used for quick calculation of hourly maintenance fluid regime.

Textbox K. Estimated ideal body weight for overweight or obese adults

Height (cm)	Estimated, IBW (kg) for adult males	Estimated IBW (kg) for adult females
150	50	45.5
160	57	52
170	66	61.5
180	75	70

Textbox L. Example of a monitoring chart for dengue

Parameters	Time and date					
Body temperature						
Respiratory rate						
Heart rate						
Blood pressure						
Pulse pressure/volume						
Capillary refill time						
Temperature of extremities						
Abdominal pain						
Vomiting						
Bleeding						

Textbox M. Choice of intravenous fluids for resuscitation

Based on the three randomized controlled trials comparing the different types of fluid resuscitation regime in dengue shock in children, there is no clear advantage to the use of colloids over crystalloids in terms of the overall outcome. However, colloids may be the preferred choice if the blood pressure has to be restored urgently, i.e. in those with pulse pressure less than 10 mm Hg. Colloids have been shown to restore the cardiac index and reduce the level of haematocrit faster than crystalloids in patients with intractable shock (*18–20*).

An ideal physiological fluid is one that resembles the extracellular and intracellular fluids compartments closely. However, the available fluids have their own limitations when used in large quantities. Therefore it is advisable to understand the limitations of these solutions to avoid their respective complications.

Crystalloids

0.9% saline ("normal" saline)
Normal plasma chloride ranges from 95 to 105 mmol/L. 0.9% Saline is a suitable option for initial fluid resuscitation, but repeated large volumes of 0.9% saline may lead to hyperchloraemic acidosis. Hyperchloraemic acidosis may aggravate or be confused with lactic acidosis from prolonged shock. Monitoring the chloride and lactate levels will help to identify this problem. When serum chloride level exceeds the normal range, it is advisable to change to other alternatives such as Ringer's Lactate.

Ringer's Lactate

Ringer's Lactate has lower sodium (131 mmol/L) and chloride (115 mmol/L) contents and an osmolality of 273 mOsm/L. It may not be suitable for resuscitation of patients with severe hyponatremia. However, it is a suitable solution after 0.9 Saline has been given and the serum chloride level has exceeded the normal range. Ringer's Lactate should probably be avoided in liver failure and in patients taking metformin where lactate metabolism may be impaired.

Colloids

The types of colloids are gelatin-based, dextran-based and starch-based solutions. One of the biggest concerns regarding their use is their impact on coagulation. Theoretically, dextrans bind to von Willebrand factor/Factor VIII complex and impair coagulation the most. However, this was not observed to have clinical significance in fluid resuscitation in dengue shock. Of all the colloids, gelatine has the least effect on coagulation but the highest risk of allergic reactions. Allergic reactions such as fever, chills and rigors have also been observed in Dextran 70. Dextran 40 can potentially cause an osmotic renal injury in hypovolaemic patients.

DENGUE CASE

ASSESSMENT

PRESUMPTIVE DIAGNOSIS

Live in/travel to dengue endemic area.
Fever and two of the following criteria:

- Anorexia and nausea
- Rash
- Aches and pains
- Warning signs
- Leukopenia
- Tourniquet test positive

Laboratory confirmed dengue
(important when no sign of plasma leakage)

WARNING SIGNS*

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargment >2 cm
- Laboratory: increase in HCT concurrent with rapid decrease in platelet count

*(requiring strict observation and medical intervention)

CLASSIFICATION

NEGATIVE

Co-existing conditions
Social circumstances

POSITIVE

NEGATIVE

DENGUE WITHOUT WARNING SIGNS

DENGUE WITH WARNING SIGNS

MANAGEMENT

Group A
(May be sent home)

Group criteria
Patients who do not have warning signs
AND
who are able:

- to tolerate adequate volumes of oral fluids
- to pass urine at least once every 6 hours

Laboratory tests

- full blood count (FBC)
- haematocrit (HCT)

Treatment
Advice for:

- adequate bed rest
- adequate fluid intake
- Paracetamol, 4 gram maximum per day in adults and accordingly in children.

Patients with stable HCT can be sent home.

Monitoring
Daily review for disease progression:

- decreasing white blood cell count
- defervescence
- warning signs (until out of critical period).

Advice for immediate return to hospital if development of any warning signs, and

- written advice for management (e.g. home care card for dengue).

Group B
(Referred for in-hospital care)

Group criteria
Patients with any of the following features:

- co-existing conditions such as pregnancy, infancy, old age, diabetes mellitus, renal failure
- social circumstances such as living alone, living far from hospital

Laboratory tests

- full blood count (FBC)
- haematocrit (HCT)

Treatment

- Encouragement for oral fluids. If not tolerated, start intravenous fluid therapy 0,9% saline or Ringer's Lactate at maintenance rate.

Monitoring
Monitor:

- temperature pattern
- volume of fluid intake and losses
- urine output (volume and frequency)
- warning signs
- HCT, white blood cell and platelet counts.

OR: Existing warning signs

Laboratory tests

- full blood count (FBC)
- haematocrit (HCT)

Treatment
Obtain reference HCT before fluid therapy. Give isotonic solutions such as 0.9 % saline, Ringer's Lactate. Start with 5–7 ml/kg/hr for 1–2 hours, then reduce to 3–5 ml/kg/hr for 2–4 hr, and then reduce to 2–3 ml/kg/hr or less according to clinical response.

Reassess clinical status and repeat HCT:

- if HCT remains the same or rises only minimally -> continue with 2–3 ml/kg/hr for another 2–4 hours;
- if worsening of vital signs and rapidly rising HCT -> increase rate to 5–10 ml/kg/hr for 1–2 hours.

Reassess clinical status, repeat HCT and review fluid infusion rates accordingly:

- reduce intravenous fluids gradually when the rate of plasma leakage decreases towards the end of the critical phase.

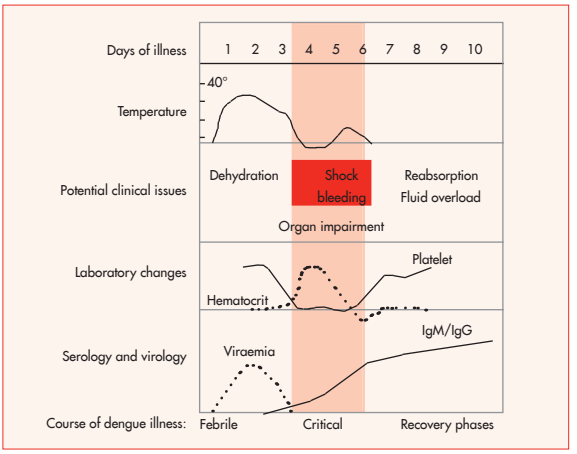
This is indicated by:

- adequate urine output and/or fluid intake
- HCT deceases below the baseline value in a stable patient.

Monitoring
Monitor:

- vital signs and peripheral perfusion (1–4 hourly until patient is out of critical phase
- urine output (4–6 hourly)
- HCT (before and after fluid replacement, then 6–12 hourly)
- blood glucose
- other organ functions (renal profile, liver profile, coagulation profile, as indicated).

MANAGEMENT



POSITIVE

SEVERE DENGUE

Group C

(Require emergency treatment)

Group criteria

Patients with any of the following features:

- severe plasma leakage with shock and/or fluid accumulation with respiratory distress
- severe bleeding
- severe organ impairment

Laboratory tests

- full blood count (FBC)
- haematocrit (HCT)
- other organ function tests as indicated

Treatment of compensated shock

Start IV fluid resuscitation with isotonic crystalloid solutions at 5–10 ml/kg/hr over 1 hour. Reassess patients' condition.

If patient improves:

- IV fluids should be reduced gradually to 5–7 ml/kg/hr for 1–2 hours, then to 3–5 ml/kg/hr for 2–4 hours, then to 2-3 ml/kg/hr for 2–4 hours and then reduced further depending on haemodynamic status;
- IV fluids can be maintained for up to 24–48 hours.

If patient is still unstable:

- check HCT after first bolus;
- **if HCT increases**/still high (>50%), repeat a second bolus of crystalloid solution at 10–20 ml/kg/hr for 1 hour;
- if there is improvement after second bolus, reduce rate to 7–10 ml/kg/hr for 1–2 hours and continue to reduce as above;
- **if HCT decreases**, this indicates bleeding and need to cross-match and transfuse blood as soon as possible.

Treatment of hypotensive shock

Initiate IV fluid resuscitation with crystalloid or colloid solution at 20 ml/kg as a bolus for 15 minutes.

If patient improves:

- give a crystalloid/colloid solution of 10 ml/kg/hr for 1 hour, then reduce gradually as above.

If patient is still unstable:

- review the HCT taken before the first bolus;
- **if HCT was low** (<40% in children and adult females, <45% in adult males) this indicates bleeding, the need to cross-match and transfuse (see above);
- **if HCT was high** compared to baseline value, change to IV colloids at 10–20 ml/kg as a second bolus over 30 minutes to 1 hour; reassess after second bolus.
- **If patient is improving** reduce the rate to 7–10ml/kg/hr for 1–2 hours, then back to IV cystalloids and reduce rates as above;
- **if patient's condition is still unstable**, repeat HCT after second bolus.
- **If HCT decreases**, this indicates bleeding (see above);
- **if HCT increases**/remains high (>50%), continue colloid infusion at 10–20 ml/kg as a third bolus over 1 hour, then reduce to 7–10 ml/kg/h 1–2 hours, then change back to crystalloid solution and reduce rate as above.

Treatment of haemorrhagic complications

Give 5–10 ml/kg of fresh packed red cells or 10–20 ml/kg of fresh whole blood.

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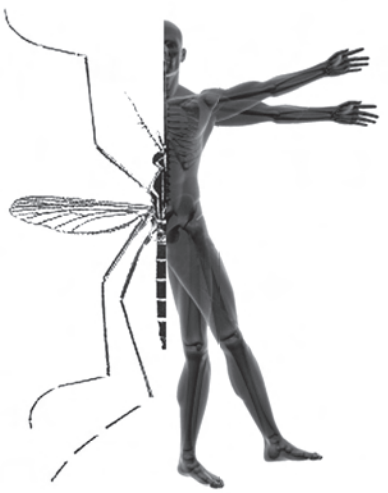


CHAPTER 3

VECTOR MANAGEMENT AND DELIVERY OF VECTOR CONTROL SERVICES

CHAPTER 3.VECTOR MANAGEMENT AND DELIVERY OF VECTOR CONTROL SERVICES

3.1 OVERVIEW

Preventing or reducing dengue virus transmission depends entirely on control of the mosquito vectors or interruption of human–vector contact.

Activities to control transmission should target *Ae. aegypti* (the main vector) in the habitats of its immature and adult stages in the household and immediate vicinity, as well as other settings where human–vector contact occurs (e.g. schools, hospitals and workplaces), unless there is sound evidence that *Ae. albopictus* or other mosquito species are the local vectors of dengue. *Ae. aegypti* proliferates in many purposely-filled household containers such as those used for domestic water storage and for decorative plants, as well as in a multiplicity of rain-filled habitats – including used tyres, discarded food and beverage containers, blocked gutters and buildings under construction. Typically, these mosquitoes do not fly far, the majority remaining within 100 metres of where they emerged. They feed almost entirely on humans, mainly during daylight hours, and both indoors and outdoors.

Integrated vector management (IVM) is the strategic approach to vector control promoted by WHO (*1*) and includes control of the vectors of dengue. Defined as "a rational decision-making process for the optimal use of resources for vector control", IVM considers five key elements in the management process, namely:

- *advocacy, social mobilization and legislation* – the promotion of these principles in development policies of all relevant agencies, organizations and civil society; the establishment or strengthening of regulatory and legislative controls for public health; and the empowerment of communities;
- *collaboration within the health sector and with other sectors* – the consideration of all options for collaboration within and between public and private sectors; planning and decision-making delegated to the lowest possible administrative level; and strengthening communication among policy-makers, managers of programmes for the control of vector-borne diseases, and other key partners;
- *integrated approach to disease control* – ensuring the rational use of available resources through the application of a multi-disease control approach; integration of non-chemical and chemical vector control methods; and integration with other disease control measures;
- *evidence-based decision-making* – adaptation of strategies and interventions to local vector ecology, epidemiology and resources, guided by operational research and subject to routine monitoring and evaluation;
- *capacity-building* – the development of essential infrastructure, financial resources and adequate human resources at national and local levels to manage IVM programmes, based on a situation analysis.

Control of *Ae. aegypti* is mainly achieved by eliminating container habitats that are favourable oviposition sites and which permit the development of the aquatic stages.

The habitats are eliminated by preventing access by mosquitoes to these containers or by frequently emptying and cleaning them, by removing the developing stages using insecticides or biological control agents, by killing the adult mosquitoes using insecticides, or by combinations of these methods.

Historically, efforts to control dengue vectors in the WHO Region of the Americas resulted in the elimination of *Ae. aegypti* populations from much of the neotropics by the 1970s. However, re-introductions followed, leading to the re-establishment of vector populations. Today, therefore, the main aim of most programmes is to reduce the densities of vector populations as much as possible and to maintain them at low levels. Where feasible, efforts may also be made to reduce the longevity of adult female mosquitoes by the use of insecticidal methods in order to lessen the risk of virus transmission.

In selecting the most appropriate vector control method, or combination of methods, consideration should be given to the local ecology and behaviour of the target species, the resources available for implementation, the cultural context in which control interventions are carried out, the feasibility of applying them in a timely manner, and the adequacy of coverage. Methods of vector control include the elimination or management of larval habitats, larviciding with insecticides, the use of biological agents and the application of adulticides.

3.2 METHODS OF VECTOR CONTROL

Ae. aegypti uses a wide range of confined larval habitats, both man-made and natural. However, it may not be feasible or cost-effective to attempt to control the immature stages in all such habitats in a community. Some man-made container habitats produce large numbers of adult mosquitoes, whereas others are less productive. Consequently, control efforts should target the habitats that are most productive and hence epidemiologically more important rather than all types of container, especially when there are major resource constraints. Such targeted strategies require a thorough understanding of the local vector ecology and the attitudes and habits of residents pertaining to the containers.

3.2.1 Environmental management

Environmental management seeks to change the environment in order to prevent or minimize vector propagation and human contact with the vector-pathogen by destroying, altering, removing or recycling non-essential containers that provide larval habitats. Such actions should be the mainstay of dengue vector control. Three types of environmental management are defined:

- *Environmental modification* – long-lasting physical transformations to reduce vector larval habitats, such as installation of a reliable piped water supply to communities, including household connections.
- *Environmental manipulation* – temporary changes to vector habitats involving the management of "essential" containers, such as frequent emptying and cleaning by scrubbing of water-storage vessels, flower vases and desert room coolers; cleaning of gutters; sheltering stored tyres from rainfall; recycling or proper disposal of discarded containers and tyres; management or removal from the

vicinity of homes of plants such as ornamental or wild bromeliads that collect water in the leaf axils.

- *Changes to human habitation or behaviour* – actions to reduce human–vector contact, such as installing mosquito screening on windows, doors and other entry points, and using mosquito nets while sleeping during daytime.

The choice of approach should be effective, practicable and appropriate to local circumstances. Actual or potentially important container types that cannot be removed from the area should be dealt with in situ. Table 3.1 summarizes the main actions used to control immature Aedes larval habitats.

Table 3.1 Environmental management actions to control immature stages of *Aedes aegypti*[a]

Larval habitat	Empty,clean and scrub weekly	Mosquito-proof cover	Store under roof	Modify design, and/or repair and clean	Use expanded polystyrene beads	Fill (with sand, soil or concrete)	Collect, recycle and dispose of	Puncture or drain
Water-storage tank or cistern		+		+	+			
Drums (150–200 litres)	+	+		+				
Flower vase filled with water	+					+		
Potted plants with saucers	+			+				
Roof gutter				+				
Animal water container	+							
Discarded food and drink containers							+	
Hollow fence posts				+		+		
Used tyres			+			+	+	
Discarded large appliances							+	
Discarded buckets (<20 litres)			+				+	+
Tree holes						+		
Rock holes						+		

[a] Adapted from *Dengue and dengue haemorrhagic fever in the Americas: guidelines for prevention and control* (2)

Improvements in, and maintenance of, urban infrastructure and basic services contribute to the reduction in available larval habitats since large *Ae. aegypti* populations are often associated with poor water supply and inadequate sanitation and waste disposal services.

3.2.1.1 Improvement of water supply and water-storage systems

Improving water supplies is a fundamental method of controlling *Aedes* vectors, especially *Ae. aegypti*. Water piped to households is preferable to water drawn from wells, communal standpipes, rooftop catchments and other water-storage systems. However, potable water must be supplied reliably so that water-storage containers that serve as larval habitats – such as drums, overhead or ground tanks and concrete jars – are not necessary. In urban areas the use of cost-recovery mechanisms such as the introduction of metered water may actually encourage household collection and storage of roof catchment rainwater that can be harvested at no cost, resulting in the continued use of storage containers. Traditional water storage practices may also persist even when reliable supplies are available. The installation of reliable piped water supplies in houses should therefore be accompanied by a communication strategy that discourages traditional storage practices.

3.2.1.2 Mosquito-proofing of water-storage containers

Water-storage containers can be designed to prevent access by mosquitoes for oviposition. Containers can be fitted with tight lids or, if rain-filled, tightly-fitted mesh screens can allow for rainwater to be harvested from roofs while keeping mosquitoes out. Removable covers should be replaced every time water is removed and should be well maintained to prevent damage that permits mosquitoes to get in and out.

Expanded polystyrene beads used on the surface of water provide a physical barrier that inhibits oviposition in storage containers from which water is drawn from below via a pipe and from which there is no risk of overflow. These beads can also be placed in septic tanks, which *Ae. aegypti* sometimes exploits.

3.2.1.3 Solid waste management

In the context of dengue vector control, "solid waste" refers mainly to non-biodegradable items of household, community and industrial waste. The benefits of reducing the amount of solid waste in urban environments extend beyond those of vector control, and applying many of the basic principles can contribute substantially to reducing the availability of *Ae. aegypti* larval habitats. Proper storage, collection and disposal of waste are essential for protecting public health. The basic rule of "reduce, reuse, recycle" is highly applicable. Efforts to reduce solid waste should be directed against discarded or non-essential containers, particularly if they have been identified in the community as important mosquito-producing containers.

Solid waste should be collected in plastic sacks and disposed of regularly. The frequency of collection is important: twice per week is recommended for housefly and rodent control in warm climates. Integration of *Ae. aegypti* control with waste management services is possible and should be encouraged.

It is also important to provide information on these activities to encourage and promote them. Globally, recycling is on the increase. This practice places value on many items previously classified as waste products, leading to growth in the recycling market and profit for both small and large-scale businesses as a consequence. But although recycling can contribute to significant economic improvements, the recycling market can potentially impact dengue vector populations. For there to be an impact, however, containers of importance must have value in the marketplace, be it real (e.g. plastics or tyres for recycling) or created (e.g. beverage container deposit laws), and advertising and promotion must be sustained.

Used tyres are common and sometimes highly productive larval habitats that may warrant special attention in urban areas. Discarded tyres should be collected, recycled or disposed of by proper incineration in waste transformation facilities (e.g. incinerators, energy-production plants, or lime kilns fitted with emission control devices). Regulation of the sale of new tyres mandating the payment of an additional deposit and return charge may also be an incentive for better management and disposal of old tyres. Tyres can be recycled in a variety of ways, including for use as shoe soles, flooring, industrial rubber gaskets or household hardware (e.g. buckets, rubbish bins). Industrially shredded tyres can be incorporated into road surfacing materials. Sanitary regulations may require that whole tyres are buried in a separate area of a landfill to avoid their rising upwards under compaction and disrupting soil cover.

3.2.1.4 Street cleansing

A reliable and regular street cleansing system that removes discarded water-bearing containers and cleans drains to ensure they do not become stagnant and breed mosquitoes will both help to reduce larval habitats of *Ae. aegypti* and remove the origin of other urban pests.

3.2.1.5 Building structures

During the planning and construction of buildings and other infrastructure, including urban renewal schemes, and through legislation and regulation, opportunities arise to modify or reduce potential larval habitats of urban disease vectors, including *Ae. aegypti*, *Culex quinquefasciatus* and *An. stephensi*. For example, under revised legislation in Singapore, roof gutters are not permitted on buildings in new developments because they are difficult to access and maintain. Moreover, property owners are required to remove existing gutters on their premises if they are unable to maintain them satisfactorily.

3.2.2 Chemical control: larvicides

Although chemicals are widely used to treat *Ae. aegypti* larval habitats, larviciding should be considered as complementary to environmental management and – except in emergencies – should be restricted to containers that cannot otherwise be eliminated or managed. Larvicides may be impractical to apply in hard-to-reach natural sites such as leaf axils and tree holes, which are common habitats of *Ae. albopictus*, or in deep wells. The difficulty of accessing indoor larval habitats of *Ae. aegypti* (e.g. water-storage containers, plant vases, saucers) to apply larvicides is a major limitation in many urban contexts.

As *Ae. aegypti* often deposits eggs in water-storage containers, the larvicides should have low toxicity to other species and should not significantly change the taste, odour or colour of the water.

The International Programme on Chemical Safety (IPCS) has assessed the toxicity of the active ingredients methoprene, pyriproxyfen and temephos and those in *Bacillus thuringiensis serovar israelensis (Bti)* to determine their safety for use as mosquito larvicides in drinking-water at dosages that are effective against *Aedes* larvae. However, the safety of the active ingredients in the final formulation varies from product to product and requires further study, as does the possible microbiological contaminants in formulations of *Bti*. *WHO's Guidelines for drinking-water quality* (*3*) provide authoritative guidance on the use of pesticides in drinking-water. Understandably, placing chemicals in domestic water, particularly drinking-water, is often viewed with suspicion and may be unacceptable in some communities.

3.2.2.1 Target area

Productive larval habitats should be treated with chemicals only if environmental management methods or other non-chemical methods cannot be easily applied or are too costly. Perifocal treatment involves the use of hand-held or power-operated equipment to spray, for example, wettable powder or emulsifiable-concentrate formulations of insecticide on larval habitats and peripheral surfaces. This will destroy existing and subsequent larval infestations in containers of non-potable water, and will kill the adult mosquitoes that frequent these sites. Perifocal treatment can be used to treat containers, irrespective of whether they hold water or are dry at the time of application. The internal and external walls of containers are sprayed until they are covered by a film of insecticide, and spraying is also extended to cover any wall within 60 cm of the container. Perifocal treatment thus has both larviciding and residual adulticiding characteristics. This method is suitable only for collections of non-potable water (such as in large piles of tyres or discarded food and beverage containers).

3.2.2.2 Insecticides

Table 3.2 lists the mosquito larvicides that are suitable for application to non-potable water containers. For treatment of drinking-water, temephos and methoprene can be applied at dosages of up to 1 mg of active ingredient (a.i.) per litre (1 ppm); pyriproxyfen can be applied at dosages up to 0.01 mg a.i. per litre (0.01 ppm) and *Bti* at1–5mg per litre

3.2.2.3 Application procedures

Hand-operated compression sprayers are suitable for applying liquid insecticides to larger larval habitats. Knapsack sprayers are also suitable, especially for delivering wettable powder formulations. A syringe or pipette can be used for treating indoor flower vases and ant traps. Granule and certain other solid formulations are applied directly by (protected) hand to confined larval habitats or by a convenient standard measure (e.g. a dessert spoon or teaspoon). When treating containers of drinking-water, sufficient insecticide should be added for the volume of the container even if the container is not full of water (e.g. 1 g of 1% temephos granules for 10 litres of container volume).

3.2.2.4 Treatment cycle

The treatment cycle will depend on the species of mosquito, seasonality of transmission, patterns of rainfall, duration of efficacy of the larvicide and types of larval habitat. Two or three application rounds carried out annually in a timely manner with proper monitoring of efficacy may suffice, especially in areas where the main transmission season is short.

3.2.2.5 Precautions

Extreme care must be taken when treating drinking-water to avoid dosages that are toxic for humans. Label instructions must always be followed when using insecticides.

Table 3.2 WHO-recommended compounds and formulations for control of mosquito larvae in container habitats[a]

Insecticide	Formulation[b]	Dosage[c]	WHO hazard classification of active ingredient[d]
Organophosphates			
Pirimiphos-methyl	EC	1	III
Temephos	EC, GR	1	U
Insect growth regulators			
Diflubenzuron	DT, GR, WP	0.02–0.25	U
rs-methoprene[e]	EC	1	U
Novaluron	EC	0.01–0.05	NA
Pyriproxyfen[e]	GR	0.01	U
Biopesticides			
Bacillus thuringiensis israelensis[e]	WG	1–5 mg/L	U
Spinosad	DT, GR, SC	0.1-0.5	U

[a] WHO recommendations on the use of pesticides in public health are valid only if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available at http://www.who.int/whopes/quality/en/. Label instructions must always be followed when using insecticides.
[b] DT = tablet for direct application; GR = granule; EC = emulsifiable concentrate; WG = water-dispersible granule; WP = wettable powder; SC = suspension concentrate.
[c] mg/L of active ingredient for control of container-breeding mosquitoes.
[d] Class II = moderately hazardous; Class III = slightly hazardous; Class U = unlikely to pose an acute hazard in normal use; NA = not available.
[e] Can be used at recommended dosages in potable water.

3.2.3 Chemical control: adulticides

Methods of chemical control that target adult vectors are intended to impact on mosquito densities, longevity and other transmission parameters. Adulticides are applied either as residual surface treatments or as space treatments.

3.2.3.1 Residual treatment

Perifocal treatment, as described above, has both adulticiding and larviciding effects. Suitable insecticides can be applied with hand-operated compression sprayers. Power sprayers can be used to treat large accumulations of discarded containers (e.g. tyre dumps) rapidly. Care must be taken not to treat containers used to store potable water.

3.2.3.2 Space sprays and their application

Space spraying is recommended for control only in emergency situations to suppress an ongoing epidemic or to prevent an incipient one. The objective of space spraying is the massive, rapid destruction of the adult vector population. However, there has been considerable controversy about the efficacy of aerosol insecticide applications during epidemics of dengue and yellow fever. Any control method that reduces the number of infective adult mosquitoes, even for a short time, should reduce virus transmission during that time, but it remains unclear whether the transient impact of space treatments is epidemiologically significant in the long run. There is no well-documented example of the effectiveness of this approach in interrupting an epidemic. Nevertheless, if space spraying is used early in an epidemic and on a sufficiently large scale, the intensity of transmission may be reduced, which would give time for the application of other vector control measures that provide longer-term control, including larviciding and community-based source reduction. Thus, if disease surveillance is sensitive enough to detect cases in the early stages of an epidemic, and if the resources are available, emergency space spraying can be initiated at the same time as source reduction measures and larviciding are intensified.

Not only insecticide susceptibility but also droplet size, application rate and indoor penetration of the insecticide are all crucial to the efficacy of this method for controlling *Ae. aegypti*. Indoor penetration of an insecticide depends on the structure of the building, whether doors and windows are left open during spraying and, when applied from vehicle-mounted equipment, residential block configuration, the route of the spray vehicle and meteorological conditions. Where indoor penetration of droplets is likely to be poor, indoor application with portable equipment will be more effective against *Ae. aegypti*. However, rates of coverage are much lower and accessibility may be difficult, particularly in large cities.

Vector populations can be suppressed over large areas by the use of space sprays released from low-flying aircraft, especially where access with ground equipment is difficult and extensive areas must be treated rapidly. Indoor penetration of insecticide droplets is again a critical factor for efficacy. In applying space sprays from the air, careful consideration must be given to meteorological conditions, especially wind speed at spray height and at ground level, and to the droplet size spectrum obtained at the flying speed of the aircraft. For all aerial spraying operations, clearance must be obtained from the civil aviation authority. For safety reasons, populated areas must usually be sprayed from twin-engined aircraft. Modern aircraft are fitted with global positioning systems so the exact position of the aircraft while the insecticide is being applied can be accurately recorded.

Target area

Since total coverage can rarely be achieved during ground applications, space spraying should focus on areas where people congregate (e.g. high-density housing, schools, hospitals) and where dengue cases have been reported or vectors are abundant. Selective space treatment up to 400 metres from houses in which dengue cases have been reported is commonly practised (and is sometimes also referred to as "perifocal spraying"). However, by the time a case is detected and a response mounted, the infection is likely to have spread to a wider area. Thorough planning is required to ensure that adequate resources (equipment, insecticide, human and financial resources) can be deployed in a timely manner to ensure proper coverage. Only if resources permit should area-wide treatment be considered.

Insecticides

Table 3.3 lists the insecticides that are suitable for space spraying as cold aerosols or thermal fogs. The choice of insecticide formulation for space spraying in and around dwellings should be based on its immediate environmental impact and the compliance of the community. Only insecticide products with high flash-points should be used for thermal fogging. Space-spraying formulations are usually oil-based, as the oil carrier inhibits evaporation of small fog droplets. Diesel fuel has been used as a carrier for thermal fogging agents, but it creates thick smoke, has a strong smell and creates oily deposits, which may lead the community to reject its use. Water-based formulations are also available, some of which contain substances that prevent rapid evaporation. Label instructions should always be followed when using insecticides.

Application procedures

Space sprays can be applied either as thermal fogs at 10–50 l/ha or as ultra-low-volume applications of undiluted or slightly diluted technical-grade insecticide in the form of a cold aerosol of droplets of controlled size (15–25 µm) at a rate of 0.5–2.0 l/ha. Portable or vehicle-mounted thermal or cold-fog generators can be used for ground application. If the target area exceeds 1000 ha or cannot be covered by ground equipment within 10 days, aerial cold fog application is sometimes used. However, several factors must first be considered – including safety, timeliness, cost, meteorological conditions, vector behaviour, biological effectiveness and availability of equipment, operational sites, and highly skilled air and ground crews. The difficulties of ensuring penetration of insecticide droplets into the resting sites of the target species are similar to those for aerosols dispensed from road vehicles. For ground applications, maps of the areas to be sprayed showing all passable roads are helpful in planning routes. The development of Geographic Information Systems (GIS) may also be helpful. A communication plan should be prepared to inform the population, encouraging them to open their doors and windows in order to improve the effectiveness of the spraying programme.

Application rates vary with the susceptibility of the target species and environmental considerations. Wind speed has a strong effect on droplet distribution and contact with insects. In most situations, a wind speed of 1–4 metres per second (approximately

3.6–15 km/h) is needed to drift droplets downwind from the line of travel. Furthermore, space sprays should be applied when there are temperature inversions – i.e. colder air closer to the ground – which occur early in the morning or in the evening when the ground temperature begins to fall. Space spray applications should correspond to the activity of the target species. *Ae. aegypti* and *Ae. albopictus* are active during the day, with peak flight activity in the morning and afternoon. For these species, spraying outdoors is therefore usually carried out in the early morning or late afternoon. Indoor treatments with portable cold or thermal fog generators are particularly effective against *Ae. aegypti* because its resting behaviour is mainly indoors. Indoor treatments are the only choice where there is no access for vehicles.

For application from vehicle-mounted equipment in areas with narrow roads and houses close to the roadside, the spray should be directed backwards from the vehicle. In areas with wide roads and buildings far from the roadside, the vehicle should be driven close to the side of the road and the spray should be directed at a right angle (downwind) to the road rather than directly behind the vehicle. More detailed information on operational guidelines for space spraying is available in the WHO manual on this subject (*5*).

Cold fog applications from large fixed-wing aircraft are made at approximately 240 km/h and 60 m above the ground, with swath spacing of 180 m. Smaller, fixed-wing aircraft are flown at slower speeds and usually lower altitudes (approximately 160 km/h, 30 m above the ground, with a swath width of 50–100 m). In emergencies, agricultural spraying aircraft can be used so long as they are fitted with rotary atomizers or other suitable nozzles calibrated for the insecticide, its formulation and the desired application rate.

Treatment cycle

When a rapid reduction in vector density is essential, such as in emergencies, space treatment should ideally be carried out every 2–3 days for 10 days. Further applications should then be made once or twice a week to sustain suppression of the adult vector population. Continuous entomological and epidemiological surveillance should be conducted, however, to determine the appropriate application schedule and the effectiveness of the control strategy.

Precautions

Operators who carry out house-to-house space spraying using portable equipment should wear face masks in addition to normal protective clothing and should operate the equipment for short periods only. Fogging with vehicle-mounted equipment in urban areas can be a traffic hazard, and spotting of vehicle paintwork may result, particularly when large droplets are used. Ultra-low-volume aerial applications should be made only by highly skilled pilots trained to undertake spraying at the proper speeds and heights. Clearance from the local civil aviation authority must be sought. Ground reconnaissance should be made before treatment and the public advised to safeguard non-target animals and beehives.

Table 3.3 Selected insecticides for cold aerosol or thermal fog application against mosquitoes[a]

Insecticide	Chemical	Dosage of active ingredient (g/ha)		WHO hazard classification of active ingredient[c]
		Cold aerosols	Thermal fogs[b]	
Fenitrothion	Organophosphate	250–300	250–300	II
Malathion	Organophosphate	112–600	500–600	III
Pirimiphos-methyl	Organophosphate	230–330	180–200	III
Bioresmethrin	Pyrethroid	5	10	U
Cyfluthrin	Pyrethroid	1–2	1–2	II
Cypermethrin	Pyrethroid	1–3	–	II
Cyphenothrin	Pyrethroid	2–5	5–10	II
d,d-trans-Cyphenothrin	Pyrethroid	1–2	2.5–5	NA
Deltamethrin	Pyrethroid	0.5–1.0	0.5 – 1.0	II
D-Phenothrin	Pyrethroid	5–20	–	U
Etofenprox	Pyrethroid	10–20	10–20	U
λ-Cyhalothrin	Pyrethroid	1.0	1.0	II
Permethrin	Pyrethroid	5	10	II
Resmethrin	Pyrethroid	2–4	4	III

[a] Adapted from: *Pesticides and their application for the control of vectors and pests of public health importance* (*6*). Label instructions must always be followed when using insecticides.
[b] The strength of the finished formulation when applied depends on the performance of the spraying equipment used.
[c] Class II = moderately hazardous; class III = slightly hazardous; class U = unlikely to pose an acute hazard in normal use; NA = not available.

3.2.4 Safe use of insecticides

All pesticides are toxic to some degree. Safety precautions for their use – including care in the handling of pesticides, safe work practices for those who apply them, and appropriate field application – should be followed. A safety plan for insecticide application can be organized along the following lines:

- Instructions on pesticide labels should be followed carefully.
- Spray operators should be provided with at least two uniforms to allow for frequent changes.
- Safety gloves, goggles and masks should be used for high-exposure activities such as machine calibration.
- Changing and washing facilities 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, preferably daily.
- Particular attention should be given to washing gloves, as wearing contaminated gloves can be dangerous.
- Spray operators should wash their hands and face 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 disposing of used insecticide containers.
- After each day's operation, any unused liquid larvicide should be disposed of safely.

- Blood cholinesterase levels should be monitored if organophosphate insecticides are used.
- Operator supervision by a well-trained individual is essential.
- During and immediately after indoor space spray operations, householders and pets must remain outside the dwelling.

WHO has published specific guidelines on use of insecticides and safety procedures (*3–7*).

3.2.5 Monitoring of insecticide susceptibility

Insecticides have been used widely for dengue vector control since their development. As a result, insecticide-resistant populations of *Ae. aegypti* have been detected in a number of countries. Operationally significant levels of resistance to organophosphates, pyrethroids, carbamates and organochlorines have been documented.

Insecticide resistance must be considered as a potentially serious threat to effective dengue vector control. Routine monitoring of insecticide susceptibility should be integral to any programme.

In countries with a history of extensive DDT use, resistance may be widespread. Also, DDT resistance may predispose to pyrethroid resistance, since both insecticides have the same target site (the voltage gated sodium channel) and both have been associated with mutations in the kdr gene in *Ae. aegypti*. Consequently, in countries such as Thailand where pyrethroids – including deltamethrin, cypermethrin and permethrin – are increasingly being used in favour of organophosphates for space spraying, pyrethroid resistance is likely to occur sooner in mosquito populations that already have this mutation. This phenomenon reinforces the importance of carrying out routine susceptibility testing at regular intervals during any control programme.

WHO kits for testing the susceptibility of adult and larval mosquitoes remain the standard methods for determining the susceptibility status of *Aedes* populations. Instructions on testing and for purchasing kits, test papers and solutions are available to order from WHO[1].

3.2.6 Individual and household protection

Clothing that minimizes skin exposure during daylight hours when mosquitoes are most active affords some protection from the bites of dengue vectors and is encouraged particularly during outbreaks. Repellents may be applied to exposed skin or to clothing. Repellents should contain DEET (N, N-diethyl- 3-methylbenzamide), IR3535 (3-[N-acetyl-N-butyl]-aminopropionic acid ethyl ester) or Icaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester). The use of repellents must be in strict accordance with label instructions. Insecticide-treated mosquito nets afford good protection for those who sleep during the day (e.g. infants, the bedridden and night-shift workers).

Where indoor biting occurs, household insecticide aerosol products, mosquito coils or other insecticide vaporizers may also reduce biting activity. Household fixtures such as window and door screens and air-conditioning can also reduce biting.

[1] Supplies for monitoring insecticide resistance in disease vectors: procedures and conditions. Geneva, World Health Organization, 2002. Accessible at: http://www.who.int/whopes/resistance/en/WHO_CDS_CPE_PVC_2001.2.pdf

3.2.7 Biological control

Biological control is based on the introduction of organisms that prey upon, parasitize, compete with or otherwise reduce populations of the target species. Against *Aedes* vectors of dengue, only certain species of larvivorous fish and predatory copepods (Copepoda: Cyclopoidea) – small freshwater crustaceans – have proved effective in operational contexts in specific container habitats, and even then seldom on a large scale. While biological control avoids chemical contamination of the environment, there may be operational limitations – such as the expense and task of rearing the organisms on a large scale, difficulty in applying them and their limited utility in aquatic sites where temperature, pH and organic pollution may exceed the narrow requirements of the organism. Biological control methods are effective only against the immature stages of vector mosquitoes in the larval habitat where they are introduced. Importantly, the biological control organisms are not resistant to desiccation, so their utility is mainly restricted to container habitats that are seldom emptied or cleaned, such as large concrete or glazed clay water-storage containers or wells. The willingness of local communities to accept the introduction of organisms into water containers is essential; community involvement is desirable in distributing the fish or copepods, and monitoring and restocking containers when necessary.

3.2.7.1 Fish

A variety of fish species have been used to eliminate mosquitoes from larger containers used to store potable water in many countries, and in open freshwater wells, concrete irrigation ditches and industrial tanks. The viviparous species *Poecilia reticulata* adapts well to these types of confined water bodies and has been most commonly used. Only native larvivorous fish should be used because exotic species may escape into natural habitats and threaten the indigenous fauna. WHO has published further information on the use of fish for mosquito control (*8*).

3.2.7.2 Predatory copepods

Various predatory copepod species have also proved effective against dengue vectors in operational settings. However, although copepod populations can survive for long periods, as with fish, reintroductions may be necessary for sustained control. A vector control programme in northern Viet Nam using copepods in large water-storage tanks, combined with source reduction, successfully eliminated *Ae. aegypti* in many communes and has prevented dengue transmission for a number of years. To date, these successes have not been replicated in other countries.

3.2.8 Towards improved tools for vector control

Some promising new dengue vector control tools are the subject of operational research but have not been sufficiently well field-tested under programmatic conditions for recommendations to be made for their use as public health interventions. In 2006, a WHO/TDR Scientific Working Group identified major streams of recommended research on dengue, including in the area of vector control (*9*).

3.2.8.1 Insecticide-treated materials

Insecticide-treated materials (ITMs), typically deployed as insecticide-treated bednets, have proved highly effective in preventing diseases transmitted by nocturnally active mosquitoes. Research on the efficacy of ITMs in controlling diurnally active *Ae. aegypti* is being encouraged. There is accumulating evidence that insecticide-treated window curtains (net curtains hung in windows, over any existing curtains if necessary) and long-lasting insecticidal fabric covers for domestic water-storage containers can reduce dengue vector densities to low levels in some communities – with prospects for reducing dengue transmission risk. Curtains also provide personal protection in the home. Although more studies are needed to confirm that transmission can be reduced by this type of intervention, ITMs appear to hold promise for dengue prevention and control. In studies in Mexico and Venezuela, ITMs (particularly curtains) were well accepted by the communities as their efficacy was reinforced by the reduction of other biting insects as well as cockroaches, houseflies and other pests (*10*).

The location or type of ITM need not be limited to those described or tested to date. Window curtains, screens, and doorway or wardrobe curtains, etc. all appear to warrant investigation in different settings. If the application of these interventions is shown to be efficacious and cost-effective, it may offer additional prospects for dengue vector control in the home, workplace, schools, hospitals and other locations, and allow for the selection of the most appropriate ITMs by the communities that will use them.

3.2.8.2 Lethal ovitraps

The ovitrap or oviposition trap used for surveillance of *Aedes* vectors can be modified to render it lethal to immature or adult populations of *Ae. aegypti*. Lethal ovitraps (which incorporate an insecticide on the oviposition substrate), autocidal ovitraps (which allow oviposition but prevent adult emergence), and sticky ovitraps (which trap the mosquito when it lands) have been used on a limited basis. Studies have shown that population densities can be reduced with sufficiently large numbers of frequently-serviced traps. Life expectancy of the vector may also potentially be shortened, thus reducing the number of vectors that become infective. In Singapore, ovitraps used as a control device reportedly eliminated *Ae. aegypti* from the international airport, but this level of success has not been repeated elsewhere (*11*). In Brazil, lethal ovitraps with deltamethrin-treated ovistrips substantially reduced adult densities of *Ae. aegypti* and produced almost 100% larval mortality during a one-month field trial (*12*). The potential advantages of lethal ovitraps for controlling Aedes vectors include their simplicity, their specificity for and effectiveness against container breeders such as *Ae. aegypti*, and the prospect of their integration with other chemical or biological control methodologies.

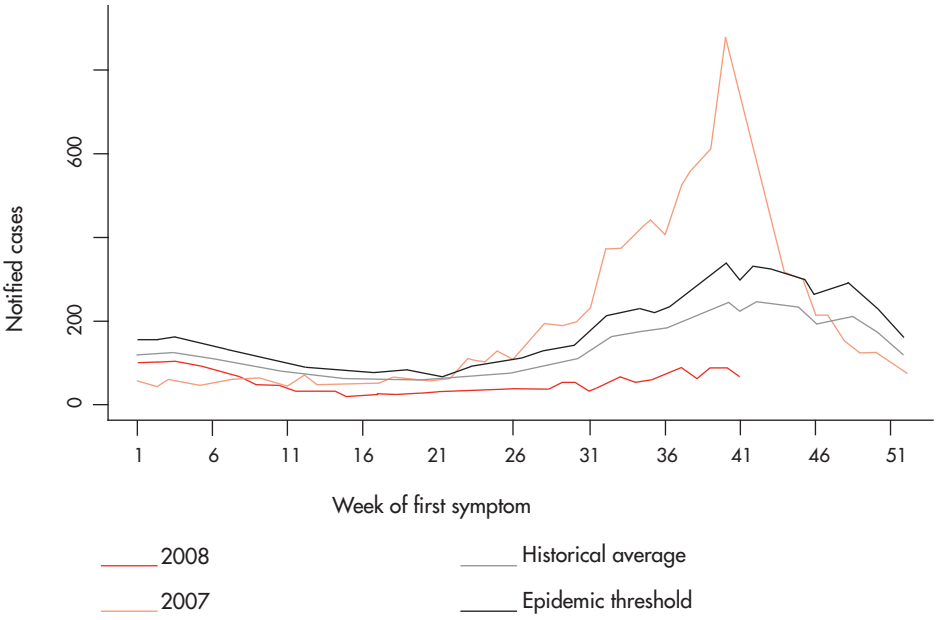
3.3 DELIVERY OF VECTOR CONTROL INTERVENTIONS

Whereas section 3.1 describes the main methods used in dengue vector control, section 3.2 focuses on the management approaches to their delivery, including intrasectoral as well as intersectoral collaboration. Table 3.4 summarizes vector surveillance and control activities and their purposes. Further details of entomological surveillance and emergency vector control are described in Chapter 5.

3.3.1 Links to epidemiological services

Vector control services should be closely linked to epidemiological services that capture and analyse the occurrence of dengue cases (temporal and spatial information). The epidemiological surveillance system should be able to differentiate between transient and seasonal increases in disease incidence and increases observed at the beginning of a dengue outbreak. One such approach is to track the occurrence of current (probable) cases and compare them with the average number of cases by week (or month) of the preceding 5–7 years, with confidence intervals set at two standard deviations above and below the average (±2 SD). This is sometimes referred to as the "endemic channel". If the number of cases reported exceeds 2 SDs above the "endemic channel" in weekly or monthly reporting, an outbreak alert is triggered. Figure 3.1 is an example from the surveillance system in Puerto Rico in 2007–2008. Such an approach is epidemiologically far more meaningful than year-to-year comparisons of cumulative totals of reported cases.

Figure 3.1 Surveillance data for dengue outbreak alerts, Puerto Rico, 2007–2008[a]



[a] Reproduced by kind permission of Centers for Disease Control and Prevention (CDC), Division of Vector-Borne Infectious Diseases, San Juan, Puerto Rico.

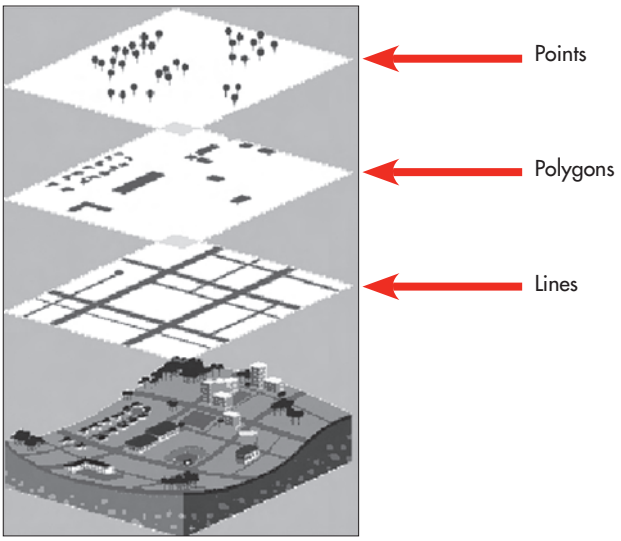
Table 3.4 Vector control services: activities and purpose

Area of activity	Specific activity	Purpose
Entomology		
Entomological surveillance	1. Presence/absence of larvae and/or pupae	To describe levels of infestation and ecology of immature development stages To monitor impact of vector control
	2. Number of pupae	To describe levels of infestation and to identify the most adult-productive container categories To focus vector control activities on the most epidemiologically important container categories to contribute to the estimation of transmission risk
	3. Relative adult abundance over time	To measure impact of vector control activities, seasonal and diel activity
	4. Insecticide susceptibility (larvae and adults)	To monitor for insecticide resistance and its management
Routine vector control operations	1. Controlling the immature stages	To reduce the vector population to levels at which transmission of virus is lowered or prevented
	2. Controlling adults	To reduce adult survival to lower vectorial capacity (for transmission of virus)
Emergency vector control operations	1. Controlling the immature stages 2. Controlling adults	To rapidly reduce vector population to slow/contain/interrupt transmission, or prevent incipient outbreak
Collaboration		
Communication and social mobilization	Design communication strategies and tools to inform and mobilize communities and other partners/sectors for vector control, personal protection, and case management.	To increase awareness and collaboration with recommended actions
Epidemiological surveillance	1. Passive surveillance data - clinical reporting of dengue cases (probable or confirmed dengue and severe dengue) - laboratory reporting on confirmed dengue cases/ serotypes. 2. Active surveillance data - case report verification - active case-finding - specific studies.	- describe trends and burden of disease - detect areas of transmission - outbreak detection/prediction - guide epidemiological stratification
Support		
Interagency coordination	Coordination between different national and international agencies involved in dengue control	To increase coordination between different players To reduce overlap of interventions and wastage of resources
Innovation	Assessment of novel techniques for use in the national context	To increase the uptake of novel techniques
Capacity-training	Rolling programme of training in techniques for vector control services	To ensure appropriate training and retraining of personnel
Monitoring and evaluation	Continuous monitoring and evaluation of vector control services according to established programme criteria and indicators	To ensure that programmes achieve what they should
Logistics	Support of vector control services	To ensure appropriate logistical support
Administration and finances	Support of vector control services	To ensure appropriate administrative and financial support

The spatial patterning of health events and disease outcomes has a long history. The development of GIS has facilitated the inclusion of a spatial component in epidemiological, entomological and environmental studies. A GIS is a collection of computer hardware, software and geographical data used for capturing, managing, analysing and displaying all forms of geographically referenced information. It allows users to choose different layers of information and to combine them according to what questions need to be answered or what data need to be analysed. Figure 3.2 describes the workings of a GIS.

Figure 3.2 Geographical information system (GIS), Singapore[a]

GIS allows the layering of health, demographic and environmental data sources to be analysed by their location on earth's surface.

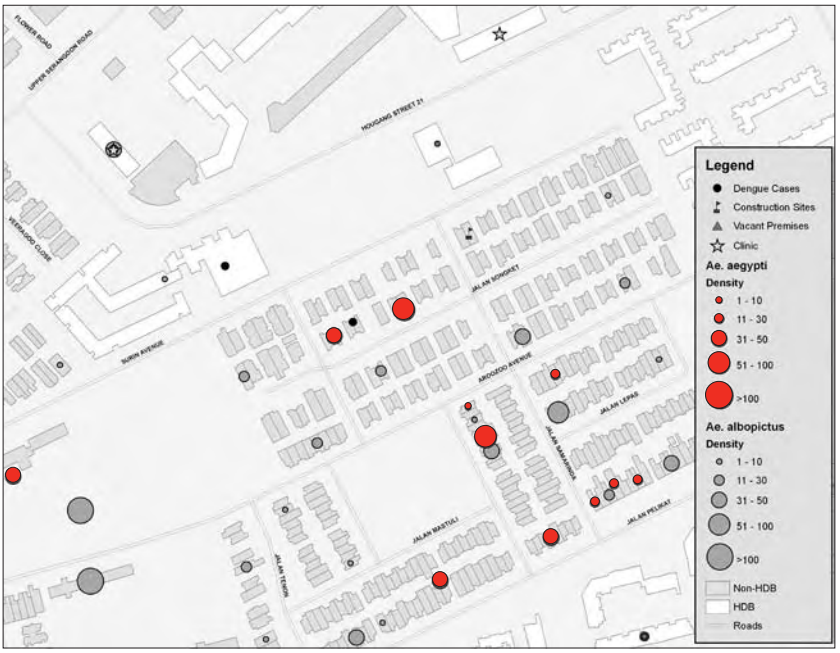


[a] Reproduced by kind permission of ESRI South Asia Pte Ltd, Singapore.

In setting up a GIS to support vector control services, data are organized in different layers to describe features such as streets, residences, buildings, train stations, schools, construction sites, shopping centres, medical clinics and electoral divisions. Above these base layers can be added entomological data, case data, virus serotype, enforcement data, demographics, weather data and so on.

Figure 3.3 shows a snapshot of a GIS map with eight layers of data used to support vector control operations in Singapore.

Figure 3.3 Snapshot of geographical information system (GIS) mapping, Singapore[a]



[a] Reproduced by kind permission of the National Environment Agency, Singapore.

GIS are widely used in public health to map diseases with different pathologies, to analyse the distribution of disease data in space and space-time, to identify risk factors and to map areas of risk. Typically, each case is located at either the residential or work address, and these locations are integrated into a GIS for mapping and analysis. Because a GIS allows epidemiologists to map environmental risk factors associated with disease vectors – such as construction sites, derelict or uninhabited premises and areas of congregation – it is especially relevant for the surveillance of vector-borne diseases such as dengue and malaria. For dengue, such mapping (epidemiological, entomological and environmental stratification) can serve to identify areas where transmission repeatedly occurs and which may warrant intensified or targeted control activities, or to stratify areas based on characteristics of larval habitats. The availability of such information in a timely manner could determine the outcome of vector control operations and even help to reduce the intensity of outbreaks. GIS technology has been found particularly useful for planning vector control operations, for managing and deploying resources for dengue control, and for presenting the dengue situation of any locality.

Free-access computer software programs are available; some software packages and maps can be downloaded from the Internet. Some dengue control programmes use hand-held global positioning system (GPS) devices and other hand-operated computer equipment to record data that are uploaded to a central database.

Where such resources are unavailable, commercial or hand-drawn maps may be used, with pins or labels indicating reported data.

3.3.2 Advocacy, social mobilization and legislation

Advocacy is a process through which groups of stakeholders can be influenced to gain support for and reduce barriers to specific initiatives or programmes. Multiple strategies, often used simultaneously, are key to the success of any advocacy effort. Strategies may include social mobilization and administrative, legislative, regulatory, legal and media advocacy (*13*). While there may be different target audiences and even different objectives for the strategies, it is the coordination of actions that leads to the achievement of the overarching goal of the strategic advocacy effort. Some actions may have a longer time frame – such as legislative and regulatory advocacy to address tyre disposal at national level – while others such as mobilizing local authorities and residents to carry out specific actions before the start of the rainy season may be time-limited.

A strategic advocacy plan usually includes one or more of the following types of advocacy (Table 3.5 shows examples of advocacy efforts in Asia and Latin America):

- *Social mobilization* – brings people together to achieve a common goal with a shared interpretation and direction.
- *Administrative advocacy* – informs authorities, decision-makers and opinion leaders of the importance of the programme, the costs and benefits of its activities and programme needs in order to enlist their support and cooperation.
- *Legislative advocacy* – uses federal/state/provincial/departmental/local legislative processes to promote legislation that addresses issues beyond the responsibility of any one governmental entity (e.g. legislation to address tyre disposal at national or regional level, the establishment of sanitary landfills, or the modification of housing designs or water catchment and storage systems).
- *Regulatory advocacy* – creates rules through which legislation is implemented (e.g. efforts to implement or modernize existing sanitary laws).
- *Legal advocacy* – uses the judicial system to enforce sanitary legislation and regulations (e.g. fines on contractors whose building construction sites persistently harbour aquatic foci of dengue vectors, or on householders and estates that fail to prevent mosquito breeding on their premises).
- *Media advocacy* – systematically engages the media to place issues of community interest on the social agenda, with a view to influencing public agendas. One such example, although not dengue-specific, is the PAHO Caribbean Media Awards for Health Journalism, an annual prize-giving event involving all mass media communication channels in the subregion.

Table 3.5 Examples of advocacy efforts in Asia and Latin America

Type of advocacy	Asia	Latin America
Social mobilization	**Malaysia**. Large-scale social mobilization started out "locally" in Johor Bahru. Singapore. Social mobilization involves the creation of "dengue prevention volunteer groups" among local grassroots organizations in the community.	**Brazil**. D-Day, a large-scale, coordinated social mobilization and media advocacy effort started out "locally" in Rio de Janeiro.
Administrative advocacy	**Malaysia**. Through administrative advocacy, activities have expanded to several other states.	**Brazil**. Through administrative advocacy D-Day is now nationwide. **Nicaragua**. After the elections, programme personnel initiated administrative advocacy to engage new personnel and elected officials in the COMBI process.
Legislative advocacy	**India**. Mumbai implements legislative measures to prevent mosquito breeding on premises. Several municipalities of Gujarat State have framed by-laws to implement similar measures. In Goa State, modified by-laws require householders to install mosquito-proof lids on their water tanks, and building construction site managers are required to prevent mosquito breeding. **Singapore**. The law requires all construction sites to have a full-time or part-time "environmental control officer" to take care of sanitation, hygiene and vector control issues of the site.	**Brazi**l. Through the national tyre recycling programme, regional tyre collection and processing centres were created. **El Salvador**. The first tyre recycling centre was modelled on the Brazil programme.
Legal advocacy	**India**. Fines are imposed on house owners on detection of mosquito breeding in cities such as Ahmedabad, Delhi, Mumbai and Surat. **Singapore**. House owners who create conditions favourable for mosquito breeding are penalized. Legislation exists to regulate sanitation.	**Cuba**. Fines are universally imposed on house owners and businesses when *Aedes* breeding is found.
Media advocacy	**Malaysia**. Media advocacy and social mobilization are two key components of the dengue programme.	**Brazil**. Media advocacy and social mobilization are two key components of D-Day.

3.3.3 Social mobilization and communication

Unlike chronic or sexually transmitted infections, vector-borne diseases require more than individual behaviour change in order to influence disease transmission. Behavioural change is required at both individual and community levels in order to reduce vector larval habitats successfully, and in turn to reduce the number of adult mosquitoes available to transmit disease. This has led to greater emphasis on social mobilization and communication activities which are fully integrated into dengue prevention and control efforts (*14*).

In order to develop an appropriate communication strategy, it is necessary to understand that communication is not just the dissemination of messages through various channels

such as interpersonal communication or the mass media. Communication is a two-way interactive process through which two or more participants (individuals or groups) create and share information in order to reach a common understanding and to identify areas of mutual agreement. This in turn allows for collective actions such as advocacy and social mobilization to be implemented. Once collective actions have been identified, social mobilization can be used to engage people's participation in achieving a specific goal through their own efforts (*15*). Social mobilization is not just a single activity; it involves all relevant segments of society (i.e. decision-makers and policy-makers, opinion leaders, bureaucrats and technocrats, professional groups, religious groups, commerce and industry, communities and individuals). It also takes into account the perceived needs of the people, embraces the critical principle of community involvement, and seeks to empower individuals and groups for action.

To date, most dengue-related communication (including more traditional information, education and communication (or IEC) efforts) and social mobilization activities have targeted individuals and communities generally defined by geographical boundaries – such as neighbourhoods, schools, and houses that fall within the radius of a confirmed dengue case. Little attention has been given to creating and sustaining a dialogue at policy level in order to address the underlying causes of increasing availability of vector larval habitats, such as ineffective refuse disposal services or an inconsistent or poor-quality water supply. Policy efforts require different communication strategies in order to engage the diverse target audience, which may include representatives from ministries of natural resources and the environment (water and sanitation), urban planning, finance and tourism, as well as municipal authorities. While many countries have a national dengue committee that may be activated during outbreaks or epidemics, these committees generally do not address the broader issues that lead to the ongoing propagation of the mosquito vectors of dengue fever.

Communication plans and strategies are often lacking, resulting in short-term information campaigns and ad hoc activities in reaction to outbreaks. In 2004, WHO published *Planning social mobilization and communication for dengue prevention and control*: a step-by-step guide (*16*) to assist programme managers in developing effective mobilization and communication strategies to promote behavioural change as part of routine vector control programming. The guide uses the COMBI (communication-for-behavioural-impact) planning methodology to focus communication and mobilization efforts on promoting and measuring changes in behaviour, and not just changes in knowledge and attitudes. This focus on behaviour rather than knowledge builds on many years of IEC efforts to increase community knowledge of dengue, the mosquito vector(s) and their larval habitats. Understanding the precise steps needed to carry out a recommended behaviour will help programmes to shift from the use of general messages that are often ignored by the target audience to messages that promote and encourage the process of behavioural change (*17*).

3.3.4 Collaboration within the health sector and with other sectors

Intersectoral collaboration among partners is a key strategy of IVM. Networking facilitates a more coordinated approach than the individual and independent efforts of different sectors, and provides a platform for partners to resolve cross-agency issues and to share best practices while reducing duplication of efforts. Networking for dengue

control also helps to leverage the strengths of partners and to synergize their efforts, thereby enhancing the effectiveness and efficiency of actions for dengue prevention and control.

3.3.4.1 Collaboration within the health sector

Control measures can be integrated with strong local health systems by transferring responsibility, authority, resources and knowledge from central to local level. However, it is critically important for the transfer of responsibility to be accompanied by the transfer of financial and technical resources. Transfer can be accomplished by offering, for instance, capacity-strengthening workshops or training courses in vector biology and control, epidemiology, and communication among other topics at the local level. At all administrative levels of government (state, provincial, departmental and local), the dengue control programme is usually part of the local health system, wherein lies the responsibility for planning, implementing, monitoring and evaluating the local programme.

Contacts, liaison and cooperative activities should be promoted within the different divisions of the health sector. This cooperation with the dengue programme is necessary since the prevention and control of dengue is not the responsibility of a single department. Regardless of whether the programme is led by the Ministry of Health, collaboration within this ministry is essential among those departments responsible for vector control and surveillance, epidemiological surveillance, clinical diagnosis and management, maternal and child health (e.g. the programme on integrated management of childhood illnesses), health education, community participation and environmental health. Entities such as national health institutes and schools of public health and medicine can also contribute by carrying out activities for which the Ministry of Health may not have resources, such as training and research projects.

3.3.4.2 Collaboration with other sectors and with the community

Dengue prevention and control necessitates an effective intersectoral approach, requiring coordination between the lead ministry, usually the Ministry of Health, and other ministries and government agencies, the private sector (including private health providers), nongovernmental organizations (NGOs) and local communities. Resource-sharing is an important aspect of this (Table 3.6). Such cooperation is critical in emergency situations when scarce or widely dispersed human and material resources must be mobilized rapidly and their use coordinated to mitigate the effects of an epidemic. The process can be facilitated by policy adjustment.

Policy adjustment

The Ministry of Health and the programme manager should seek mutual agreement with other ministries, sectors or municipal governments – and even the adjustment of existing policies and practices – to place public health centrally among the goals of those bodies (administrative advocacy). For instance, the public works sector could be encouraged to give priority to improvements in water supply for those communities at highest risk of dengue.

Table 3.6 Selected examples of potential intersectoral actions

Sector	Issue	Rationale
Public sector		
Ministry of the Environment	Solid waste management, water supply, promotion of healthy public policies	Discarded containers and household water-storage containers are larval habitats.
Ministry of Education	Incorporation of environmental health issues and activities in school curricula	Empowerment of children with knowledge of health risks and skills to carry out actions to manage the environment. This is often an entry point for community action.
Municipal authority	Urban infrastructure and planning, including water and sanitation services	Urban development and infrastructure can be designed and managed to avoid creation of larval habitats (e.g. sufficient resources dedicated to the collection and disposal of refuse, and to a reliable and good quality water system).
Ministry of Public Works	Street stormwater drainage systems, underground service units for telephones, etc. Housing structure and water-storage containers.	Design of water run-off drainage systems and manholes that do not create underground mosquito larval habitats. Design structures that avoid the creation of mosquito larval habitats (e.g. roofing design, water run-off and catchment systems).
Ministry of Tourism	Reduction of economic losses associated with dengue outbreaks	Involvement of the hospitality sector in routine vector control actions and environmental management.
Ministry of Finance and Planning	Legislative framework	Provision of the legislative and regulatory framework to support environmental management actions and sound use of insecticides.
Private sector	Stewardship, particularly in industrial and manufacturing sectors	Consumer packaging and petrochemical industries, and manufacturers of tyres and water-storage tanks are examples of how the private sector may contribute – indirectly or directly – to larval habitats and to potential solutions, especially in the context of "social or environmental responsibility". Small-scale tyre businesses may have limited means to protect used tyres stored on the premises.
Nongovernmental organizations	Mobilization of community action	NGOs are able to mobilize resources and actions at community level on issues of common interest.

Potential roles of government ministries

Public works. The ministry responsible for public works and its municipal counterparts are responsible for providing dependable water supply, sanitation and solid waste management services to all planned communities. The dimensions and quality of those services have a direct bearing on the availability of larval habitats. Additionally, through the adoption and enforcement of housing and building codes (legislative and regulatory advocacy), a municipality may mandate the provision of utilities such as piped water or

sewerage connections for individual households and rainwater run-off control for new housing developments, or it may prohibit the construction of open groundwater wells. Such opportunities are prescient when planning urban redevelopment schemes and because of the benefits of reduced risk of dengue and of mosquitoes and other pests.

Education. The Ministry of Education should be a key partner as it is responsible not only for educating children and young people but also for inculcating social norms which include appropriate hygiene behaviours. Where dengue prevention and control involve a health communication component targeted at schoolchildren, the Ministry of Health can work closely with the Ministry of Education to develop, communicate and impart appropriate messages and skills for behaviour change. Such messages and skills should ideally be integrated into existing curricula to ensure long-term continuity (*18*).

Health education models can be jointly developed, tested and evaluated for different age groups. Research programmes in universities and colleges can be encouraged to include components that generate information of direct importance (e.g. vector biology and control, case management) or of indirect importance (e.g. improved water supplies, promotion of community sanitation, waste characterization studies, analysis of cost and cost-effectiveness of interventions) to aid evidence-based decision-making.

Tourism. Coordination with the Ministry of Tourism can facilitate the timely communication of outbreak or epidemic alert messages to tourists and to the hotel industry so that actions can be taken to reduce the risk of exposure to infection.

Environment. The ministry responsible for the environment can help the Ministry of Health to gather information on ecosystems and habitats in and around cities and smaller communities at high risk of dengue so as to aid in programme planning. In at least one country (Singapore), the Ministry of the Environment has direct responsibility for dengue vector control and promotes healthy public policies that include sound management of public health pesticides.

Collaboration with nongovernmental organizations

NGOs can play important roles in promoting and implementing environmental management for dengue vector control, most often involving health communication on reduction of sources and improvement of housing. Community NGOs – which may be informal neighbourhood groups such as private volunteer organizations, religious groups and environmental and social action groups – should be identified as potential partners.

With appropriate orientation and guidance, particularly on source reduction methods, NGOs can collect discarded containers (e.g. tyres, food and drink containers), or can clean drains and culverts, remove abandoned vehicles and roadside litter, and fill tree and rock holes. During outbreaks NGOs may be influential in mobilizing householders and other community members to eliminate important larval habitats of the vector or to manage the containers in ways that do not allow mosquito emergence (e.g. by emptying and cleaning water-storage containers at weekly intervals). NGOs may also encourage public cooperation and acceptance of space spraying and larvicide application measures.

Communities organize themselves in many ways, so there is no prescribed formula for interaction. However, social mobilization initiatives must be socially and culturally sensitive and should be developed between partners in a spirit of mutual respect. There are many examples of voluntary organizations and women's associations taking the lead in providing money, advertising and political support to successful community-based campaigns for source reduction or in organizing regular household activities to reduce mosquito populations.

Collaboration with industry and the private sector

Collaboration with industry and the private sector can advance the manufacture and utilization of mosquito-proof designs of water-storage containers and room-coolers, and can promote the collection and recycling of used tyres, plastic, aluminium, glass and other containers. In the construction industry, architects' associations can help to promote the design and building of mosquito-proof and otherwise healthy houses and workplaces.

3.3.5 Integrated approaches to control

Instead of targeting only the vector or vectors of dengue, there may be opportunities to integrate *Aedes* control with control of pests or vectors of other diseases. Addressing two or more public health problems simultaneously may improve cost-effectiveness and may help promote public acceptance and involvement in the programme. For example, control of *Ae. aegypti* in some urban areas can be combined with control of *Culex quinquefasciatus*, the latter species usually being a much greater nuisance to the public and an important vector of lymphatic filariasis in many tropical environments. Collection of solid waste as an environmental management component of *Aedes* control programmes need not be restricted just to the container sources of *Aedes* production but can also include items that are associated with filth flies and rodents. Moreover, given that urban yellow fever and chikungunya viruses are also transmitted by *Ae. aegypti*, and chikungunya by *Ae. albopictus* as well, their control is an effective way of reducing the risk of outbreaks of these diseases in addition to dengue. In many cities in India, *Ae. aegypti* and the malaria vector *Anopheles stephensi* share common larval habitats and can be targeted simultaneously.

3.3.6 Strengthening capacity

In vector control, as in other areas of public health, staffing levels and capacity-strengthening are important. In particular, public health entomologists, vector control personnel, environmental specialists, social scientists and communication specialists play pivotal roles.

3.3.6.1 Social scientists and communication specialists

Because social mobilization and communication are often the least planned and most under-funded elements of the dengue prevention and control programme, it is even more important to use resources in a targeted and cost-effective manner. This can be accomplished by working with social scientists who have expertise in using behavioural change theories in programme development, and with communication specialists who

have a background preferably in health communication (i.e. the use of communication strategies to inform and influence individual and community decisions that enhance health). This will require that the dengue programme includes in its annual budget an allocation for social mobilization and communication activities in order to integrate these specialists into routine programme planning.

Most ministries of health have health promotion or health education departments, and it is increasingly common to find communication included in them. However, communication is frequently viewed as use of the mass media, and therefore the communication specialists may be individuals with backgrounds in public relations, journalism or mass media who have limited knowledge of the principles of health promotion or behavioural change. In this case it is even more important to involve a health promotion specialist or a social scientist to ensure that the messages focus on appropriate and feasible behaviours that target the principal vector larval habitats, and that the impact of the social mobilization and communication activities are evaluated for changes in behaviour and not just for changes in knowledge or attitudes. Health promotion and health education personnel can generally be found at the central and state or provincial levels, while at the local level this role may be filled by a nurse or social worker if a person qualified in communication is not part of the health clinic staffing.

Ongoing training and practice in communication at all levels is critical for ensuring appropriate and effective interpersonal communication between vector control staff and householders, between the dengue programme manager and vector control staff, and between the dengue programme manager and partners within and outside the health sector. When and how to use the mass media at national, regional and local levels can be determined in collaboration with the communications specialist. Using the media may entail training sessions in public speaking for key spokespersons within the programme as well as for epidemiologists and medical personnel who may also be required to speak to the media. Training in how to work with the media, particularly during an outbreak or epidemic, is vital to ensure that accurate and useful information is shared with the broader community through the media, to avoid sending mixed messages about what is expected of the community during the outbreak and to reduce the chance of misinterpretation or sensationalization of information (particularly the number of cases of dengue).

3.3.6.2 Public health entomologists and vector control and environmental management personnel

The skills for managing, implementing, monitoring and evaluating the programme must be determined in accordance with availability of resources, programme objectives and intervention strategies. Training activities, including in-service training, should be tailored to the needs of the various groups of personnel. WHO has published guidance on needs assessment for all components of the dengue control programme (*19*).

Whether or not vector control activities are aligned with centralized or decentralized health systems, and whether they are distinct from or integrated with other health sector

activities, the available skills at any given level of administrative responsibility should be commensurate with those responsibilities. This is equally important for the purposes of financial and operational planning – including workforce planning and strategic, technical guidance – as well as for essential physical infrastructure.

3.3.7 Operational research

Operational research should be oriented to the priority needs of the programme in order to generate the evidence base for adaptation of strategies and interventions. This may include, for example, studies on the ecology of the vector, the efficacy, effectiveness and cost-effectiveness of existing and promising new vector control methods, formative research on relevant cultural practices, and guidance for engaging communities in programme activities.

3.3.8 Monitoring and evaluation

Regular monitoring of the delivery of dengue prevention and control services and evaluation of the impact of interventions are important activities for effective programme management. Suitable indicators should be identified to measure the progress of implementation, as well as output and outcome indicators. Table 3.7 gives examples of good and bad practice in dengue prevention and control.

Table 3.7 Dengue prevention and control: examples of good and bad practice

Activity	Good practice	Bad practice
Environmental management	Determining local ecology of immature stages as a basis for choosing the most appropriate interventions Integrating dengue control with sanitation, solid waste disposal, water supply services and other vector and pest control programmes	Investing disproportionately in chemical control methods when affordable and more sustainable environmental management solutions are available. Responding primarily to outbreaks and not investing in sustained vector control measures.
Chemical control	Using insecticides judiciously	Using chemical control methods without evaluating efficacy and cost-effectiveness, and without monitoring local vector susceptibility.
Municipal services	Intersectoral collaboration on urban development or renewal to minimize availability of larval habitats of dengue vectors and vectors of other diseases	Social marketing of larvicides for water-storage containers, with consequent low coverage rates.
Entomological monitoring and surveillance	Monitoring of vector populations to target control in time and space, and to provide feedback for programme adjustment	Generating routine entomological surveillance data that are not analysed or utilized in a timely and efficient manner for management decision-making.

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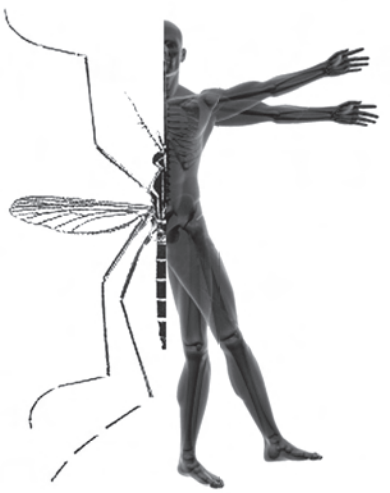


CHAPTER 4

LABORATORY DIAGNOSIS AND DIAGNOSTIC TESTS

CHAPTER 4. LABORATORY DIAGNOSIS AND DIAGNOSTIC TESTS

4.1 OVERVIEW

Efficient and accurate diagnosis of dengue is of primary importance for clinical care (i.e. early detection of severe cases, case confirmation and differential diagnosis with other infectious diseases), surveillance activities, outbreak control, pathogenesis, academic research, vaccine development, and clinical trials.

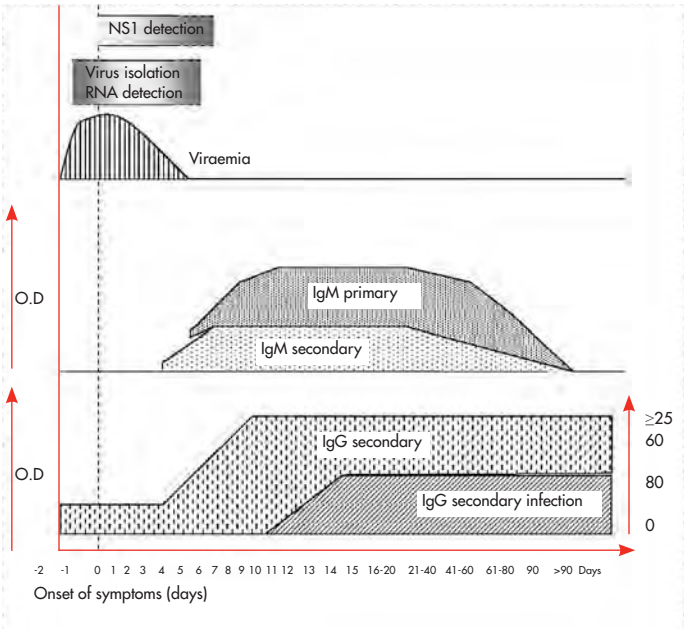
Laboratory diagnosis methods for confirming dengue virus infection may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques. After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days. During the early stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis.

Antibody response to infection differs according to the immune status of the host (*1*). When dengue infection occurs in persons who have not previously been infected with a flavivirus or immunized with a flavivirus vaccine (e.g. for yellow fever, Japanese encephalitis, tick-borne encephalitis), the patients develop a primary antibody response characterized by a slow increase of specific antibodies. IgM antibodies are the first immunoglobulin isotype to appear. These antibodies are detectable in 50% of patients by days 3-5 after onset of illness, increasing to 80% by day 5 and 99% by day 10 (Figure 4.1). IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2–3 months. Anti-dengue serum IgG is generally detectable at low titres at the end of the first week of illness, increasing slowly thereafter, with serum IgG still detectable after several months, and probably even for life (*2–4*) .

During a secondary dengue infection (a dengue infection in a host that has previously been infected by a dengue virus, or sometimes after non-dengue flavivirus vaccination or infection), antibody titres rise rapidly and react broadly against many flaviviruses. The dominant immunoglobulin isotype is IgG which is detectable at high levels, even in the acute phase, and persists for periods lasting from 10 months to life. Early convalescent stage IgM levels are significantly lower in secondary infections than in primary ones and may be undetectable in some cases, depending on the test used (*5*). To distinguish primary and secondary dengue infections, IgM/IgG antibody ratios are now more commonly used than the haemagglutination-inhibition test (HI) (*6–8*).

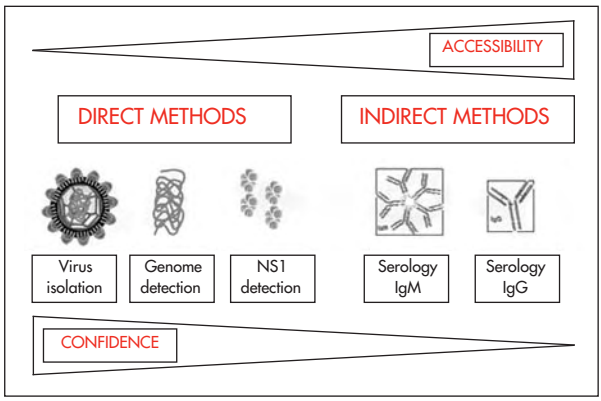
A range of laboratory diagnostic methods has been developed to support patient management and disease control. The choice of diagnostic method depends on the purpose for which the testing is done (e.g. clinical diagnosis, epidemiological survey, vaccine development), the type of laboratory facilities and technical expertise available, costs, and the time of sample collection.

Figure 4.1 Approximate time-line of primary and secondary dengue virus infections and the diagnostic methods that can be used to detect infection



In general, tests with high sensitivity and specificity require more complex technologies and technical expertise, while rapid tests may compromise sensitivity and specificity for the ease of performance and speed. Virus isolation and nucleic acid detection are more labour-intensive and costly but are also more specific than antibody detection using serologic methods. Figure 4.2 shows a general inverse relationship between the ease of use or accessibility of a diagnostic method and the confidence in the results of the test.

Figure 4.2 Comparison of diagnostic tests according to their accessibility and confidence



4.2 CONSIDERATIONS IN THE CHOICE OF DIAGNOSTIC METHODS

4.2.1 Clinical management

Dengue virus infection produces a broad spectrum of symptoms, many of which are non-specific. Thus, a diagnosis based only on clinical symptoms is unreliable. Early laboratory confirmation of clinical diagnosis may be valuable because some patients progress over a short period from mild to severe disease and sometimes to death. Early intervention may be life-saving.

Before day 5 of illness, during the febrile period, dengue infections may be diagnosed by virus isolation in cell culture, by detection of viral RNA by nucleic acid amplification tests (NAAT), or by detection of viral antigens by ELISA or rapid tests. Virus isolation in cell culture is usually performed only in laboratories with the necessary infrastructure and technical expertise. For virus culture, it is important to keep blood samples cooled or frozen to preserve the viability of the virus during transport from the patient to the laboratory. The isolation and identification of dengue viruses in cell cultures usually takes several days. Nucleic acid detection assays with excellent performance characteristics may identify dengue viral RNA within 24–48 hours. However, these tests require expensive equipment and reagents and, in order to avoid contamination, tests must observe quality control procedures and must be performed by experienced technicians. NS1 antigen detection kits now becoming commercially available can be used in laboratories with limited equipment and yield results within a few hours. Rapid dengue antigen detection tests can be used in field settings and provide results in less than an hour. Currently, these assays are not type-specific, are expensive and are under evaluation for diagnostic accuracy and cost-effectiveness in multiple settings. Table 4.1 summarizes various dengue diagnostic methods and their costs.

Table 4.1 Summary of operating characteristics and comparative costs of dengue diagnostic methods (*9*)

Diagnostic methods	Diagnosis of acute infection	Time to results	Specimen	Time of collection after onset of symptoms	Facilities	Cost
Viral isolation and serotype identification	Confirmed	1–2 weeks	Whole blood, serum, tissues	1–5 days	Mosquito or cell culture facilities, BSL-2/BSL-3[a] laboratory, fluorescence microscope or molecular biology equipment	$$$
Nucleic acid detection	Confirmed	1 or 2 days	Tissues, whole blood, serum, plasma	1–5 days	BSL-2 laboratory, equipment for molecular biology	$$$
Antigen detection	Not yet determined Confirmed	1 day >1 day	Serum Tissue for immuno-chemistry	1–6 days NA	ELISA facilities Facilities for histology	$$ $$$
IgM ELISA	Probable	1–2 days	Serum, plasma, whole blood	After 5 days	ELISA facilities	$
IgM rapid test		30 minutes			No additional supplies	
IgG (paired sera) by ELISA, HI or neutralization test	Confirmed	7 days or more	Serum, plasma, whole blood	Acute sera, 1–5 days; convalescent after 15 days	ELISA facilities BSL-2 laboratory for neutralization assay	$

[a] Requirements may vary according to each country's national policies.

After day 5, dengue viruses and antigens disappear from the blood coincident with the appearance of specific antibodies. NS1 antigen may be detected in some patients for a few days after defervescence. Dengue serologic tests are more available in dengue-endemic countries than are virological tests. Specimen transport is not a problem as immunoglobulins are stable at tropical room temperatures.

For serology, the time of specimen collection is more flexible than that for virus isolation or RNA detection because an antibody response can be measured by comparing a sample collected during the acute stage of illness with samples collected weeks or months later. Low levels of a detectable dengue IgM response – or the absence of it – in some secondary infections reduces the diagnostic accuracy of IgM ELISA tests. Results of rapid tests may be available within less than one hour. Reliance on rapid tests to diagnose dengue infections should be approached with caution, however, since the performance of all commercial tests has not yet been evaluated by reference laboratories (*10*).

A four-fold or greater increase in antibody levels measured by IgG ELISA or by haemagglutination inhibition (HI) test in paired sera indicates an acute or recent flavivirus infection. However, waiting for the convalescent serum collected at the time of patient discharge is not very useful for diagnosis and clinical management and provides only a retrospective result.

4.2.1.1 Differential diagnosis

Dengue fever can easily be confused with non-dengue illnesses, particularly in non-epidemic situations. Depending on the geographical origin of the patient, other etiologies – including non-dengue flavivirus infections – should be ruled out. These include yellow fever, Japanese encephalitis, St Louis encephalitis, Zika, and West Nile, alphaviruses (such as Sinbis and chikungunya), and other causes of fever such as malaria, leptospirosis, typhoid, Rickettsial diseases (*Rickettsia prowazeki, R. mooseri, R. conori, R. rickettsi, Orientia tsutsugamushi, Coxiella burneti, etc.*), measles, enteroviruses, influenza and influenza-like illnesses, haemorrhagic fevers (Arenaviridae: Junin, etc.; Filoviridae: Marburg, Ebola; Bunyaviridae: hantaviruses, Crimean-Congo haemorrhagic fever, etc.).

Both the identification of virus/viral RNA/viral antigen and the detection of an antibody response are preferable for dengue diagnosis to either approach alone (see Table 4.2).

Table 4.2 Interpretation of dengue diagnostic tests [adapted from Dengue and Control (DENCO) study]

Highly suggestive	Confirmed
One of the following: 1. IgM + in a single serum sample 2. IgG + in a single serum sample with a HI titre of 1280 or greater	One of the following: 1. PCR + 2. Virus culture + 3. IgM seroconversion in paired sera 4. IgG seroconversion in paired sera or fourfold IgG titer increase in paired sera

Unfortunately, an ideal diagnostic test that permits early and rapid diagnosis, is affordable for different health systems, is easy to perform, and has a robust performance, is not yet available.

4.2.2 Outbreak investigations

During outbreaks some patients may be seen presenting with fever with or without rash during the acute illness stage; some others may present with signs of plasma leakage or shock, and others with signs of haemorrhages, while still others may be observed during the convalescent phase.

One of the priorities in a suspected outbreak is to identify the causative agent so that appropriate public health measures can be taken and physicians can be encouraged to initiate appropriate acute illness management. In such cases, the rapidity and specificity of diagnostic tests is more important than test sensitivity. Samples collected from febrile patients could be tested by nucleic acid methods in a well-equipped laboratory or a broader spectrum of laboratories using an ELISA-based dengue antigen detection kit. If specimens are collected after day 5 of illness, commercial IgM ELISA or sensitive dengue IgM rapid tests may suggest a dengue outbreak, but results are preferably confirmed with reliable serological tests performed in a reference laboratory with broad arbovirus diagnostic capability. Serological assays may be used to determine the extent of outbreaks.

4.2.3 Surveillance

Dengue surveillance systems aim to detect the circulation of specific viruses in the human or mosquito populations. The diagnostic tools used should be sensitive, specific and affordable for the country. Laboratories responsible for surveillance are usually national and/or reference laboratories capable of performing diagnostic tests as described above for dengue and for a broad range of other etiologies.

4.2.4 Vaccine trials

Vaccine trials are performed in order to measure vaccine safety and efficacy in vaccinated persons. The plaque reduction and neutralization test (PRNT) and the microneutralization assays are commonly used to measure protection correlates.

Following primary infections in non-flavivirus immunes, neutralizing antibodies as measured by PRNT may be relatively or completely specific to the infecting virus type (*11,12*). This assay is the most reliable means of measuring the titre of neutralizing antibodies in the serum of an infected individual as a measure of the level of protection against an infecting virus. The assay is based on the principle that neutralizing antibodies inactivate the virus so that it is no longer able to infect and replicate in target cells.

After a second dengue virus infection, high-titre neutralizing antibodies are produced against at least two, and often all four, dengue viruses as well as against non-dengue flaviviruses. This cross reactivity results from memory B-cells which produce antibodies directed at virion epitopes shared by dengue viruses. During the early convalescent stage following sequential dengue infections, the highest neutralizing antibody titre is often directed against the first infecting virus and not the most recent one. This phenomenon is referred to as "original antigenic sin" (*13*).

The disadvantages of PRNT are that it is labour-intensive. A number of laboratories recently developed high through-put neutralization tests that can be used in large-scale surveillance studies and vaccine trials. Variable results have been observed in PRNTs performed in different laboratories. Variations can be minimized if tests are performed on standard cell lines using the same virus strains and the same temperature and time for incubation of virus with antibody. Input virus should be carefully calculated to avoid plaque overlap. Cell lines of mammalian origin, such as VERO cells, are recommended for the production of seed viruses for use in PRNT.

The microneutralization assay is based on the same principle as PRNT. Variable methods exist. In one, instead of counting the number of plaques per well, viral antigen is stained using a labelled antibody and the quantity of antigen measured colorimetrically. The test may measure nucleic acid using PCR. The microneutralization assay was designed to use smaller amounts of reagents and for testing larger numbers of samples. In viral antigen detection tests the spread of virus throughout the cells is not limited because, in PRNTs using semisolid overlays, the time after infection must be standardized to avoid measuring growth after many cycles of replication. Since not all viruses grow at the same rate, the incubation periods are virus-specific. As with standard PRNTs, antibodies measured by micromethods from individuals with secondary infections may react broadly with all four dengue viruses.

Table 4.3 Advantages and limitations of dengue diagnostic methods (*9*)

Indications	Diagnostic Tests	Advantages	Limitations
Diagnosis of acute dengue infection	Nucleic acid detection	•Most sensitive and specific •Possible to identify serotype •Early appearance (pre-antibody), so opportunity to impact on patient management	•Potential false positive due to contamination •Expensive •Needs expertise and expensive laboratory equipment •Not possible to differentiate between primary and secondary infection
	Isolation in cell culture and identification using immuno-fluorescence	•Specific •Possible to identify serotype by using specific antibodies	•Need expertise and facility for cell culture and fluorescent microscopy •Takes more than 1 week •Not possible to differentiate between primary and secondary infection
	Antigen detection in clinical specimens	•Easy to perform •Opportunity for early diagnosis may impact on patient treatment	•Not as sensitive as virus isolation or RNA detection
	Serologic tests: IgM tests Seroconversion: 4-fold rise in HI or ELISA IgG titres between acute and convalescent samples	•Useful for confirmation of acute infection •Least expensive •Easy to perform •Can distinguish between primary and secondary infection	•May miss cases because IgM levels may be low or undetectable in some secondary infections •Need two samples •Delay in confirming diagnosis
Surveillance and outbreak identification; Monitor effectiveness of interventions	IgM detection	•Identify probable dengue cases •Easy to perform for case detection in sentinel laboratories	•May miss cases because IgM levels may be low in secondary infections
	Viral isolation and RNA detection	•Confirm cases •Identify serotypes	•Can be performed only in reference laboratories •Need acute samples

In drug trials, patients should have confirmed etiological diagnosis (see Table 4.2 for highly suggestive and confirmed diagnosis).

Table 4.3 summarizes the advantages and limitations of each of the diagnostic methods for each purpose.

4.3 CURRENT DENGUE DIAGNOSTIC METHODS

4.3.1 Virus isolation

Specimens for virus isolation should be collected early in the course of the infection, during the period of viraemia (usually before day 5). Virus may be recovered from serum, plasma and peripheral blood mononuclear cells and attempts may be made from tissues collected at autopsy (e.g. liver, lung, lymph nodes, thymus, bone marrow). Because dengue virus is heat-labile, specimens awaiting transport to the laboratory should be kept in a refrigerator or packed in wet ice. For storage up to 24 hours, specimens should be kept at between +4 °C and +8 °C. For longer storage, specimens should be frozen at -70 °C in a deep-freezer or stored in a liquid nitrogen container. Storage even for short periods at –20 °C is not recommended.

Cell culture is the most widely used method for dengue virus isolation. The mosquito cell line C6/36 (cloned from *Ae. albopictus*) or AP61 (cell line from *Ae. pseudoscutellaris*) are the host cells of choice for routine isolation of dengue virus. Since not all wild type dengue viruses induce a cytopathic effect in mosquito cell lines, cell cultures must be screened for specific evidence of infection by an antigen detection immunofluorescence assay using serotype-specific monoclonal antibodies and flavivirus group-reactive or dengue complex-reactive monoclonal antibodies. Several mammalian cell cultures, such as Vero, LLCMK2, and BHK21, may also be used but are less efficient. Virus isolation followed by an immunofluorescence assay for confirmation generally requires 1–2 weeks and is possible only if the specimen is properly transported and stored to preserve the viability of the virus in it.

When no other methods are available, clinical specimens may also be inoculated by intracranial route in suckling mice or intrathoracic inoculation of mosquitoes. Newborn animals can develop encephalitis symptoms but with some dengue strains mice may exhibit no signs of illness. Virus antigen is detected in mouse brain or mosquito head squashes by staining with anti-dengue antibodies.

4.3.2 Nucleic acid detection

RNA is heat-labile and therefore specimens for nucleic acid detection must be handled and stored according to the procedures described for virus isolation.

4.3.2.1 RT-PCR

Since the 1990s, several reverse transcriptase-polymerase chain reaction (RT-PCR) assays have been developed. They offer better sensitivity compared to virus isolation

with a much more rapid turnaround time. In situ RT-PCR offers the ability to detect dengue RNA in paraffin-embedded tissues.

All nucleic acid detection assays involve three basic steps: nucleic acid extraction and purification, amplification of the nucleic acid, and detection and characterization of the amplified product. Extraction and purification of viral RNA from the specimen can be done by traditional liquid phase separation methods (e.g. phenol, chloroform) but has been gradually replaced by silica-based commercial kits (beads or columns) that are more reproducible and faster, especially since they can be automated using robotics systems. Many laboratories utilize a nested RT-PCR assay, using universal dengue primers targeting the C/prM region of the genome for an initial reverse transcription and amplification step, followed by a nested PCR amplification that is serotype-specific (*14*). A combination of the four serotype-specific oligonucleotide primers in a single reaction tube (one-step multiplex RT-PCR) is an interesting alternative to the nested RT-PCR (*15*). The products of these reactions are separated by electrophoresis on an agarose gel, and the amplification products are visualized as bands of different molecular weights in the agarose gel using ethidium bromide dye, and compared with standard molecular weight markers. In this assay design, dengue serotypes are identified by the size of their bands.

Compared to virus isolation, the sensitivity of the RT-PCR methods varies from 80% to 100% and depends on the region of the genome targeted by the primers, the approach used to amplify or detect the PCR products (e.g. one-step RT-PCR versus two-step RT-PCR), and the method employed for subtyping (e.g. nested PCR, blot hybridization with specific DNA probes, restriction site-specific PCR, sequence analysis, etc.). To avoid false positive results due to non-specific amplification, it is important to target regions of the genome that are specific to dengue and not conserved among flavi- or other related viruses. False-positive results may also occur as a result of contamination by amplicons from previous amplifications. This can be prevented by physical separation of different steps of the procedure and by adhering to stringent protocols for decontamination.

4.3.2.2 Real-time RT-PCR

The real-time RT-PCR assay is a one step assay system used to quantitate viral RNA and using primer pairs and probes that are specific to each dengue serotype. The use of a fluorescent probe enables the detection of the reaction products in real time, in a specialized PCR machine, without the need for electrophoresis. Many real-time RT-PCR assays have been developed employing TaqMan or SYBR Green technologies. The TaqMan real-time PCR is highly specific due to the sequence-specific hybridization of the probe. Nevertheless, primers and probes reported in publications may not be able to detect all dengue virus strains: the sensitivity of the primers and probes depends on their homology with the targeted gene sequence of the particular virus analyzed. The SYBR green real-time RT-PCR has the advantage of simplicity in primer design and uses universal RT-PCR protocols but is theoretically less specific.

Real-time RT-PCR assays are either "singleplex" (i.e. detecting only one serotype at a time) or "multiplex" (i.e. able to identify all four serotypes from a single sample). The multiplex assays have the advantage that a single reaction can determine all four serotypes without the potential for introduction of contamination during manipulation of the sample. However the multiplex real-time RT-PCR assays, although faster, are currently less sensitive than nested RT-PCR assays. An advantage of this method is the ability to

determine viral titre in a clinical sample, which may be used to study the pathogenesis of dengue disease (*16*).

4.3.2.3 Isothermal amplification methods

The NASBA (nucleic acid sequence based amplification) assay is an isothermal RNA-specific amplification assay that does not require thermal cycling instrumentation. The initial stage is a reverse transcription in which the single-stranded RNA target is copied into a double-stranded DNA molecule that serves as a template for RNA transcription. Detection of the amplified RNA is accomplished either by electrochemiluminescence or in real-time with fluorescent-labelled molecular beacon probes. NASBA has been adapted to dengue virus detection with sensitivity near that of virus isolation in cell cultures and may be a useful method for studying dengue infections in field studies (*17*).

Loop mediated amplification methods have also been described but their performance compared to other nucleic acid amplification methods are not known (*18*).

4.3.3 Detection of antigens

Until recently, detection of dengue antigens in acute-phase serum was rare in patients with secondary infections because such patients had pre-existing virus-IgG antibody immunocomplexes. New developments in ELISA and dot blot assays directed to the envelop/membrane (E/M) antigen and the non-structural protein 1 (NS1) demonstrated that high concentrations of these antigens in the form of immune complexes could be detected in patients with both primary and secondary dengue infections up to nine days after the onset of illness.

The NS1 glycoprotein is produced by all flaviviruses and is secreted from mammalian cells. NS1 produces a very strong humoral response. Many studies have been directed at using the detection of NS1 to make an early diagnosis of dengue virus infection. Commercial kits for the detection of NS1 antigen are now available, though they do not differentiate between dengue serotypes. Their performance and utility are currently being evaluated by laboratories worldwide, including the WHO/TDR/PDVI laboratory network.

Fluorescent antibody, immunoperoxidase and avidin-biotin enzyme assays allow detection of dengue virus antigen in acetone-fixed leucocytes and in snap-frozen or formalin-fixed tissues collected at autopsy.

4.3.4 Serological tests

4.3.4.1 MAC-ELISA

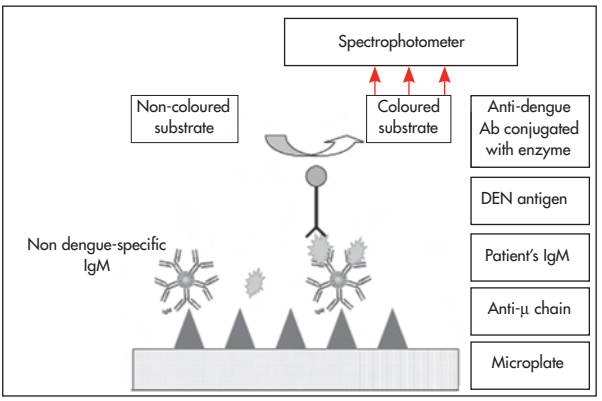
For the IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) total IgM in patients' sera is captured by anti-μ chain specific antibodies (specific to human IgM) coated onto a microplate. Dengue-specific antigens, from one to four serotypes (DEN-1, -2, -3, and -4), are bound to the captured anti-dengue IgM antibodies and are detected by monoclonal or polyclonal dengue antibodies directly or indirectly conjugated with an

enzyme that will transform a non-coloured substrate into coloured products. The optical density is measured by spectrophotometer.

Serum, blood on filter paper and saliva, but not urine, can be used for detection of IgM if samples are taken within the appropriate time frame (five days or more after the onset of fever). Serum specimens may be tested at a single dilution or at multiple dilutions. Most of the antigens used for this assay are derived from the dengue virus envelope protein (usually virus-infected cell culture supernatants or suckling mouse brain preparations). MAC-ELISA has good sensitivity and specificity but only when used five or more days after the onset of fever. Different commercial kits (ELISA or rapid tests) are available but have variable sensitivity and specificity. A WHO/TDR/PDVI laboratory network recently evaluated selected commercial ELISAs and first-generation rapid diagnostic tests, finding that ELISAs generally performed better than rapid tests.

Cross-reactivity with other circulating flaviviruses such as Japanese encephalitis, St Louis encephalitis and yellow fever, does not seem to be a problem but some false positives were obtained in sera from patients with malaria, leptospirosis and past dengue infection (*10*). These limitations have to be taken into account when using the tests in regions where these pathogens co-circulate. It is recommended that tests be evaluated against a panel of sera from relevant diseases in a particular region before being released to the market. It is not possible to use IgM assays to identify dengue serotypes as these antibodies are broadly cross-reactive even following primary infections. Recently, some authors have described MAC-ELISA (Figure 4.3) that could allow serotype determination but further evaluations are required (*19*).

Figure 4.3 Principle of a MAC-ELISA test



4.3.4.2 IgG ELISA

The IgG ELISA is used for the detection of recent or past dengue infections (if paired sera are collected within the correct time frame). This assay uses the same antigens as the

MAC-ELISA. The use of E/M-specific capture IgG ELISA (GAC) allows detection of IgG antibodies over a period of 10 months after the infection. IgG antibodies are lifelong as measured by E/M antigen-coated indirect IgG ELISA, but a fourfold or greater increase in IgG antibodies in acute and convalescent paired sera can be used to document recent infections. Test results correlate well with the haemagglutination-inhibition test. An ELISA inhibition method (EIM) to detect IgG dengue antibodies (*20*) is also used for the serological diagnosis and surveillance of dengue cases. This system is based in the competition for the antigen sites by IgG dengue antibodies in the sample and the conjugated human IgG anti-dengue.

This method can be used to detect IgG antibodies in serum or plasma and filter-paper stored blood samples and permits identification of a case as a primary or secondary dengue infection (*20,21,22*). In general, IgG ELISA lacks specificity within the flavivirus serocomplex groups. Following viral infections, newly produced antibodies are less avid than antibodies produced months or years after infection.

Antibody avidity is used in a few laboratories to discriminate primary and secondary dengue infections. Such tests are not in wide use and are not available commercially.

4.3.4.3 IgM/IgG ratio

A dengue virus E/M protein-specific IgM/IgG ratio can be used to distinguish primary from secondary dengue virus infections. IgM capture and IgG capture ELISAs are the most common assays for this purpose. In some laboratories, dengue infection is defined as primary if the IgM/IgG OD ratio is greater than 1.2 (using patient's sera at 1/100 dilution) or 1.4 (using patient's sera at 1/20 dilutions). The infection is secondary if the ratio is less than 1.2 or 1.4. This algorithm has also been adopted by some commercial vendors. However, ratios may vary between laboratories, thus indicating the need for better standardization of test performance (*8*).

4.3.4.4 IgA

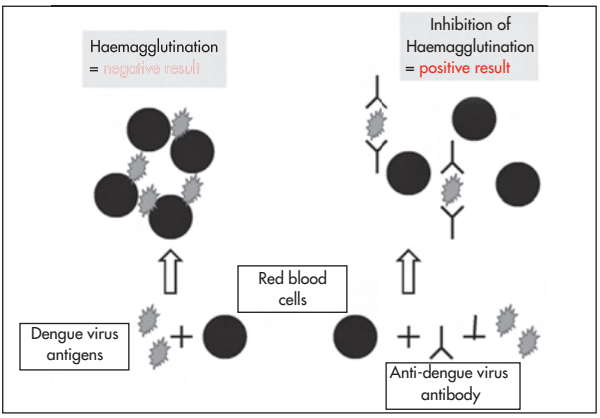
Positive detection for serum anti-dengue IgA as measured by anti-dengue virus IgA capture ELISA (AAC-ELISA) often occurs one day after that for IgM. The IgA titre peaks around day 8 after onset of fever and decreases rapidly until it is undetectable by day 40. No differences in IgA titres were found by authors between patients with primary or secondary infections. Even though IgA values are generally lower than IgM, both in serum and saliva, the two methods could be performed together to help in interpreting dengue serology (*22,23*). This approach is not used very often and requires additional evaluation.

4.3.4.5 Haemagglutination-inhibition test

The haemagglutination-inhibition (HI) test (see Figure 4.4) is based on the ability of dengue antigens to agglutinate red blood cells (RBC) of ganders or trypsinized human O RBC. Anti-dengue antibodies in sera can inhibit this agglutination and the potency of this inhibition is measured in an HI test. Serum samples are treated with acetone or kaolin to remove non-specific inhibitors of haemagglutination, and then adsorbed with gander or trypsinized type O human RBC to remove non-specific agglutinins. Each batch

of antigens and RBC is optimized. PH optima of each dengue haemagglutinin requires the use of multiple different pH buffers for each serotype. Optimally the HI test requires paired sera obtained upon hospital admission (acute) and discharge (convalescent) or paired sera with an interval of more than seven days. The assay does not discriminate between infections by closely related flaviviruses (e.g. between dengue virus and Japanese encephalitis virus or West Nile virus) nor between immunoglobulin isotypes. The response to a primary infection is characterized by the low level of antibodies in the acute-phase serum drawn before day 5 and a slow elevation of HI antibody titres thereafter. During secondary dengue infections HI antibody titres rise rapidly, usually exceeding 1:1280. Values below this are generally observed in convalescent sera from patients with primary responses.

Figure 4.4 Haemagglutination-inhibition assay



4.3.5 Haematological tests

Platelets and haematocrit values are commonly measured during the acute stages of dengue infection. These should be performed carefully using standardized protocols, reagents and equipment.

A drop of the platelet count below 100 000 per µL may be observed in dengue fever but it is a constant feature of dengue haemorrhagic fever. Thrombocytopaenia is usually observed in the period between day 3 and day 8 following the onset of illness.

Haemoconcentration, as estimated by an increase in haematocrit of 20% or more compared with convalescent values, is suggestive of hypovolaemia due to vascular permeability and plasma leakage.

4.4 FUTURE TEST DEVELOPMENTS

Microsphere-based immunoassays (MIAs) are becoming increasingly popular as a serological option for the laboratory diagnosis of many diseases. This technology is based on the covalent bonding of antigen or antibody to microspheres or beads. Detection methods include lasers to elicit fluorescence of varying wavelengths. This technology is attractive as it is faster than the MAC-ELISA and has potential for multiplexing serological tests designed to identify antibody responses to several viruses. MIAs can also be used to detect viruses.

Rapid advances in biosensor technology using mass spectrometry have led to the development of powerful systems that can provide rapid discrimination of biological components in complex mixtures. The mass spectra that are produced can be considered a specific fingerprint or molecular profile of the bacteria or virus analysed. The software system built into the instrument identifies and quantifies the pathogen in a given sample by comparing the resulting mass spectra with those in a database of infectious agents, and thus allows the rapid identification of many thousands of types of bacteria and viruses. Additionally, these tools can recognize a previously unidentified organism in the sample and describe how it is related to those encountered previously. This could be useful in determining not only dengue serotypes but also dengue genotypes during an outbreak. Identification kits for infectious agents are available in 96-well format and can be designed to meet specific requirements. Samples are processed for DNA extraction, PCR amplification, mass spectrometry and computer analysis.

Microarray technology makes it possible to screen a sample for many different nucleic acid fragments corresponding to different viruses in parallel. The genetic material must be amplified before hybridization to the microarray, and amplification strategy can target conserved sequences as well as random-based ones. Short oligonucleotides attached on the microarray slide give a relatively exact sequence identification, while longer DNA fragments give a higher tolerance for mismatches and thus an improved ability to detect diverged strains. A laser-based scanner is commonly used as a reader to detect amplified fragments labelled with fluorescent dyes. Microarray could be a useful technology to test, at the same time, dengue virus and other arboviruses circulating in the region and all the pathogens responsible for dengue-like symptoms.

Other approaches have been tested but are still in the early stages of development and evaluation. For instance, the luminescence-based techniques are becoming increasingly popular owing to their high sensitivity, low background, wide dynamic range and relatively inexpensive instrumentation.

4.5 QUALITY ASSURANCE

Many laboratories use in-house assays. The main weakness of these assays is the lack of standardization of protocols, so results cannot be compared or analysed in aggregate. It is important for national or reference centres to organize quality assurance programmes to ensure the proficiency of laboratory staff in performing the assays and to produce reference materials for quality control of test kits and assays.

For nucleic acid amplification assays, precautions need to be established to prevent contamination of patient materials. Controls and proficiency-testing are necessary to ensure a high degree of confidence (*24*).

4.6 BIOSAFETY ISSUES

The collection and processing of blood and other specimens place health care workers at risk of exposure to potentially infectious material. To minimize the risk of infection, safe laboratory techniques (i.e. use of personal protective equipment, appropriate containers for collecting and transporting samples, etc.) must be practised as described in WHO's *Laboratory biosafety manual* (*25*).

4.7 ORGANIZATION OF LABORATORY SERVICES

In a disease-endemic country, it is important to organize laboratory services in the context of patients' needs and disease control strategies. Appropriate resources should be allocated and training provided. A model is proposed in Table 4.4. Examples of good and bad practice can be found in Table 4.5.

Table 4.4 Proposed model for organization of laboratory services

Dengue diagnostic tests	Primary health centres	District centres	Reference centre
- Virus culture			+
- Nucleic acid detection			+
- Antigen detection		+	+
• ELISA		+	+
• Rapid tests	+	+	+
- Serology			
• ELISA		+	+
• Rapid tests	+	+	+
Functions			
- Training and supervision		+	+
- Quality assurance	+	+	+
- Surveillance activities		+	+
- Outbreak investigations			+
- Referral of problem specimens	+	+	+
- Investigation of problem specimens			+

Table 4.5 Dengue laboratory diagnosis: examples of good and bad practice

	Good practice	Bad practice
1. When to use a test	Consider the purpose of the testing before making a selection (e.g. for outbreak investigations versus clinical diagnosis)	Use of inappropriate test leading to misinterpretation of results
2. How to use a test	Strictly follow manufacturer's recommendations or updated Standard Operational Procedures with Good Laboratory Practices	Not following the manufacturer's recommendations Not following the written laboratory procedures for each test, or modifying the procedures without prior validation
3. Laboratory issues	System of quality management instituted in the laboratory	Results not reliable or accurate because no quality control was used in the assay, or personnel are not proficient, or equipment not calibrated Records not properly kept Use of unvalidated test kits Mixing reagents from different test kits or test lots False positive results due to contamination
	Paired serum samples should be tested in the same assay to determine if there is a four-fold rise in titre	Over-interpretation and misinterpretation of test results Acute samples not re-tested together with convalescent samples

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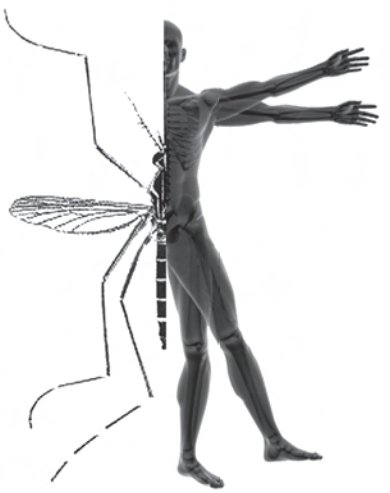

CHAPTER 5

SURVEILLANCE, EMERGENCY PREPAREDNESS AND RESPONSE

CHAPTER 5. SURVEILLANCE, EMERGENCY PREPAREDNESS AND RESPONSE

5. 1 OVERVIEW

The prevention of and response to dengue and other arboviruses involve developing and implementing preparedness plans. These should include early warning systems, epidemiological, entomological and environmental surveillance, laboratory support, clinical case management, vector control, environmental controls, risk communication and social mobilization. Sustainable solutions to dengue control require political will and leadership in order to respond effectively to the needs of preparedness planning and epidemic response (including insight into community responses to dengue, and applied research to devise, test and evaluate new approaches and technologies). National partnerships involving government bodies, research institutions and the private sector, as well as international collaborations, are needed for comprehensive plans and programmes for dengue epidemic preparedness and response.

This chapter provides an overview of the key areas of preparedness planning, epidemic detection and emergency response for dengue containment and control in endemic countries. It gives guidance to countries at risk of dengue introduction through the movement of infected persons or vectors. Strategies are suggested for preparedness, alert and response at local, national and international levels. Table 5.2 provides examples of good and bad practice for dengue surveillance.

5.2 DENGUE SURVEILLANCE

The three major components of dengue surveillance – disease surveillance, vector surveillance and monitoring of environmental and social risks – are presented below.

5.2.1 Disease (epidemiological) surveillance

Epidemiological surveillance is the ongoing systematic collection, recording, analysis, interpretation and dissemination of data reflecting the current health status of a community or population so that action may be taken to prevent or control a disease. Surveillance is a critical component of any dengue prevention and control programme as it provides the information necessary for risk assessment, epidemic response and programme evaluation. Surveillance can utilize both passive and active data collection processes. Depending on the circumstances under investigation, surveillance uses a wide variety of data sources to enhance and expand the epidemiological picture of transmission risk.

5.2.1.1 Purpose and objectives of the dengue surveillance system

The purpose(s) of the surveillance system should be explicitly and clearly described and should include the intended uses of the system. Specific objectives and performance indicators (expected results) should be set for each administrative level of the health system and for other agencies involved in dengue response within countries, as well as for international and global surveillance. The objectives of the surveillance system will guide the type of surveillance conducted, the timing of surveillance activities, the

signal(s) and thresholds for investigation and response, the specific surveillance methods used and the balance between the desired attributes of the surveillance system.

The overall objectives of public health surveillance (*1*) which are most applicable to dengue are as follows:

- detect epidemics quickly for early intervention;
- measure the burden of disease and provide data for the assessment of the social and economic impact of dengue on the affected community;
- monitor trends in the distribution and spread of dengue over time and geographically;
- evaluate the effectiveness of dengue prevention and control programmes;
- facilitate planning and resource allocation on the basis of lessons learned from programme evaluation.

In setting up a surveillance system, consideration should be given to balancing the risk of an epidemic occurring, the value of early intervention in reducing the medical, social and economic impact of the disease, and the finite resources available for investigation and control. The usefulness of the surveillance system depends on its attributes – the relative importance given to the timeliness of signal detection, and the system's sensitivity, positive predictive value (PPV) and negative predictive value (NPV) for detecting epidemics. Most importantly, the system must ensure that surveillance is linked to response and to the ability to control or limit an epidemic by:

- Timeliness is the speed with which cases or alerts are detected and reported.
- Sensitivity is the proportion of cases or alerts occurring in the geographical area or population detected by the system. Sensitivity is measured against a "gold standard", often laboratory confirmation, to determine the proportion of dengue signals detected by the surveillance system against all real or confirmed cases or alerts.
- The PPV is the probability of a dengue signal being a confirmed case or epidemic and is dependent on the background prevalence of the disease in the population.
- The NPV is the probability that the surveillance system does not yield a dengue signal when no epidemic is occurring.

The quality of data is measured by its completeness (accuracy of information, missing data, etc) and representativeness. Representativeness reflects whether the surveillance system accurately describes the distribution of cases in time (seasonality), place (geographical distribution), and the population at risk of dengue. Data may not be representative when case ascertainment is incomplete (e.g. in a sentinel surveillance system or a statistically-based sample).

The main objective of dengue disease surveillance is usually to detect and forecast epidemic activity. Surveillance activities should ideally include the surveillance of human cases of the disease, laboratory-based surveillance, vector surveillance, and the monitoring of environmental risk factors for dengue epidemics. Effective surveillance of dengue also requires the appropriate level of investment of financial and human resources,

and training and tools to ensure that increased dengue transmission is detected early and that the response is appropriate. Dengue activity may require consultation with, or notification to, WHO under the International Health Regulations (2005) (*2*), depending on the risk assessment. For instance, dengue activity may warrant communication with WHO in cases such as the first confirmation of locally-acquired dengue in a previously unaffected area, a new serotype, the importation of dengue vectors, atypical clinical presentations, and excessively high case-fatality rates.

Depending on whether epidemic detection or trend monitoring, or both, are the main purposes of the surveillance system, different system attributes should be emphasized. Where the risk of a dengue epidemic or the introduction of dengue is high, the surveillance system should aim for timeliness, high sensitivity and a low threshold for investigation, bearing in mind that a higher proportion of signals detected will prove to be false positives and that follow-up investigations are likely to be resource-intensive.

Monitoring dengue incidence and prevalence over time establishes a baseline measurement of the background rate of disease so that an unexpected rise in the number of cases or the proportion of severe cases will trigger an alert and further investigation, intervention and prevention measures. Early warning of an epidemic enables health services to allocate human and material resources more effectively, to alert clinicians to the need to diagnose and properly treat dengue cases, and to better engage the community in prevention and control activities, thereby reducing transmission and improving clinical outcomes.

5.2.1.2 Early warning systems

Early warning systems for dengue can include event-based surveillance, case-based reporting of human disease or aggregated data reporting, and/or the surveillance of risk factors for human disease.

Event-based surveillance
Event-based surveillance is designed to rapidly detect unusual or unexpected disease events (signals) such as disease clusters (e.g. of unexplained fever or acute haemorrhagic fever syndrome) and unexplained deaths, and may include events related to potential exposure for humans (e.g. climatic conditions favourable to oviposition and subsequent maturation of mosquitoes) (*3,4*). Event-based surveillance uses reports generated by the media and other open sources of information, or via key informants in the affected community, as well as reports provided through established surveillance systems. Unlike classical surveillance systems, event-based surveillance uses unstructured and ad hoc reporting of events rather than the routine collection of data and automated thresholds for action (*5*). To be effective, event-based surveillance requires that a public health unit or team is designated to sort incoming information, confirm each reported event, and trigger an immediate response, as appropriate.

Case-based surveillance and the surveillance of dengue risk factors
The utility of traditional dengue surveillance in triggering rapid response is strengthened by the availability and real-time collection, analysis and interpretation of linked clinical, laboratory and epidemiological data using established case definitions of infection and disease. The surveillance case definitions for dengue and severe dengue are currently under review.

The threshold for alert and response will vary according to the operational level affected (local, national, international) and whether the affected area is endemic for dengue or at risk of its introduction. For example, at the local level a single dengue case may trigger action. At state or provincial level, the increase in reported cases above an established baseline for the same week or month in preceding years, or increasing vector density, may indicate impending epidemic activity. At national level, the detection of changes in virus serotype, subtype or genotype distribution, clinical severity or seropositivity rate, or the introduction of a dengue vector into a new ecological niche, are signals that should be investigated without delay.

Well-defined triggers (indicators) should be identified and established before the emergency occurs in order to intensify surveillance and follow-up, and to initiate the emergency response. The following indicators for epidemic detection – which use the deviation of reported dengue cases or laboratory results from the "average" – have been documented, although these indicators have been tested mostly in one specific setting only (*6*):

- "Excess of dengue cases" has been used successfully in Puerto Rico to predict every epidemic since 1982, including those involving DEN-4 in 1982, DEN-4 in 1986-87, DEN-2 in 1994, DEN-4 in 1998 and DEN-3 in 2001.
- "Virus isolation rate in excess of routine virological testing rate" measured as the increased proportion of blood samples positive for dengue viruses in the low season for dengue has proved useful in Puerto Rico (*7,8,9*).
- The appearance of a new dengue serotype can be used as an early warning signal but does not predict the occurrence of an immediate epidemic (*10*).
- "Fever alert" – i.e. an unexpected and unusual increase in the number of fever cases – may be a useful signal of increased dengue activity in locations where the frequency and differential diagnosis of acute febrile illness is limited. A fever alert is thus useful when used to monitor febrile syndromes such as dengue, malaria, leptospirosis and West Nile fever (*11,12*).
- In specific sites, additional indicators such as the "excess of non-malaria fever cases" in malaria-endemic areas may provide complementary information and should be further evaluated. It has already been tested in French Guiana (*13*).

The routine reporting of dengue cases or dengue-like syndromes can use passive, active or enhanced surveillance. "Passive surveillance" is the routine reporting of diseases, by which notification is usually initiated by health care providers (*14*). A particular challenge of passive surveillance systems is continuously to motivate health care providers to report cases in accordance with standardized case definitions. Unfortunately, in many dengue-endemic countries, surveillance is only passive and relies on reports by local physicians who may not consider dengue in their differential diagnosis and/or fail to report cases quickly and routinely. As a result, an epidemic has often reached or passed its peak before it is recognized, and opportunities for control are missed. Designating dengue as a notifiable disease that is covered by public health legislation is one way to increase compliance with reporting.

In "active surveillance", the disease report is initiated by the health authority which systematically asks health care providers about the disease of interest. This involves outreach by public health authorities to stimulate reporting; examples include regular telephone calls or visits to laboratories and hospitals to collect data in a timely fashion.

"Sentinel surveillance", or the study of disease rates in a specific cohort, geographic area or population subgroup, is a form of active surveillance that is useful for answering specific epidemiological questions. Sentinel surveillance may be very effective at determining disease trends locally, but because sentinel sites may not represent the general population or the general incidence of dengue, they may have limited value in determining national disease patterns and trends. Sentinel surveillance may also be used to detect a "first case" (as the word "sentinel" suggests), such as testing for dengue in a neurology ward to detect cases of dengue-related neurological disease, or post-mortem testing for dengue-related deaths. A system to monitor the severe end of the disease spectrum is, therefore, an important component of an early warning system to detect epidemic activity. Detailed clinical protocols should be developed to ensure that adequate clinical, haematological and pathological data and samples are obtained. Depending on laboratory capacity, the type of samples collected and the circumstances of specimen collection, storage and transport, samples may also be processed for virus isolation and/or viral RNA by PCR.

"Enhanced surveillance" is a form of active surveillance usually in response to an epidemic alert. The passive surveillance system is "enhanced" so that it becomes active during the alert period and/or it is extended to municipalities or jurisdictions that are beyond the affected area and that would not normally be included in routine surveillance. Table 5.1 presents examples of dengue surveillance systems and the types of specimens and laboratory tests required.

5.2.1.3 Laboratory support for surveillance

The effective prevention and control of epidemic dengue requires an active laboratory-based disease surveillance programme that can provide early warning of impending epidemic transmission. Laboratory methods should include both serological and virological diagnosis. Quality control for laboratory diagnostics should be provided by a national dengue reference laboratory and/or an international reference laboratory. At a minimum, diagnostic laboratories should be capable of performing IgM antibody-capture ELISA (MAC-ELISA) as a front-line screening test for current or recent dengue infection. Confirmatory tests are needed to exclude cross-reactivity with other flaviviruses, and because IgM will not be detectable in the first few days after onset of illness (especially in primary dengue infections) and may persist for several weeks after infection (Chapter 4)

Sentinel laboratory surveillance systems provide high-quality virological and serological data and are useful for detecting rare diseases (e.g. introduced dengue), but data that are not linked to clinical and epidemiological information must be interpreted with caution. Data that may be provided include: participating laboratory identifier, onset of illness, specimen collection date, patient identifier, organism, patient's sex, date of birth or age, place of residence, specimen source, clinical diagnosis and the method of diagnosis. There are certain biases inherent in de-linked laboratory surveillance data (e.g. the number of participating laboratories may vary over time and some jurisdictions may have a larger number of participating laboratories than others). Also, changes in diagnostic practices – particularly the introduction of new testing methodologies – may affect laboratory reports. The ability of laboratory tests to distinguish acute infection from past infection without a clinical history must also be taken into account when interpreting the data. Although changes in incidence cannot be determined with precision from such data, general trends can be observed (e.g. with respect to seasonality and the age-sex distribution of dengue cases).

Table 5.1 Components of dengue surveillance[a,b]

Type of surveillance	Samples[b]	Approach
Event-based surveillance	Blood from all or representative cases of dengue-like illness, taken 3–15 days after onset of illness	Once the details of the reported event are validated, a rapid response team investigates the event as an outbreak Cases detected through active case-finding for acute febrile illness and/or laboratory confirmation during outbreak investigations can also constitute an event.
Fever alert system	Blood samples from representative cases of acute febrile illness	Increased febrile illness in the community is investigated immediately; samples are tested as above.
Routine reporting (passive surveillance)	Blood from clinical cases of dengue-like illness	Highly dependent on clinicians considering dengue in the differential diagnosis and on the availability of diagnostic support Ideally, clinical, laboratory and epidemiological data are linked. Reporting is based on case definitions and case classification (e.g. laboratory-confirmed, probable, or suspected cases).
Sentinel clinic and physician network	Blood from all or representative cases of dengue-like illness, taken 3–15 days after onset of illness	Representative samples or purposeful sampling taken year-round or at specific times of the year are processed weekly for IgM antibodies, and for virus isolation and/or PCR if available.
Sentinel hospital surveillance system	Blood and tissue samples taken during hospitalization and/or at death	According to the outcome of interest, all severe disease and all viral syndromes with fatal outcome are investigated immediately and tested as above.
Sentinel laboratory-based surveillance	Blood and other clinical samples	Usually there are no denominator data. Hence changes in incidence cannot be determined. General trends can be observed if the same laboratories report regularly (e.g. with respect to seasonality and the age and sex distribution of patients).
National notifiable disease surveillance system	Blood from cases of dengue-like illness and/or clinical samples from cases that test positive for dengue	Mandatory reporting of, for instance, laboratory-confirmed cases by time, place, person ± risk factor data. May also include probable and suspected dengue cases, depending on the sensitivity of the surveillance system.

[a] Source: adapted from Gubler (*15*)
[b] Emphasis should be placed on the inter-epidemic period using a simplified case definition. After an epidemic begins and after the virus serotype(s) is known, the case definition should be made more specific and surveillance should be focused on severe disease.

5.2.2 Entomological surveillance

5.2.2.1 Overview

Entomological surveillance is used for operational (and research) purposes to determine changes in geographical distribution of vectors, for monitoring and evaluating control programmes, for obtaining relative measurements of the vector population over time, and for facilitating appropriate and timely decisions regarding interventions. There are a number of methods for monitoring dengue vectors (mostly for *Ae. aegypti*). However, the selection and use of method requires a clear understanding of the surveillance objectives, the availability of skills and resources, and in some instances the level of infestation.

Surveillance may serve to identify areas of high-density infestation or periods of mosquito population increase. In areas where the vector is no longer present, entomological surveillance is critical in order to detect new introductions rapidly before they become widespread and difficult to eliminate. Monitoring of the vector population's susceptibility to insecticide should also be an integral part of any programme that uses insecticides.

This section describes a variety of sampling methods but by no means provides an exhaustive list of all available methods.

5.2.2.2 Sampling larvae and pupae

For reasons of practicality and reproducibility, the most common survey methodologies employ larval (active immatures, including pupae) sampling procedures rather than egg or adult collections. The basic sampling unit is the house or premise, which is systematically searched for water-holding containers.

Containers are examined for the presence of mosquito larvae, pupae, and larval and pupal skins. Depending on the objectives of the survey, the search may be terminated as soon as aedine larvae are found, or it may be continued until all containers have been examined. Laboratory examination is usually necessary to confirm the species. The following three indices are commonly used to record *Ae. aegypti* infestation levels:

- House (premise) index (HI) – i.e. percentage of houses infested with larvae and/or pupae.

$$HI = \frac{\text{Infested houses X 100}}{\text{houses inspected}}$$

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

$$CI = \frac{\text{Containers positive X 100}}{\text{containers inspected}}$$

- Breteau index (BI) – i.e. number of positive containers per 100 houses inspected.

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

The house index has been used most widely for measuring population levels, but it does not take into account the number of positive containers or the productivity of those containers. Similarly, the container index provides information only on the proportion of water-holding containers that are positive. The Breteau index establishes a relationship between positive containers and houses, and is considered to be the most informative, but again there is no accommodation of container productivity. Nevertheless, in the course of gathering the basic information for calculating the Breteau index, it is possible (and highly desirable) to obtain a profile of the larval habitat characteristics by simultaneously recording the relative abundance of the various container types either as potential or actual sites of mosquito production (e.g. the number of positive drums per 100 houses, the number of positive tyres per 100 houses). These data are particularly relevant for focusing larval control efforts on the management or elimination of the most common habitats and for the orientation of educational messages for community-based initiatives (see also the section below on pupal/demographic surveys). A problem here is that the most common container (e.g. the drinks bottle) is often not the most productive (*16*).

It should be noted that larval indices are a poor indication of adult production. For instance, the rate of emergence of adult mosquitoes from rainwater drums is likely to differ markedly from the rate from discarded cans or house plants, yet the larval survey registers them only as positive or negative. The implication is that for localities with similar larval indices but different container profiles, adult abundance and hence transmission potentials may be quite different.

5.2.2.3 Pupal/demographic surveys

If the classes of containers with the highest rates of adult mosquito emergence are known in a community, their selective targeting for source reduction (e.g. elimination) or other vector control interventions can be the basis for the optimized use of limited resources (*17*). The pupal/demographic survey is a method of identifying these most epidemiologically important types of containers and may therefore be considered an operational research tool (Chapter 3). Unlike the traditional *Stegomyia (Aedes)* indices described above, pupal/demographic surveys measure the total number of pupae in different classes of containers in a given community. Such surveys are far more labour-intensive than the above-mentioned larval surveys and are not envisaged for the routine monitoring of *Ae. aegypti* populations.

The collection of demographic data enables the calculation of the ratio between the numbers of pupae (a proxy for adult mosquitoes) and persons in the community. There is growing evidence that, together with other epidemiological parameters such as dengue serotype-specific seroconversion rates and temperature, it is possible to determine how much vector control is needed in a specific location to inhibit virus transmission. This remains an important area for research with potential for public health application. Similar methods have been used to measure total populations of larvae.

5.2.2.4 Passive collection of larvae and pupae

Funnel traps have been used for sampling *Aedes* species and other container-breeding organisms in sites with poor or difficult access, such as wells (*18*). The funnel trap comprises a weighted funnel attached to a bottle that inverts on entry to and exit from the water surface where it floats. The device collects organisms such as fish, copepods,

mosquitoes, ostracods and tadpoles as they return to the surface. Calibration of the device, using known numbers of *Ae. aegypti* larvae, enables the size of the larval population to be estimated (*19*). In some locations the device has focused attention on the importance of subterranean habitats and harbourages during winter or in dry conditions (*20*). The funnel trap captures a lower proportion of pupae because they are less active than larvae.

Quantification of the funnel trap allows results to be compared with larval counts in other containers and allows estimates to be made of the relative importance of the various types of containers. However, there is no way to relate funnel trap captures to the risk of transmission because there is no direct relationship between larval densities and density-dependent larval survival.

5.2.2.5 Sampling the adult mosquito population

Adult vector sampling can provide valuable data for studies of seasonal population trends or evaluation of adulticiding measures. However, results are less reproducible than those obtained from sampling of immature stages. The methods for collection of adult mosquitoes also tend to be labour-intensive and depend heavily on the collector's proficiency and skill.

5.2.2.6 Landing collections

Although landing collections on humans are a sensitive means of detecting low-level infestations and for studying the biting times and places of host attraction, the method is both labour-intensive and expensive. Moreover, the method poses safety and ethical issues in areas endemic for disease. Both male and female *Ae. aegypti* are attracted to humans. Because adult mosquitoes, especially males, have low dispersal rates, their presence can be a reliable indicator of proximity to hidden larval habitats. Rates of capture, typically using hand nets or aspirators as mosquitoes approach or land on the collector, are usually expressed in terms of landing rates per man-hour.

5.2.2.7 Resting collections

During periods of inactivity, adult *Ae. aegypti* typically rests indoors, especially in bedrooms, and mostly in dark places such as clothes closets and other hidden sites. Resting collections involve the systematic searching of these sites with the aid of a flashlight and the capture of adults using mouth- or battery-powered aspirators and hand-held nets. Backpack aspirators powered by rechargeable 12-volt batteries have proven to be an efficient and effective alternative means of collecting resting adult mosquitoes in and around human habitation. Following a standard collection routine, densities are recorded as the number of adult mosquitoes per house (females, males, or both) or the number of adults per man-hour of effort. Where infestation levels are low, the percentage of houses positive for adults is sometimes used.

5.2.2.8 Sticky trap collections

Various sticky trap devices have been used for sampling adult *Ae. aegypti*. They may be designed to be visually attractive, odour-baited, or both, or are simply located at constricted access points through which adult mosquitoes pass (e.g. at points of exit and entry from subterranean habitats such as keyholes in service manhole covers in roads).

Age and viral infection have been determined in adult mosquitoes collected with sticky traps though mainly in a research context.

5.2.2.9 Sampling the ovipositing population

Oviposition traps
These devices, also known as "ovitraps", constitute a sensitive and economical method for detecting the presence of *Ae. aegypti* and *Ae. albopictus* in situations where infestations are low and larval surveys are generally unproductive (e.g. when the Breteau index is <5). They have proved especially useful for the early detection of new infestations in areas from which the mosquito has been eliminated. For this reason, oviposition traps are useful for surveillance at international ports of entry which, in accordance with international sanitary codes, should be kept free of vector foci.

The standard ovitrap is a wide-mouth 0.5 litre glass jar painted black on the outside and equipped with a hardboard or wooden paddle that is clipped vertically to the inside with its rough side facing inwards. The jar is partially filled with clean water and is appropriately placed in a rain-sheltered site – usually outdoors and close to habitation.

Ovitraps are usually serviced weekly and the paddles are examined for the presence of *Ae. aegypti* eggs. The percentage of positive ovitraps provides the simplest index of infestation levels. In more detailed studies, all the eggs on each paddle are counted and the mean number of eggs per ovitrap is calculated. Ovitraps with plant germination paper as a substrate for egg deposition can also be used. For accurate interpretation, field records must indicate the location of each ovitrap and its condition at the time of servicing. If a trap is flooded, dry, missing, or overturned, the data should be discarded. Ovitraps are inexpensive and it is possible to install and service them over large areas relatively quickly. They can also be used by people without specialized training.

An "enhanced CDC ovitrap" is considerably more attractive to ovipositing females and yields many more *Ae. aegypti* eggs than the standard version. In this double ovitrap method, one jar contains an olfactory attractant made from a "standardized" 7-day-old hay infusion, while the other contains a 10% dilution of the same infusion. Unlike the original version, with which positivity rates and egg counts are seldom sufficiently high, the enhanced ovitrap has proved suitable for monitoring changes in the adult female populations daily rather than weekly and has been successfully used to assess the impact of adulticidal space spraying on adult females. Alternatives to hay infusions have also been used to improve the attraction of standard ovitraps.

While ovitraps can be used to monitor changes in oviposition activity over time, comparisons between areas are not reliable because the availability of larval habitats in which females can choose to lay eggs will differ. Similarly, it can be misleading to monitor and interpret ovitrap data over time in a given area where vector control interventions include source reduction measures.

Tyre section larvitraps

Tyre section larvitraps of various designs have also been used for monitoring oviposition activity. The simplest of these is a water-filled radial section of a tyre. A prerequisite for any tyre section larvitrap is that it facilitates either visual inspection of the water in situ or the ready transfer of the contents to another container for examination. Tyre larvitraps differ functionally from ovitraps in that water level fluctuations caused by rainfall induce the hatching of eggs, and it is the larvae that are counted rather than the eggs deposited on the inner surfaces of the trap. The usefulness of tyre section larvitraps as an alternative to the ovitrap for early detection of new infestations and for surveillance of low-density vector populations has been well demonstrated.

Insecticide susceptibility testing

The initial and continued susceptibility of the vector to specific insecticides is of fundamental importance for the success of larviciding or adulticiding operations. The development of resistance may lead to failure of the control programme unless it is carefully monitored and a timely decision is made to use alternative insecticides or control strategies.

Standard WHO bioassay procedures and kits are available for determining the susceptibility or resistance of mosquito larvae and adults to insecticides. Biochemical and immunological techniques are also available for testing individual mosquitoes but are not widely used by programmes.

Sampling strategies

Only in exceptional conditions are larval surveys of every house (i.e. census) warranted. Such situations arise when the objective is one of vector eradication and larval infestation levels have been reduced to very low levels (HI = <1.0%). At this point it is necessary to locate and control every infested and potentially infested container, or to verify that eradication has indeed been achieved, or to ensure that re-infestation has not occurred. In other situations, the number of houses to be inspected should be based on considerations of available resources, the desired level of precision of the results, and the total number of houses in the locality. This is contrary to the routine procedures employed in many vector control programmes in which eradication campaign methodologies have persisted and entomological data are collected from every house immediately prior to insecticide treatment – usually by the person who administered the treatment. Such practices, if used solely for measuring infestation levels, are wasteful and are likely to result in poor-quality reporting due to conflicts of worker interest and the tedious nature of the work. Whenever possible, it is recommended that a different team or individual should conduct the entomological evaluation, or that the two tasks should be performed separately. The sample size for routine surveys can be calculated by statistical methods based on the expected level of infestation and the desired level of confidence in the results.

Several sampling procedures that eliminate or minimize bias can be applied equally well to the selection of houses for larval, adult, ovitrap, or knowledge-attitude-practice (KAP) surveys. These are as follows:

- Systematic sampling applies to every "n"th house (where "n" equals an agreed number) throughout a community or along linear transects through the community. For example, if a sample of 5% of the houses is to be inspected, every 20th house (= 100/5) would be inspected. This is a practical option for rapid assessment of infestation levels, especially in areas where there is no house-numbering system. All areas of the locality are well represented.

- Simple random sampling means that houses to be selected are obtained from a list of random numbers (either from tables of random numbers in a statistical text book or from a calculator or a computer-generated list). This is a more laborious process since detailed house maps or lists of street addresses are a prerequisite for selecting the houses. Many statistical tests require random sampling. Unfortunately, although every house has an equal chance of being selected, some areas of the locality are usually under-represented and others are over-represented.
- Stratified random sampling minimizes the problem of under-representation and over-representation by subdividing the localities into sectors or "strata". These are usually based on identified risk factors – such as areas with houses without a piped water supply, areas not served by sanitation services, and densely-populated areas. A simple random sample is taken from each stratum, with the number of houses inspected being in proportion to the number of houses in each stratum.
- Cluster sampling may be conducted in large cities or geographical areas where it may be difficult or impossible to use random or systematic sampling because of limitations of time, money and personnel, or because of other logistical constraints. In these circumstances, the sample may be selected in two stages in order to minimize the resources needed for the survey. The first stage is obtained by simple or stratified random sampling of population groups or clusters (e.g. city blocks, villages, or administrative districts). Having identified these clusters, simple or stratified random sampling procedures are again applied to identify the specific houses within each cluster for inclusion in the survey.

5.2.2.10 Frequency of sampling

The frequency of sampling depends on the frequency and expected duration of the control measures. For programmes that use larvicides, it is important to monitor the duration of efficacy to ensure that intervals between cycles of treatment are optimal. This may vary from weeks to months. For programmes using integrated strategies, such frequent intervals for routine assessment of the impact of the measures applied may be unnecessary. This is especially true when the effect of some of the nonchemical intervention strategies exceed the impact of residual insecticides (e.g. larvivorous fish in large storage containers of potable water, source reduction, or mosquito-proofing of containers). On the other hand, rapid feedback at monthly intervals is desirable to evaluate and guide community action activities, indicating sectors that need more attention and activities that need to be reinforced. For specific research studies, it may be necessary to sample on a weekly, daily or even on an hourly basis (e.g. to determine the diel pattern of host-seeking activity).

5.2.3 Monitoring environmental and social risks

In addition to the evaluation of aspects directly pertaining to vector densities and distribution, community-oriented, integrated vector management strategies require that other parameters be measured or periodically monitored.

Various factors have been determined to influence a community's vulnerability to dengue epidemics. The distribution and density of the human population, settlement characteristics, conditions of land tenure, housing styles, education, and socio-economic

status are all interrelated and fundamentally important for planning and for assessing dengue risk. Knowledge of changes in the distribution of water supply services and their quality and reliability over time, as well as knowledge of domestic water storage practices and solid waste disposal services, are of particular relevance. This type of information helps in establishing ecological profiles that can be of value for planning targeted source reduction or management activities and for organizing epidemic intervention measures.

Some of these data sets are generated by the health sector, while others are derived from external sources. In most cases, annual or even less frequent updates will suffice for programme management purposes. However, in the case of meteorological data, especially rainfall patterns, a more frequent analysis (such as weekly or monthly) is warranted if the data are to be of predictive value in determining seasonal trends and short-term fluctuations of the vector population.

5.3 DENGUE PREPAREDNESS PLANNING AND RESPONSE

5.3.1 Overview

This section provides an overview of the elements of a comprehensive dengue prevention and control programme. Emergency preparedness and anticipated response (contingency) planning must be explicitly included in dengue surveillance and control policies and should be reviewed regularly. Emergency preparedness and response are often overlooked by programme managers and policy-makers.

When the dynamics of dengue activity are known, the timing of response activities can be adjusted to maximize their effectiveness. In dengue-endemic areas, activities can be grouped into those that should occur continuously, those that should occur during an epidemic, and those that should be carried out in the post-epidemic period. Different activities and approaches may be required in areas where dengue occurs sporadically and in dengue-free areas at risk of transmission. In epidemic-prone areas, a multisectoral dengue action committee should be convened with the responsibility of coordinating the response.

The two major components of the emergency response to a dengue outbreak are: (i) emergency vector control to curtail transmission of the dengue virus as rapidly as possible and (ii) early diagnosis and the appropriate clinical case management of severe dengue to minimize the number of dengue-associated deaths. These responses should occur concurrently.

5.3.2 Dengue emergency response planning

There are three levels of dengue emergency response planning *(21)*, namely:

- Ongoing prevention – where there is no current dengue activity in the area;
- Response to sporadic cases or other risk indicators – where there is no epidemic dengue activity in the area but either (i) sporadic cases are being reported through the surveillance system or (ii) there is increased virus activity or the introduction of serotypes that have not previously or recently been recorded in the area;

- Outbreak response – where there is epidemiological evidence of epidemic activity. The best response to a dengue epidemic is preparedness to prevent infections, thereby reducing ongoing transmission, and to minimize its effects on those who become ill.

The dengue emergency response plan should clearly articulate its aims, objectives and scope, the lead (coordinating) agency, the organizational links with other agencies that have direct responsibility for implementing aspects of the plan, and the "support" agencies (e.g. social welfare) that may be more involved in the recovery phase after an epidemic. Each agency should be assigned specific roles and responsibilities under the plan, and costs and resources should be identified. Hard copies of the plan should be distributed to all response and supporting agencies and should be available electronically if possible. The plan should ideally include a monitoring and evaluation framework with performance indicators for each of the response and support agencies as well as overall indicators of the plan's effectiveness.

Clear triggers for the activation, escalation and deactivation should be written into the plan and reviewed as required. Wherever possible, the plan should be tested through exercises.

Some emergency response plans emphasize the temporal aspects of the response (i.e. the alert phase, declaration of an emergency, and the emergency phase). As mentioned earlier in this chapter, there may be reluctance on the part of public health officials to implement a dengue emergency response plan until the case count and fatality numbers are elevated. It is recommended that the plan should include objective criteria for defining an epidemic on the basis of specific local data and not general concepts.

While plans have frequently been prepared in dengue-endemic countries, they are seldom validated. Once the dengue emergency response plan has been drafted and approved by participating groups, it is important to conduct simulations or "table top" exercises. Since the emergency response is usually multisectoral, exercises provide opportunities for all partners to participate and better understand their roles, responsibilities, channels of command and communication, and to ensure the availability of the human resources, equipment and supplies needed for a rapid emergency response. Formal debriefing sessions should take place with the partners after exercises and after epidemics. Lessons learned should be incorporated into a revised emergency response plan.

5.3.2.1 Priority areas for emergency response plans

Rigau-Pérez and Clark (*7*) have identified 10 priority areas for dengue emergency response planning, namely:

1. Establishing a multisectoral dengue action committee.
2. Formalizing an emergency action plan.
3. Enhancing disease surveillance.
4. Diagnostic laboratory testing.
5. Enhancing vector surveillance and control.
6. Protecting special populations.
7. Ensuring appropriate patient care.

8. Educating the community and relevant professional groups about the current procedures used for dengue control by the responsible authorities in their jurisdiction (local, provincial and national governments, as appropriate) as well as their roles and responsibilities in dengue prevention and control.
9. Investigating the epidemic.
10. Managing the mass media.

For endemic countries, the overall aim of a dengue emergency response plan is to reduce the risk of dengue epidemics and to strengthen control measures for any future epidemics – thereby minimizing the clinical, social and economic impact of the disease.

For receptive countries (i.e. dengue vectors present without circulating virus), risk management plans should focus on strategies for risk reduction. These should include rapid investigation of sporadic cases (clinically suspected or laboratory confirmed) to determine whether they are imported or locally-acquired, monitoring of vectors and their abundance (particularly in regions with recorded or suspected cases), social mobilization, and environmental management efforts. Once a locally acquired case is confirmed, the response may be escalated to epidemic response to prevent further spread and/or interruption of transmission.

In countries at risk of the introduction of dengue vectors, the focus of activities may be on entomological surveillance at ports of entry and education of the health care community about the risk of dengue in travellers, and its diagnosis and reporting requirements.

5.3.2.2 Establishment of a multisectoral dengue action committee

If the prevention and control of dengue are to be effective and sustainable, a multisectoral, multidisciplinary and multilevel approach is required. It is not possible for a single government agency to control the causes and consequences of dengue epidemics and to protect population health. For this reason it is recommended that countries establish a multisectoral dengue action committee. The committee must have solid funding and a designated national coordinator with the political mandate to make policy and financial decisions and to coordinate the multisectoral preparedness and response strategy at local, state and national levels.

Depending on the epidemiological situation in each country, membership of the dengue action committee may include, but need not be limited to, heads of government agencies (e.g. health, ambulance services, agriculture, emergency services, customs and immigration, port health authorities, telecommunications, media, education, environment, water, solid waste disposal) and NGOs, and must also include relevant private-sector groups (e.g. industrial, commercial, private education, labour unions). For countries at lower risk of dengue transmission, the dengue action committee may be constituted within the affected jurisdiction (e.g. local government area, state, province). A major responsibility of the dengue action committee is to develop the dengue emergency response plan, review it regularly, and update it as necessary on the basis of the lessons learned from its implementation or simulation. Members of the committee should communicate regularly with their stakeholders – including local government, health care providers in the public and private sectors, vector control personnel, laboratory scientists, industry groups and community representatives.

5.3.3 Risk assessment

5.3.3.1 Populations at risk

Because of their rapid expansion and long duration, dengue epidemics reduce the productive capacity and economic development of many sections of society. Some groups need special attention because of their dependence on others who take care of them and their immediate surroundings (e.g. older or incapacitated persons who live alone or in institutions, travellers, pre-school children in day care programmes, students, migrant workers and soldiers). If not properly screened or air-conditioned, health care settings may also be at high risk for dengue transmission.

Migrant workers living in poorly constructed and maintained facilities can be particularly at risk of the transmission or introduction of dengue.

A study on knowledge of and attitudes towards dengue, conducted in the Caribbean by the Assessments of Impacts and Adaptations to Climate Change (AIACC) project, indicated that the most vulnerable to the disease were the poor who lived in informal or squatter settlements. These typically lacked basic community infrastructure, including access to piped water and adequate garbage disposal, and lacked both the organization needed for collective action against the threat of a dengue fever epidemic and the understanding about how they could contribute to preventive actions to mitigate the risk (*22*). Communication for Behavioural Impact (COMBI) for dengue prevention and control needs to be tailored to the various populations at risk and delivered in a culturally appropriate way to ensure sustainability (*23*).

5.3.3.2 Cross-border spread and ports of entry

The rapid spread of dengue since the 1970s has been attributed to increasing urbanization and the increasing use of (often disposable) man-made containers (*24*) that are ideal larval habitats for dengue mosquitoes. In addition, international trade in, and inadequate disposal of, vehicle tyres similarly provide larval habitats, and international air travel results in the movement of viraemic individuals and vectors over long distances. Increased international travel and trade provide ideal means for infected human transport of dengue viruses and/or vectors, resulting in a frequent exchange of dengue viruses among endemic countries, the risk of dengue introduction to receptive areas, and the spread of vectors into new ecological niches. The international movement of human cases, vectors which carry infection, or goods that are contaminated, may cause international disease spread and require notification to WHO under Article 9 of the 2005 International Health Regulations (*2*).

In areas that are highly receptive to dengue, a single imported case can start an epidemic. Because of the risk of a viraemic traveller initiating an outbreak, surveillance for clinical cases of dengue is very important since it enables action to be taken promptly to reduce the risk of local transmission.

Countries should undertake a dengue risk assessment as the basis for preparedness plans. Dengue-related risks will vary considerably both within and between countries. Risk assessment is conducted so that each country can match the level of risk (e.g. risk of epidemics or risk of virus introduction) with appropriate activities and investment and

thus avoid introducing costly and demanding measures that are not justified by the epidemiological situation.

5.3.3.3 Vector ecology

As part of their risk assessment, countries at risk of dengue should conduct entomological investigations to identify and map the distribution of potentially competent dengue vectors.

5.3.3.4 Ensuring appropriate patient care

A case of severe dengue requires careful observation and repeated laboratory tests throughout the illness (Chapters 2 and 4). It is imperative that medical and nursing staff understand the rationale and priorities for patient care under epidemic conditions.

The principal burden that dengue epidemics create for affected countries is not the number of deaths but the enormous number of hospitalizations and days of illness. Providing care for an elevated number of dengue cases requires criteria for triage, trained physicians and nursing personnel, beds, supplies and equipment, and training guidelines for treatment and patient isolation. Isolation refers not only to routine precautions for manipulation of blood and other body fluids but also to the use of (insecticide-treated) mosquito nets to prevent mosquitoes from biting viraemic (febrile) patients and subsequently spreading the virus within the community. It is essential to train professionals in the early detection of cases and to educate the community to seek medical attention when dengue symptoms appear. Planning for sufficient provisions during a dengue epidemic can be guided by hospitalization rates in previous outbreaks.

5.3.3.5 Communication for Behavioural Impact and Risk Communication

Primary prevention is the most effective measure in dengue prevention and control since no vaccine is currently available. An intensive COMBI programme (*23*) that ensures accurate and timely information for the public should be implemented concurrently with vector control activities in order to engage the community in practices that reduce dengue transmission.

A weakness of some current dengue prevention strategies is that they are reactive rather than anticipatory. They may often be implemented late, thus reducing the opportunities for preventing transmission and controlling the epidemic. In general, such reactive strategies lead only to short-term behavioural change and fail to institutionalize the idea of community and personal responsibility for dengue prevention and control in partnership with government efforts.

Public education must continue to reinforce how important it is for people to seek medical attention if they have dengue symptoms, and should stress the need to reduce larval habitats and the options for personal protection.

Epidemics are frequently marked by uncertainty, confusion and a sense of urgency. During an epidemic the aim of public risk communication, generally through the media, is to build trust. It does this by announcing the epidemic early, providing accurate information, communicating openly and honestly with the public (transparency), and

providing specific information about what people can do to make themselves and their community safer. This gives people a sense of control over their own health and safety, which in turn allows them to react to the risk with more reasoned responses (*25*). In endemic countries, involving the media before the occurrence of the seasonal increase in dengue enhances the opportunity to increase public awareness of the disease and of the personal and community actions that can be taken to mitigate the risk.

5.3.4 Identifying outbreaks and triggers for an epidemic response

As indicated in previous sections, the more sensitive, extensive, responsive and functional the dengue surveillance system, the better will be the information that is generated. Information about an impending increase in the incidence of dengue will provide valuable time to make final preparations and implement the contingency plan. The public health community is increasingly observing the implementation of emergency responses to diseases such as avian influenza in preparation for pandemic influenza. These can serve as examples for the preparation of dengue contingency plans.

In areas where a comprehensive surveillance system exists, detailed information about when and where dengue outbreaks/epidemics occurred in the past can be a useful guide to the potential magnitude and severity of future epidemics. If there is no surveillance system in place, the "early warning" element of the programme and the valuable time needed to make preparations for the response are lost. In such a situation, the emergency may rapidly overwhelm the public health and medical care agencies without warning.

5.4 PROGRAMME ASSESSMENT

Dengue programme assessment is part of risk assessment. It aims to identify strengths and weaknesses in programmatic and public health infrastructure that can reduce or increase vulnerability to respond to dengue. Programme assessment should review areas such as the dengue emergency response plan, human resource planning (including training), the effectiveness of the dengue surveillance system in providing early warning, the effectiveness of the vector control programme, laboratory capacity, stockpiling and applied research needs (*26*). In order to develop a meaningful preparedness plan during the inter-epidemic period, it is important to estimate the population at risk, expected admission rates, the equipment, supplies and personnel required for vector control and patient management, and to document the location of resources.

5.4.1 Human resource planning

In addition to human resource planning for surge situations such as epidemics, the education of health care workers should draw attention to the importance of laboratory testing and case notification. Many health care providers fail to report dengue activity until they receive a positive laboratory diagnosis because they are unaware that prompt vector control measures can be initiated as soon as suspected cases are notified. Some doctors may not request tests during an outbreak because they may be confident of their ability to diagnose dengue clinically. Other doctors may not be aware of the value of laboratory confirmation or may not be familiar with the tests available, the timing of investigations, and the problems inherent in interpreting laboratory results when more

than one flavivirus is circulating or the patient has been exposed to dengue or other flaviviruses in the past.

Physicians, nurses and laboratory staff should receive regular clinical training in the management of dengue patients. This must include specific training on aspects of emergency response.

Clinicians' education in the emergency response to a dengue epidemic relates principally to raising awareness of the spectrum of disease and the essentials and complexities of treatment. It is important to emphasize that treatment of dengue consists of appropriate hydration and the administration of paracetamol/acetaminophen to control pain and fever (never acetylsalicylic acid [aspirin]). Physicians must be able to distinguish between typical and atypical dengue syndromes, and access to laboratory diagnosis is useful. Contact should be made with national and regional reference laboratories for diagnostic support and verification functions prior to the occurrence of an epidemic. Epidemics also offer unique opportunities to identify risk factors for the disease through applied research.

Clinicians are often the first point of contact for community education and social mobilization. From initial contact with medical and public health personnel, the patients and their household members should be given orientation in eliminating adult mosquitoes and water-holding containers in and around their residence to avoid mosquito bites.

5.4.2 Assessment of the surveillance system

Dengue surveillance systems should be adapted to country needs and resources. Priority should be given to: (i) improving routine reporting using standardized case definitions, (ii) improving laboratory support for diagnosis through greater access to laboratory services, standardized test procedures, access to more sophisticated diagnostics for virus tracking and quality assurance, and (iii) introducing active surveillance as a complementary measure. At all times, appropriate and timely data analysis and response at the lowest operational level possible are crucial, as is data sharing in the system. This may mean that core capacities in surveillance and response need to be developed in accordance with the International Health Regulations (2005) (*2*). Reporting of dengue should be consistent both within and between countries, and reported cases should have a defined severity level (Chapter 1). Once a vaccine becomes available, effective disease surveillance will be critical in determining priority areas for vaccine use and evaluating vaccine effectiveness in reducing dengue incidence.

5.4.3 Assessment of diagnostic resources

A detailed description of the laboratory diagnosis of dengue is provided in Chapter 4. Resource planning and assessment should include auditing of, for instance: the availability of diagnostic laboratories and staff trained in dengue diagnosis, verification and reference functions; reagents and laboratory equipment (including stockpiling for epidemics); quality assurance; and the continuous application of appropriate levels of biosafety. The capacity to perform dengue virus genotyping, whether locally or at an international dengue reference laboratory, is particularly important for tracking genetically the potential origins of outbreaks and the international spread of the virus.

5.4.4 Assessment of resources for clinical care

Chapter 2 summarizes current international best practice for the clinical case management of dengue. Hospital emergency response plans for dengue should include a detailed resource plan that covers anticipated hospital bed occupancy, including high-dependency care beds, staffing levels and surge capacity needs, and stock management (intravenous fluids, drug supplies etc). Similar assessments should be conducted for outpatient care in the public and private sectors in dengue-endemic areas.

5.4.5 Assessment of vector control resources

The adequacy of equipment for insecticide application and the availability of insecticides should be part of the needs assessment of the vector programme.

5.4.6 Information products

Successful dengue control programmes have integrated vector control with public health education and community involvement in risk reduction activities. Public education on dengue is often promoted through posters in a variety of public places, including public transport facilities, advertisements in newspapers, dengue messages on radio and other media channels, and through face-to-face communication. Affected communities are encouraged to eliminate containers of stagnant water that can potentially provide larval habitats in homes and neighbourhoods. Grassroots groups, community volunteers, schools and the private sector should be updated regularly for active engagement of the public in dengue prevention and control.

Personal protective measures include the application of topical repellents for the skin (personal insect repellents), use of household aerosol insecticides, or use of insecticide-treated bednets for persons sleeping during the daylight hours. However, personal protective measures come at a cost, thereby limiting their possible use by the poorest, who are the most vulnerable.

Public education must also continue to reinforce the importance of people seeking medical attention if they have dengue symptoms.

Table 5.2 Dengue surveillance: examples of good and bad practice

	Good practice	Bad practice
1	Integrating disease surveillance, vector surveillance and monitoring of environmental and social risks	No information exchange on data and analysis between agencies involved in disease surveillance, vector surveillance and the monitoring of environmental and social risks
2	Considering surveillance always in the context of monitoring and a planned response	Data collection in surveillance for academic purposes only
3	Combining event-based and case-based surveillance data	Relying on case-based surveillance data only
4	Defining and implementing surveillance indicators with the aim of an early response to dengue cases and outbreaks	No defined indicators for surveillance
5	Defining, agreeing on and reacting to triggers for early outbreak response	Reacting to dengue outbreaks only when outbreaks are evident
6	Unrestricted and timely information-sharing about dengue cases and risks nationally and with the international community	Not sharing or suppressing information about dengue cases and risks
7	Development of dengue preparedness plans with components for emergency situations, including simulation exercises	Relying on ad hoc planning for dengue prevention and control
8	Identifying the membership of an intersectoral dengue action committee at national or district level	Awaiting dengue outbreaks to define the dengue action committee
9	Integration of a combination of evidence-based interventions for dengue prevention and control in the dengue preparedness plan	Planning for interventions without considering efficacy and community effectiveness
10	Linking preparedness planning to surge capacity in health care, delivering appropriate triage and treatment of severe cases	Not incorporating health care delivery, including surge capacity, in preparedness plans
11	Assessment of dengue surveillance systems including preparedness plans nationally and internationally	Not examining and updating dengue surveillance systems and preparedness plans
12	Integrating communities in dengue prevention and control using appropriate methodology (e.g. COMBI)	Exclusively top-down approach to dengue outbreak response

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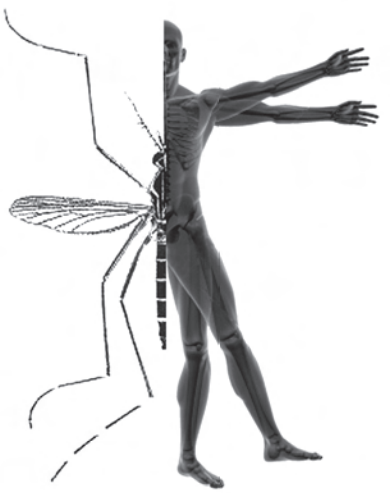

CHAPTER 6

NEW AVENUES

CHAPTER 6. NEW AVENUES

6.1 OVERVIEW

Primary prevention of dengue is currently possible only with vector control and personal protection from the bites of infected mosquitoes. However, the development of vaccines and drugs has the potential to change this. This chapter describes the current development of vaccines (section 6.2) and drugs (section 6.3).

6.2 DENGUE VACCINES

6.2.1 Overview

Despite formidable challenges to developing tetravalent dengue vaccines, significant progress has been made in recent years and the pace towards clinical efficacy trials has accelerated substantially (*1,2,3,4*). Box 6.1 summarizes the complexity of developing a dengue vaccine. Triggered by the continued unchecked spread of dengue worldwide, there has been renewed interest in dengue by researchers, funding agencies, policy-makers and vaccine manufacturers alike. The creation of public–private partnerships for product development has facilitated the process. Recent studies of the burden of disease have quantified the cost of dengue both to the public sector and to households and have demonstrated the potential cost-effectiveness of a dengue vaccine (*5,6,7,8*). The vaccine pipeline is now sufficiently advanced for it to be possible to have a first-generation dengue vaccine licensed within the next five to seven years (*3*). In addition, a number of diverse candidates are at earlier stages of evaluation and could become second-generation vaccines.

6.2.2 Product development

A primary immunological mechanism that confers protection from dengue illness is virus neutralization through antibodies, and all current dengue vaccine candidates aim to elicit high levels of neutralizing antibody. The increasing co-circulation of the four dengue virus types means that a vaccine is needed that protects against all four of them; hence, the vaccine needs to be tetravalent. Moreover, the induction of protective, neutralizing antibody responses against all four serotypes of dengue virus simultaneously should avoid the theoretical concern of vaccine-induced antibody-dependent enhancement in vaccine recipients. Dengue vaccines in development are of four types: live attenuated viruses, chimeric live attenuated viruses, inactivated or sub-unit vaccines, and nucleic acid-based vaccines.

Live attenuated vaccines (LAVs) can induce durable humoral and cellular immune responses since they most closely mimic a natural infection. Several parameters are crucial for LAVs:

- The viruses must be sufficiently attenuated and viral replication reduced so that viraemia is low and symptoms of illness are minimal.
- Transmission of the viruses by mosquitoes is reduced or eliminated.

- The viruses should replicate well in cell culture and be sufficiently immunogenic to provide long-lasting immunity in humans, so that low doses can be used.
- A balanced immune response to all four dengue viruses must be elicited.
- The genetic basis for attenuation must be known and must be stable (*4*).

Several live attenuated vaccines are in advanced stages of development. One is a chimeric tetravalent vaccine in which the structural genes (prM and E) of each of the four dengue viruses were inserted individually to replace those of yellow fever virus in the backbone of the yellow fever 17D vaccine. Thus, the nonstructural genes of yellow fever are provided to allow replication of the chimeric virus, and attenuation is imparted by the yellow fever portion of the chimera. Monovalent vaccines, as well as tetravalent mixtures of all four viruses, have been given to human volunteers of varying ages in phase 1 and phase 2 trials in both non-endemic and endemic regions. At least two doses were required to achieve high rates of tetravalent neutralizing antibodies, and somewhat higher seroconversion rates were observed in subjects with pre-existing immunity to yellow fever (*2*).

Another comprises strains of the four serotypes of the dengue virus, each attenuated by passage in primary dog kidney cells and initially prepared as candidate vaccines in fetal rhesus monkey lung cells (FRhL). Each attenuated dengue virus was rederived by transfecting cells with purified viral RNA. Original and rederived attenuated viruses have been extensively tested both individually and in tetravalent formulation in phase 1 and phase 2 trials in human volunteers of different ages. Tetravalent anti-DEN neutralizing antibodies are raised to high rates following the administration of at least two doses at an interval of six months, especially if volunteers have previously been exposed to flaviviruses.

Other vaccine candidates in phase 1 testing include live attenuated viruses. Here, vaccine development is being approached in two ways: (i) via direct removal of 30 nucleotides in the 3′ untranslated region of DEN-1 and DEN-4, and (ii) the construction of chimeric viruses consisting of DEN-2 and DEN-3 structural genes in the non-structural backbone of the DEN-4 strain with 30 nucleotides deleted from the 3′ untranslated region. Satisfactory attenuation, immunogenicity and protection have been obtained in rhesus monkeys for each of the four dengue viruses in this way (*4*). Phase 1 testing has taken place with all four monovalent vaccines.

Dengue vaccines in advanced preclinical development include DEN-DEN chimeras. In this vaccine, the prM and E protein genes of DEN-1, DEN-3 and DEN-4 were each inserted into the infectious clone of PDK-passaged, attenuated DEN-2 (PDK53). The three attenuating mutations are located outside the structural protein genes of PDK53 and appear to be quite stable. The tetravalent vaccine produced by combining the four chimeric dengue viruses is protective when administered to mice. Monkey challenge experiments have been conducted but preparations for clinical trials are underway.

Several DNA vaccines designed to deliver structural dengue viral genes into cells have been generated, and a monovalent DEN-1 DNA vaccine is currently undergoing phase 1 testing. In addition, candidate vaccines that have successfully protected monkeys from viraemic challenge include recombinant 80% envelope protein from the four DEN serotypes in conjunction with DEN-2 NS1 administered with several new-generation adjuvants. There is also work in progress on subunit vaccines based on domain III

of the E protein, which is considered to be the principal neutralizing epitope region of the virus, employing different strategies to increase immunogenicity. A tetravalent replication-defective-recombinant adenovirus (cAdVaX) has also been prepared, as have formalin-inactivated vaccines of the four dengue viruses. Finally, prime-boost strategies combining vaccines of distinct formulations are under investigation.

6.2.3 Challenges

Since dengue is caused by four serologically related viruses, the first major problem in developing a dengue vaccine is to develop not just one immunogen but four immunogens that will induce a protective immune response against all four viruses simultaneously. Therefore, the vaccine must be tetravalent. Interference between the four vaccine viruses must be avoided or overcome, and neutralizing titres to all four viruses need to be achieved regardless of the previous immune status of the vaccinated individuals. Thus, the tetravalent formulations must balance viral interference with long-lasting immunogenicity and reactogenicity.

The second issue is the lack of a validated correlate of protection since the mechanism of protective immunity against DEN infection is not fully understood. A wealth of data suggests that neutralizing antibodies are the main effector of protection against DEN virus infection. However, neither the precise antibody titres nor neutralizing epitopes nor the contributions of other immune mechanisms to protection have been defined. Further studies are necessary to elucidate the mechanism of protective immunity so that correlates of protection can be established to demonstrate that the candidate vaccines induce a protective immune response (*9*).

The dengue viruses are arboviruses whose normal transmission cycle involves mosquitoes (most commonly *Ae. aegypti* and *Ae. albopictus*) with humans as the vertebrate host, without relying on other animal reservoirs. Herein lies the third problem. Two animal models (mice and non-human primates) are used to evaluate candidate vaccines, but neither of these faithfully recapitulates both the disease outcome and the immune response in humans. Mice are often used as a small animal model to evaluate initially the ability of candidate vaccines to induce a protective immune response, and good progress has been made recently in developing mouse models for DEN virus infection and disease. However, the results are not always predictive of what will happen in higher animal species – i.e. a candidate vaccine that protects mice may not be as effective in other animal models. The second animal model is the non-human primate, and a variety of species have been used as models to evaluate candidate dengue vaccines. Unfortunately, non-human primates do not present clinical disease but do demonstrate viraemia (originally measured as infectivity, now normally measured by real-time RT-PCR and immunological parameters as proxies). Clearly, the mouse and non-human primate models must be used to evaluate candidate dengue vaccines before they are tested in humans. However, unexpected discordance has occasionally been observed between preclinical and clinical outcomes (*10*).

The fourth challenge for the development of dengue vaccines is the potential for immune enhancement, including antibody-dependent enhancement. It is clear that an infection by one dengue virus leads to lifelong protective immunity against the infecting virus, i.e. homotypic immunity. However, many studies have shown that some secondary DEN infections (i.e. infection by one dengue virus followed by infection by a different DEN serotype) can lead to severe disease (DHF/DSS) and that anti-DEN antibodies passively

transferred from mothers to infants increase the risk of DHF/DSS in the infants for a certain period of time. Thus, there is theoretically a danger that a dengue vaccine could potentially cause severe disease (including DHF or DSS) in vaccine recipients if solid immunity was not established against all four serotypes. It should be emphasized that, to date, there is no evidence that a vaccine recipient who has received a candidate vaccine has subsequently succumbed to severe disease. Rather, vaccine recipients have shown evidence of immunity for varying lengths of time. This may be influenced by the candidate vaccine. Also vaccine recipients may have been exposed to less symptomatic DEN infections than control groups, although this is based on small numbers thus far (*11*). Nonetheless, the risk of immune enhancement by a candidate vaccine must be evaluated through prolonged follow-up of vaccinated cohorts.

Box 6.1 The complexity of developing a dengue vaccine

Development
• Need for a tetravalent vaccine with not just one but four immunogens that will give a balanced immune response whereby a protective long-lasting immunity is induced against all four viruses simultaneously (balancing viral interference, immunogenicity, and reactogenicity). • Lack of immune correlate of protection since the mechanism of protective immunity against DEN infection is only partially understood. It is assumed that neutralizing antibodies are the main effector of protection against DEN infection. • Lack of a suitable animal model that recapitulates human disease and can be used to evaluate candidate vaccines. • Potential immunopathogenesis, including antibody-dependent enhancement
Implementation
• Need for long-term follow-up. • Need for testing in both Asia and the Americas. • Ideally, can be tested against all four DEN serotypes. • The exact location, timing and serotype/genotype composition of dengue epidemics varies from year to year and is somewhat unpredictable.

6.2.4 Implementation

The clinical evaluation of candidate vaccines for dengue has several unique aspects, some of which are related to the above-mentioned challenges. Dengue illness has many clinical manifestations and poses diagnostic challenges. Ideally, a vaccine should be efficacious against all forms of dengue illness, ranging from febrile illnesses to severe forms such as DHF and DSS. Trials need to be large enough to address a vaccine's impact on the different clinical forms of dengue. Phase 4, or post-marketing, trials will be particularly important for dengue for the same reason. Likewise, long-term evaluation of volunteers will be required to demonstrate lack of evidence for immune enhancement/severe disease. Trials need to take place in multiple countries – particularly in both Asia and the Americas – due to the distinct epidemiological characteristics and the viruses circulating in each region. The greatest burden of disease is found in distinct age groups in different countries, and the methodology of capturing clinical events may differ by age group and between countries. The epidemic peak varies somewhat in timing and exact location from year to year even in endemic countries; thus, long-term dengue surveillance data about the potential vaccine testing

site(s) is crucial and, even so, the unpredictability of timing and location adds a level of complexity to calculations of trial sample size. To facilitate the vaccine development process, WHO has developed guidelines for the clinical evaluation of dengue vaccines (*12*). The Paediatric Dengue Vaccine Initiative (PDVI) supports the establishment of field sites for the testing of vaccines.

6.2.5 Vaccine utilization for dengue control

Additional work is required to bring a vaccine from licensing to programmatic use in dengue-endemic areas. Depending on cost-effectiveness and the outcome of financial and operational analysis, countries may decide to introduce dengue vaccines into the national immunization programmes for routine administration. If the vaccine is to be used for infants, the dengue vaccination will need to be carried out on a schedule compatible with other vaccines. Interference between dengue vaccine and other vaccines likely to be given in the same time period must be ruled out. If the vaccine is to be delivered to older age groups, proper contact points will need to be established to deliver the vaccine effectively and to ensure post-marketing surveillance. In addition, vaccine presentation, packaging and stability requirements should be compatible with large-scale use. WHO has produced generic guidelines to guide national authorities in their decision-making on the introduction of new vaccines (*13*).

To maximize the effect of vaccination, the potential impact of a vaccine on dengue transmission needs to be studied (e.g. the role of herd immunity). A number of modelling approaches are being taken to address this and other similar issues, as well as the characteristics of the mosquito population transmitting the virus and climatographic parameters.

Given the complexity of dengue and dengue vaccines, it is imperative to continue scientific research that is directed at improving our understanding of the immune response in both natural DEN infections and vaccinees (e.g. defining neutralizing and potentially enhancing epitopes, improving animal models) alongside vaccine development and evaluation.

6.3 DENGUE ANTIVIRAL DRUGS

6.3.1 Overview

The search for dengue antivirals is a new endeavour that is gaining momentum due to both increased interest in dengue and substantial progress in the structural biology of dengue virus. Furthermore, extensive drug discovery efforts in HIV and HCV have taught us important lessons that encourage similar strategies to be adopted for dengue. Since HCV and dengue virus are members of the Flaviviridae family, the intensive work on HCV antivirals – especially those that target the RNA-dependent RNA polymerase – can benefit the search for dengue antivirals (*14*). The rationale for dengue antivirals arises from clinical studies that have noted that the quantity of virus circulating in the blood of patients who develop DHF and DSS is higher by around 1–2 logs compared with patients suffering from the milder dengue fever. Similar differences in viral load have been observed in animal models of ADE (*15,16,17*). This observation suggests that progress to serious dengue disease and adverse morbidity may be reversed by administering potent and safe small molecule compounds that target essential steps in

virus replication early during the disease, thereby lowering the viral load substantially. This hypothesis requires field-testing when a suitable anti-dengue agent is discovered.

6.3.2 Product development

The life cycle of dengue virus readily shows that the steps involved in virus entry, membrane fusion, RNA genome replication, assembly and ultimate release from the infected cell can be targeted by small molecules (*18*). The importance of targets such as the viral protease and polymerase have been studied by reverse genetics using infectious clones to validate them as targets for drug discovery. On the basis of success in the HIV and HCV fields in finding small molecules that target viral enzymes that are essential for virus replication in infected cells, academic institutions and non-profit pharmaceutical enterprises and consortia are progressing in their search for antiviral compounds active against these targets in dengue virus. The field is also benefiting from new insights afforded by x-ray crystallography and cryo-electron microscopy data. In the past five years alone, seven new 3-D structures of dengue proteins have been solved and nine structures of other flavivirus proteins have become available. Based on these, a number of *in silico* and high through-put screens have been and are being undertaken, yielding several lead compounds so far (*19*). Currently the most advanced targets are the NS3/NS2B protease and NS5 RNA-dependent RNA polymerase, which have undergone high through-put screening and lead compound optimization. New targets – including E, NS3 helicase, and NS5 methyltransferase – are being explored (*20*), and others will soon be added to the list. Recent advances in understanding the mechanism of membrane fusion during DEN infection of target cells has opened up new possibilities for designing novel antiviral strategies that target the fusion step as well (*21*).

Screening efforts are continuing and will increase. They include the proprietary libraries of pharmaceutical and biotechnology companies, focused libraries of compounds synthesized to specific targets (e.g. protease inhibitors), designed libraries based on structural information, natural products and approved drugs. Different approaches have been taken to screening compounds, such as high through-put screening of both target protein activity and viral replication in cultured cells, high through-put docking *in silico*, and fragment-based screening using NMR and x-ray crystallography. Distinct classes of inhibitors are being explored, such as substrate- and transition-state-based as well as non-substrate-based inhibitors. Reporter replicons based on yellow fever or DEN infectious clones have facilitated screening in cell culture for both primary and secondary screens (*22,23*). Early preclinical *in vivo* testing can be conducted in certain mouse models of dengue infection. For instance, tissue and cellular tropism of DEN in AG129 mice (mice of the 129 background lacking interferon α/β and γ receptors) is similar to that in humans (*24,25*), and AG129 mice are being used as the first step in preclinical testing of candidate antivirals (*26*). Alternative approaches, such as interfering with viral replication using peptide-conjugated phosphorodiamidate morpholino oligomers (P-PMOs), have also been proposed and are effective in cell culture (*27,28*). Initial studies of such compounds in mice against the related West Nile virus have been reported (*29*). Other nucleotide-based approaches, including RNAi methodologies, have been investigated in the exploratory phase (*30*). In addition, cellular targets are under consideration, as is the idea of incorporating multiple targets in one formulation so as to delay the possible development of resistance. A further approach is to target key disease manifestations. As more is understood about the mechanism of severe dengue disease, animal models that reproduce specific manifestations may be useful for testing anti-dengue drugs. For instance, the AG129 mouse demonstrates a vascular leak early-

death phenotype when infected with DEN-2 strain D2S10 (*31*). It can also reproduce antibody-dependent enhancement of infection and disease (*17*) and is currently being used to test compounds that target disease phenotypes such as vascular leak.

6.3.3 Challenges

There are numerous requirements for an anti-dengue drug (Box 6.2). The minimal target product profile includes oral route of administration, frequency of dosing of once per day, stability in the face of heat and humidity, a long shelf-life, and low/reasonable cost of goods and ease of formulation to allow a reasonably priced product (*18*). With respect to clinical efficacy, the drug must be active against all serotypes, reduce symptoms, and reduce the incidence of severe disease. Furthermore, a safe drug may be evaluated for prophylactic as well as paediatric use after proper trials.

Box 6.2 The challenge of developing dengue antivirals

Development
• Several potential viral targets, of which the most advanced are NS3/NS2B protease and NS5 polymerase; work in progress on E, NS3 helicase, and NS5 methyltransferase. • Must be active against all serotypes. • Must be effective in both primary and secondary DEN infections. • Must be active orally, stable to heat and humidity, have a long shelf-life, and have low/reasonable production costs. • Exploration of cellular targets? • Good safety profile, including few or no secondary effects. • Useful in infants, children and adults.
Implementation
• Need for rapid point-of-care diagnostic tool to apply antiviral most effectively. • Short window of viraemia. • Possible development of resistance: use cocktails of multiple drugs to avoid this eventuality. • Must be tested in acute DEN infection, and a prophylactic trial is not an option.

6.3.4 Implementation

One of the biggest challenges in testing antivirals is that to be effective the drug(s) should presumably be delivered early after onset of symptoms. However, the vast majority of dengue patients present late during their illness, perhaps on days 3 or 4 of fever. Even during clinical studies, patients do not present to study staff much earlier. In this respect, a trial of dengue antivirals would have to capture patients presenting to primary care facilities, where it would be possible to enrol patients during the first two days after the onset of symptoms, whereas hospital admission is more likely to occur on days 3 or 4 or later. However, the earlier the presentation with fever, the less likely it is that the diagnosis will be dengue. Although prospective dengue diagnosis before treatment in a clinical study would be ideal, this would potentially be problematic as it would require rapid RT-PCR testing at or near the site of the study. The availability of new serological reagents such as NS1 antigen capture tests may make early point-of-care diagnosis

possible. It would also be ideal to test reactions between an anti-dengue drug and other drugs often used to treat dengue patients, such as paracetamol.

It is likely that a multicentre trial of a candidate dengue antiviral drug would be indicated, which raises a series of issues. For instance, the treatment that dengue cases receive in hospitals is known to have a dramatic effect on the disease outcome, and the way clinicians treat dengue cases varies greatly both within and between institutions. In addition, getting physicians to document parameters and outcomes carefully in busy clinical situations is often difficult – though not impossible – and is clearly essential for any trial. A robust study design with a single protocol followed meticulously at all sites would solve the problem but would be challenging to achieve.

The greatest burden of dengue in most countries is in children, although in some areas older age groups are also significantly affected. The target population for dengue drugs would be all age groups but, because of the problematic nature of clinical trials in children, a drug trial may be better performed in the first instance in adults (>15 years of age).

Although vaccines and drugs for dengue pose significant challenges during both product development and field-testing, tremendous strides have been made recently in both areas.

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