Guidelines for Prevention and Control of Dengue
Guidelines for prevention and control of Dengue Fever and Dengue Haemorrhagic Fever

1. Introduction 1
2. Causative agent 1
3. Epidemiology 1
4. Clinical manifestations 4
5. Laboratory diagnosis 6
6. Management 7
7. Immunity 11
8. Vaccine 11
9. Surveillance in DF/DHF 11
10. Prevention and control of DF/DHF 13
11. Guidelines for collection, storage, transportation and laboratory testing of samples for Dengue and Dengue Haemorrhagic Fever 13
12. Notification 17

ANNEXURE

Prevention and control of Dengue fever 19
1. Introduction

Dengue fever is the most important mosquito spread viral disease and a major international public health concern. It is a self limiting disease found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas. Dengue Haemorrhagic Fever (DHF), a potentially lethal complication, was first recognized in 1950s during the dengue epidemic in Philippines and Thailand but today DHF affects most Asian countries and is a leading cause of childhood deaths. In India, first major outbreak associated with haemorrhagic manifestation occurred in Calcutta in 1963 followed by a major outbreak in Delhi in 1996 and 2003.

2. Causative agent

DF/DHF is caused by dengue virus which belongs to genus Flavivirus family Flaviviridae and includes serotypes 1, 2, 3 and 4 (Den-1, Den-2, Den-3 and Den-4). When a person has had classic dengue (i.e. infection by one serotype), a second infection later by another serotype increases the likelihood of suffering from DHF.

3. Epidemiology

3.1 Geographical distribution

The global prevalence of dengue has grown significantly in recent decades. The disease is now endemic in more than 100 countries in South-east Asia, Western Pacific, Eastern Mediterranean, Africa, the Americas. South-east Asia and Western Pacific are most seriously affected. Before 1970 only nine countries had experienced DHF epidemic, a number that had increased more than four-fold by 1995. Some 2500 million people are now at risk from dengue. WHO currently estimates there may be 50 million cases of dengue infection worldwide every year with around 24,000 deaths.
3.2 Situation in India

India is also endemic for Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF). Every year cases of DF and/or DHF are reported (Table-1A & 1B). In 1996, there was a large outbreak of DF and DHF. Cases and deaths were reported from various parts of the country viz. Delhi 10,252 cases and 423 deaths, Haryana 1631 cases and 54 deaths and Maharashtra 3068 cases and 5 deaths. In total, 16517 cases and 545 deaths were reported from all over the country. After 1996 there was a decline in cases of DF and DHF as depicted in the Table. Again in the year, 2003 an outbreak of DF and DHF was reported from various parts of the

Table 1A: Dengue cases and deaths from 1996 - 2000

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>D</td>
<td>C</td>
<td>D</td>
<td>C</td>
</tr>
<tr>
<td>1.</td>
<td>Andhra Pradesh</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Bihar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>Chandigarh</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>Delhi</td>
<td>10252</td>
<td>423</td>
<td>273</td>
<td>1</td>
<td>333</td>
</tr>
<tr>
<td>5.</td>
<td>Goa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>Gujarat</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td>Haryana</td>
<td>1631</td>
<td>54</td>
<td>54</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>8.</td>
<td>Karnataka</td>
<td>123</td>
<td>5</td>
<td>262</td>
<td>4</td>
<td>115</td>
</tr>
<tr>
<td>9.</td>
<td>Kerala</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>10.</td>
<td>Maharashtra</td>
<td>3068</td>
<td>5</td>
<td>249</td>
<td>5</td>
<td>193</td>
</tr>
<tr>
<td>11.</td>
<td>Orissa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>12.</td>
<td>Sikkim</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13.</td>
<td>Punjab</td>
<td>806</td>
<td>32</td>
<td>23</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>14.</td>
<td>Rajasthan</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>15.</td>
<td>Tamil Nadu</td>
<td>491</td>
<td>16</td>
<td>264</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>16.</td>
<td>Pondicherry</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17.</td>
<td>Uttar Pradesh</td>
<td>146</td>
<td>10</td>
<td>29</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>18.</td>
<td>D&amp;N Haveli</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19.</td>
<td>West Bengal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>16517</td>
<td>545</td>
<td>1177</td>
<td>36</td>
<td>707</td>
</tr>
</tbody>
</table>
Table 1B: Dengue cases and deaths from 2001 - 2005

<table>
<thead>
<tr>
<th>S. No.</th>
<th>State</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005 (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>D</td>
<td>C</td>
<td>D</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>Andhra Pradesh</td>
<td>1</td>
<td>0</td>
<td>61</td>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>Bihar</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Chandigarh</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Delhi</td>
<td>322</td>
<td>3</td>
<td>45</td>
<td>2</td>
<td>2882</td>
</tr>
<tr>
<td>5</td>
<td>Goa</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>Gujarat</td>
<td>69</td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>249</td>
</tr>
<tr>
<td>7</td>
<td>Haryana</td>
<td>260</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>8</td>
<td>Karnataka</td>
<td>220</td>
<td>0</td>
<td>428</td>
<td>1</td>
<td>1226</td>
</tr>
<tr>
<td>9</td>
<td>Kerala</td>
<td>41</td>
<td>0</td>
<td>219</td>
<td>2</td>
<td>3546</td>
</tr>
<tr>
<td>10</td>
<td>Maharashtra</td>
<td>54</td>
<td>2</td>
<td>370</td>
<td>18</td>
<td>772</td>
</tr>
<tr>
<td>11</td>
<td>Orissa</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>Sikkim</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>Punjab</td>
<td>49</td>
<td>0</td>
<td>27</td>
<td>2</td>
<td>848</td>
</tr>
<tr>
<td>14</td>
<td>Rajasthan</td>
<td>1452</td>
<td>35</td>
<td>325</td>
<td>5</td>
<td>685</td>
</tr>
<tr>
<td>15</td>
<td>Tamil Nadu</td>
<td>816</td>
<td>8</td>
<td>392</td>
<td>0</td>
<td>1600</td>
</tr>
<tr>
<td>16</td>
<td>Pondicherry</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>17</td>
<td>Uttar Pradesh</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>738</td>
</tr>
<tr>
<td>18</td>
<td>D&amp;N Haveli</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>West Bengal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3306</td>
<td>53</td>
<td>1926</td>
<td>33</td>
<td>12754</td>
</tr>
</tbody>
</table>

country especially Delhi, Kerala, Karnataka, Punjab, Tamil Nadu, Uttar Pradesh & Maharashtra. In 2003, a total of 12754 cases and 215 deaths were reported from the country. This year, too, the country witnessed (year 2005), a large outbreak of Dengue fever reporting about 10824 cases and 87 deaths (Table 1B).

3.3 Transmission
The infection is transmitted by the bite of an infected female mosquito – Aedes Aegypti. Mosquitoes generally acquire the virus while feeding on the blood of
an infected person. After virus incubation for 8 – 10 days, an infected mosquito is capable of transmitting the virus to susceptible individuals for the rest of its life and the virus is maintained in nature through trans ovarian transmission in mosquitoes.

Humans are the main host of the virus, although studies have shown that in some parts of the world monkeys may become infected and perhaps serve as a source of virus for uninfected. The virus circulates in the blood of infected humans for two to seven days.

### 3.4 Vectors of transmission
Aedes Aegypti is the main vector of dengue transmission in India. Dengue outbreaks have also been attributed to Aedes albopictus. The mosquito is a peri-domestic and domestic breeder. Mosquito breeding can occur in any water-storage containers, such as desert coolers, flower vases, coconut shells, construction sites, overhead uncovered or partially covered water tanks, discarded buckets, tyres, utensils and large containers used for collecting rain water which are not emptied and cleaned periodically. The mosquitoes rest indoors on various objects, in closets and other dark places. Outside, they rest where it is cool and shady. Aedes mosquito can fly up to a limited distance of 400 metres but can spread over vast distances mechanically in various types of vehicles used by man. The outbreaks of DF/DHF are most likely to occur in post-monsoon period when the breeding of the mosquitoes is highest.

### 3.5 High risk areas
Usually urban areas, having high population density, poor sanitation and large number of desert coolers, flower vases, construction sites, overhead tanks, discarded buckets, tyres, utensils etc. which promote mosquito breeding, are at high risk. Dengue fever/DHF can also occur in rural areas where the environment is friendly for mosquito breeding like storage water for cattle feeding and drinking, cement cisterns, coconut shells, underground cemented water sumps, discarded tins, tyres, bottles etc. which are not emptied and changed periodically.

### 4. Clinical manifestations
The incubation period of dengue fever is usually 5 – 6 days, but may vary from 3 to 10 days. Dengue fever affects infants, young children and adults, but seldom
causes death. The clinical features of dengue fever vary according to the age of the patient. Infants and young children may have a non-specific febrile illness with rash. Older children and adults may have either a mild febrile illness or classical disease with abrupt onset of high fever, severe headache, severe muscle and joint pain (break bone fever), rash and other haemorrhagic manifestation and leucopenia.

DHF is a potentially deadly complication that is characterized by high fever, accompanied by headache, anorexia, vomiting and abdominal pain. A haemorrhagic diathesis is commonly demonstrated by scattered fine petechiae on the extremities, face, trunk and in the axillae. A tourniquet test may be positive. Bleeding from nose, gums and gastrointestinal tract may be found. Haematuria is rare. The liver is usually enlarged, soft and tender.

In mild DHF cases, all signs and symptoms abate after the fever subsides. In moderate cases, spontaneous mucocutaneous bleeding, nasal bleeding and GI bleeding usually occurs. In severe cases patient’s condition may suddenly deteriorate after a few days (2 – 7 days) of fever, varying degree of circulatory disturbances occur and the patient may rapidly go into a critical state of shock (Dengue Shock Syndrome) and die within 12 – 24 hours, or quickly recover following appropriate volume replacement therapy. Prolonged shock is often complicated by metabolic acidosis and severe bleeding, which indicates poor prognosis and the DHF case fatality rate can exceed 20%. With modern intensive supportive therapy such rate can be reduced to less than 1%. A person with dengue should only take paracetamol as antipyretic and analgesic. However, aspirin should be avoided as it increases bleeding tendency.

A major cause of deaths due to DHF is leakage of plasma in the pleural and abdominal cavities leading to hypovolaemic shock. Continuous monitoring of haematocrit value and platelet count is essential for diagnosis and case management. The time course relationship between the fall in platelet count and a rise in haematocrit level appears to be unique to DHF. These changes occur before the subsidence of fever and before the onset of shock and are correlated with the disease severity.
Encephalitic signs associated with intracranial haemorrhage, metabolic and electrolyte disturbances, and hepatic failure (a form of Reye’s syndrome) may occur. They are uncommon but carry a grave prognosis.

Early in febrile illness, the differential diagnosis includes a wide spectrum of viral and bacterial infections e.g. enteric fever. The presence of marked thrombocytopenia with concurrent haemoconcentration differentiates DHF/DSS from other syndromes such as endotoxic shock from bacterial or meningococcaemia. In patients with severe bleeding, evidence of pleural effusion and/or hypoproteinaemia may indicate plasma leakage.

**Alarming signs in Dengue**
- Minute spots on the skin suggesting bleeding within the skin
- Nose bleeds and gum bleeds, hemetemesis
- Abdominal pain and/or passage of black tarry stool
- Refusal to food or drink
- Abnormal behaviour or drowsiness
- Difficulty in breathing or cold hands and feet, reduced amount of urine being passed

5. **Laboratory diagnosis**

5.1 **Serological diagnosis**
The diagnosis of DF/DHF can be confirmed by serological tests. The tests include detection of IgM antibodies which appear around the end of first week of onset of symptoms and are detectable for 1 – 3 months after the acute episode. Detection of IgM antibodies, demonstration of sero-conversion of IgM antibody or a four fold difference in titre of IgG antibody in paired sera taken at an interval of ten days or more is confirmatory. High IgG antibodies early in the course of illness indicate previous infection.

5.2 **Isolation/detection of virus/antigen**
Virus isolation can be done by inoculation in, tissue culture or mosquitoes or suckling mice and further identification by using fluorescent antibody test or
other tests. Viral genomic sequences can be detected in CSF, serum or autopsy tissue sample by using PCR (Polymerase Chain Reaction).

6. Management

6.1 Management of Dengue fever
Management of dengue fever is symptomatic with bed rest, antipyretic and analgesic. Liberal fluids intake viz. home available fluids eg. Juices, rice water, kanji, fruit juices, plain water etc. or ORS solution are recommended for patient with excessive sweating, nausea, vomiting, or diarrhoea to prevent dehydration.

6.2 Management of DHF
Prognosis in DHF depends on early recognition of plasma leakage which can be monitored by rise in haematocrit level. The frequent assessment needs to be done by serial haematocrit levels whenever feasible. In areas, where facilities for estimation of haematocrit do not exist serial vital signs along with urinary output chart needs to be maintained. Most critical period is around 3rd day of illness when the patient apparently starts feeling better and becomes afebrile but needs close observation regarding development of complications. Indications for admission are – rise in haematocrit of 20% or more, a single haematocrit value of more than 40%, platelets count of 50,000/cmm or less, spontaneous haemorrhage, signs and symptoms of shock, oliguria and circum-oral cyanosis.

For DHF patients, antipyretics can be given; salicylates and non-steroid anti-inflammatory drugs (NSAIDS) should not be given. Plenty of oral fluids is needed to avoid dehydration (due to high fever and vomiting). In case of massive plasma leakage in grade-III DHF, replacement shall be done judiciously. The required volume should be charted on 2 – 3 hourly basis and the rate of fluid replacement should be adjusted throughout 24 – 48 hours period of leakage by serial haematocrit levels and frequent assessment of vital signs such as urine output to avoid over infusion (Figure 1). Excessive volume replacement and continuation after leakage stops will cause massive pleural effusion, ascites, and pulmonary congestions/oedema when re-absorption of the extravasated plasma occurs in the convalescent stage.
Figure 1: Volume replacement flow chart for a patient with DHF and a > 20% increase in Haematocrit

5% fluid deficit

Initiate intravenous therapy: 5% glucose in physiological saline (6-7 mlkg⁻¹h⁻¹)

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

- Reduce intravenous therapy (5 mlkg⁻¹h⁻¹)
  - Improvement
  - Reduce intravenous therapy (3 mlkg⁻¹h⁻¹)

- Further improvement

- Discontinue intravenous therapy after 24 – 48 hours

- Vital signs and haematocrit stable adequate diuresis

- No Improvement
  - Haematocrit or pulse rate rises, pulse pressure falls below 20 mmHg (2.7kPa), urine output falls

- Increase intravenous therapy (10 mlkg⁻¹h⁻¹)
  - No Improvement

- Increase intravenous therapy (15 mlkg⁻¹h⁻¹)

- Unstable vital signs
  - Urine output falls, signs of shock

- Establish central venous access and urinary catheter, administer rapid fluid bolus
  - Haematocrit rises (or distress)
  - Intravenous colloid therapy
  - Blood transfusion

- Improvement
Management of Dengue Shock Syndrome (DSS)
DSS patients present with shock. Shock is a medical emergency. Volume replacement is the most important treatment measure and immediate administration of intravenous fluids to expand plasma volume is essential (Figure 2). Close observation with good nursing care is imperative. Blood transfusion should be given in case with significant haemorrhage. Fresh frozen plasma/ or concentrated platelet transfusion may be given when disseminated intravascular coagulation causes massive bleeding. Hyponatraemia and metabolic acidosis occurs occasionally in DHF/DSS patients. Electolyte levels and blood gases should be determined periodically in severely ill patients and corrected. Sedatives may be given in agitated child. Oxygen therapy should be provided to all patients in shock/hypotension.

Management during febrile phase is similar to DF with antipyretic and analgesic. Besides this fluid and electrolyte replacement by IV fluids, plasma expanders, if clinically indicated results in favorable outcomes. In some cases fresh frozen plasma is indicated and in rare cases like patient with severe shock or massive bleeding, blood transfusion is required. Amount of fluid given should be constantly monitored. Judicious volume replacement is mandatory as the plasma loss is only for 24 to 48 hours and is more rapid around the time of defervescence and/or shock. Haematocrit determination is essential for monitoring the rate of IV fluid infusion and to check overload (which has been recognized as a common problem).

Isotonic solution (0.9% sodium chloride, also known as normal saline) or a compound solution of sodium lactate is preferred. Saline with or without glucose can be used depending upon availability. Glucose solution without saline does not provide the salt required to restore electrolyte balance and is not recommended.

Transfusion of platelets does not change the course of the illness and is not recommended. Blood transfusion may be indicated in patients with severe shock, massive bleeding and DIC. Any evidence of swelling, shortness of breath or puffiness may indicate fluid overload. Adoption of appropriate standardized clinical management practices can effectively reduce DHF case fatality rates.
Figure 2: Volume replacement flow chart for a patient with DSS

Unstable vital signs
Urine output falls, signs of shock

Immediate, rapid volume replacement: *10 – 20 ml kg$^{-1}$
Ringer’s lactate, Ringer’s acetate or 5% glucose diluted in
physiological saline, as intravenous bolus (repeat if necessary)

- Improvement
  - Adjust intravenous therapy
    - As per figure 1

- No improvement
  - Oxygen

Haematocrit falls

- Blood transfusion (10 ml kg$^{-1}$ if haematocrit is still >35%)

Haematocrit rises

- 10 – 20 ml kg$^{-1}$ plasma, plasma substitutes or 5% albumin, as
  intravenous bolus (repeat if necessary)

Criteria for discharge in DF/DHF/DSS
- Absence of fever for 24 hours without antipyretics.
- Visible improvement in clinical picture and stable haematocrit.
- Three days after recovery from shock.
- Platelet count greater than 50,000/cu.mm.
- No respiratory distress from pleural effusion.
7. **Immunity**

Infection with one serotype provides life-long homologous immunity but does not provide protection against other serotypes, and instead may exacerbate subsequent infection.

8. **Vaccine**

No effective vaccine is available for dengue. Research into dengue vaccines focuses on the use of live attenuated or inactivated vaccines, infectious clone-derived vaccines, immunogens vector by various recombinant systems, sub unit immunogens and nucleic acid vaccine.

Among these intensive and stringent laboratory studies conducted for live attenuated tetravalent vaccine in Thailand. This vaccine was evaluated in animal models and phase-1 clinical trial of this vaccine was recently completed in Thailand. After two doses, sero conversion to all four serotypes was demonstrated in most vaccinated volunteers and antiviral activity remained quite stable for at least a year.

In order to promote the evaluation of live attenuated vaccines in clinical trials, a group of WHO experts has been developing guidelines for the safety of dengue vaccine. These guidelines could help public health officials to make decisions about conducting dengue vaccine trials in their countries.

9. **Surveillance in DF/DHF**

Surveillance is a pre-requisite for monitoring the dengue situation in the area and should be carried out regularly for early detection of an impending outbreak and to initiate timely preventive and control measures. Surveillance should include epidemiological, entomological and laboratory parameters.

9.1 **Epidemiological surveillance**

The epidemiological surveillance should include the following:

- Fever surveillance
- Diagnosis based on standard case definition
• Reporting of DF/DHF cases to state health authorities
• During an outbreak situation, if 5 to 10% of the samples collected from clinically diagnosed cases and found positive by laboratory, other would be considered epidemiologically linked cases. However, atypical cases (doubtful) samples must be sent for laboratory confirmation.

The peripheral health staff should be alerted to report increase of clustering of acute febrile illness compatible with the case definition of DF/DHF. Such increase of cases should be investigated locally, including entomological investigation to check for vector density in the area.

9.2 Vector surveillance
Larval surveillance during the pre monsoon and monsoon is important to find out the extent of prevalence of vectors in certain selected high risk localities/areas (from where dengue cases have been reported earlier). House index, container index and breteau index are usually used. Adult mosquito surveillance will help in finding out the susceptibility status to insecticides.

A number of indices have been described and are currently used to monitor the vector population, minimum 50 houses to be surveyed in a locality to calculate the following indices:
1. **House index** – Percentage of houses positive for larvae of Aedes aegypti.
2. **Breteau index** – Number of positive containers for Aedes aegypti per 100 houses.
3. **Container index** – Percentage of water containers positive for Aedes breeding. It is mostly utilized for drawing vector control strategy.

### Epidemiological interpretation of various entomological indices

<table>
<thead>
<tr>
<th>Entomological indices</th>
<th>High risk of transmission</th>
<th>Low risk of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breteau index</td>
<td>&gt; 50</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>House index</td>
<td>&gt; 10%</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>
However, a single water container found positive for aedes breeding warrants for immediate action for source reduction.

9.3 Laboratory surveillance
Laboratory surveillance confirms the clinical diagnosis and provide report to the public health authority. The laboratory should receive selected samples from Sentinel Hospitals from fever of unknown origin for serological surveillance and viral detection for setting up of early warning signals for timely institution of preventive and control measures. The objective of the laboratory surveillance is to detect the introduction of the virus, the new strain or serotype of dengue virus and to detect any unusual increase in the spread of dengue transmission.

10. Prevention and control of DF/DHF
The only method of controlling or preventing dengue fever and DHF is to combat the vector mosquitoes. In India, Aedes Aegypti breeds primarily in man made container like water cooler, earthenware jars, concrete cisterns used for domestic water storage, discarded plastic food containers, used automobile tyres and other items that collect rain water.

Vector control can be implemented using environmental management. Proper solid waste disposal and improved water storage practices, including covering containers can prevent access by egg-laying female mosquitoes. These methods should be encouraged through community-based programmes.

The detailed guidelines for vector control formulated by Directorate of NVBDCP are annexed.

11. Guidelines for collection, storage, transportation and laboratory testing of samples for Dengue and Dengue Haemorrhagic Fever

11.1 Case definition description

Clinical case of Dengue fever
The clinical case description of Dengue fever is an acute febrile illness of 2-7 days duration with 2 or more of the following:
Headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestation and leucopenia.

**Case definition of DHF**
Haemorrhagic tendencies seen with one or more of the following:

- Positive tourniquet test
- Petechiae, ecchymoses or purpura
- Bleeding from buccal mucosa, gastrointestinal tract, injection site, nasal bleeding or others
- Haematemesis or malaena,
  And thrombocytopenia (< 1 lakh per mm³)
And evidence of plasma leakage manifested by one or more of the following

- ≥20% rise in average hematocrit for age and sex
- Signs of plasma leakage (pleural effusion, ascites, hypoproteinaemis)

**Dengue Shock Syndrome**
DHF with circulatory failure manifested by rapid and weak pulse, narrow pulse pressure, cold and clammy skin, restlessness or lethargy, altered sensorium.

**Suspect case**  A case compatible with the clinical description

**Probable case:** A case compatible with clinical description with one or more of the following:

- Supportive serology (HI antibodies titre of ≥1280)
- Presence of confirmed case in the area during the same period

**Confirmed case:** A case compatible with clinical description and laboratory confirmed by one or more of the following.

- Specific IgM antibody detected in single serum
- Sero-conversion of IgM
- Four fold difference in titre of IgG antibody in paired sera
- Virus isolation from plasma, serum, CSF or autopsy tissue
- Detection of viral genomic sequence in serum, CSF or autopsy tissue by PCR
- Detection of viral antigen in autopsy tissue by immunohistochemistry or immunofluorescence or in serum samples by EIA
11.2 Laboratory Diagnosis of Dengue Fever/DHF

- **Hematological criteria for diagnosis**
  Thrombocytopenia (100,000 cells or less per mm3)
  Haemoconcentration (>20% rise in average hematocrit for age and sex)

- **Microbiological diagnosis**
  A definite diagnosis of dengue infection can be made by:
  i) Isolation of the virus
  ii) Demonstration of viral antigen or RNA in the tissue or serum
  iii) Demonstration of IgM antibodies or a rising titre of IgG antibodies in paired sera against dengue virus i.e. serological diagnosis
  iv) Molecular diagnosis (PCR), serotyping & genotyping.

(i) **Isolation of the virus**
Virus isolation can be done by inoculation of clinical material in tissue culture, mosquitoes or suckling mice and further detection is performed using fluorescent antibody test or haemagglutination inhibition test.

(ii) **Demonstration of virus antigen or RNA**
Viral antigen can be demonstrated by doing direct fluorescent antibody test using specific monoclonal antibodies for dengue virus.

Viral RNA or genomic sequence can also be detected in autopsy specimen, serum, CSF or culture supernatant by doing Polymerase Chain Reaction (PCR) and gene sequencing.

(iii) **Serological diagnosis**
Detection of IgM antibodies: IgM antibodies against dengue virus appears around 5 days after onset of symptoms and are detectable for 1-3 months after the acute episode. The tests employed are IgM capture ELISA test and Rapid IgM strip test. IgM capture ELISA test kit is available from NIV Pune and commercial sources and Rapid IgM Strip Test kit is available commercially.

Detection of IgG antibodies: IgG antibodies appear later than IgM antibodies in primary infection of dengue and persist at high level for 30 – 40 days before
declining to levels found in past infection and persist for life. Detection of four fold or greater increase/fall in IgG titre in paired serum samples taken at an interval of 10 – 14 days confirms the diagnosis of dengue. Test employed are IgG ELISA for dengue and Haemagglutination Inhibition (HI) Test.

11.3 Collection, storage and transportation of samples for Dengue and DHF
Proper collection, processing, storage and transportation of the specimens is an essential aspect of the laboratory diagnosis.

11.3.1 Collection of samples for serology

Sample: Blood in plain vial/Serum

Time of collection

1st Sample: 5 days after onset of illness for IgM detection as these antibodies appear at this time
2nd Sample: At least 7 to 14 days after the first sample or, in the event of a fatality, at the time of death.

11.3.2 Collection of samples for isolation & molecular diagnosis

Samples: Serum
Plasma
Whole blood (washed buffy coat)
Autopsy tissues - liver, spleen, lymph nodes & thymus
Mosquitoes collected in nature

Time of collection: Within first five days of illness

Blood collection in tubes or vials
• Aseptically collect 4-5 ml of venous blood.
• Allow blood to clot at room temperature, centrifuge at 2000 rpm to separate serum. Collect the serum in clean dry vial
• Use adhesive tape marked with pencil, indelible ink, or a typewritten self
adhesive label to identify the container. The name of the patient, identification number and date of collection must be indicated on the label.

- All clinical samples should accompany the clinical information as per proforma. (Annexure)

### 11.4 Transportation of samples

- Transport specimens to the laboratory at 2 – 8°C (ice box) as soon as possible. Do not freeze whole blood, as haemolysis may interfere with serology test results.
- If more than 24-hour delay is expected before specimens can be submitted to the laboratory, the serum should be separated from the red blood cells and stored at refrigerated temperature.
- Samples for virus isolation and molecular diagnosis should always be stored frozen.

### 12. Notification

Dengue has become a major international public health concern in recent years. Dengue/ DHF is widely prevalent in India and all four dengue serotypes are known to exist. Dengue infections have the potential of rapid spread resulting in an acute public health problem. Therefore, special attention is required to be paid for its surveillance, prevention and control. Health authorities should take appropriate and anticipatory actions to prevent DF/DHF outbreak and should it occur, they must be geared up and be in a state of epidemic preparedness to minimize the impact of outbreak in terms of morbidity and mortality.

Cases of DF/DHF must be reported monthly to Directorate of National Vector Borne Disease Control Programme (NVBDCP), 22 Sham Nath Marg, Delhi – 110054; through the concerned state nodal officer. A report may be endorsed to the Director, National Institute of Communicable Diseases (NICD), 22-Sham Nath Marg, Delhi – 110054; Phone: 23971272, 23913148; Fax : 23922677; Telegram: COMDIS, Delhi.

If there is a sudden increase of clustering of cases or deaths due to DF/DHF, it must be reported immediately to the district health office or to the immediate supervisor. The district health office must inform the concerned health officer by the quickest mode of communication.
**Proforma for a Case of Dengue Fever/DHF/Dengue Shock Syndrome**

Name of the Hospital/Institution: 

Date: 

MRD/CR No.: 

Case Investigation:

Name: 

Age: 

Sex: M/F 

Father's/Mother's Name 

Address 

Whether visited any other area during last one month 

Any other person ill with fever in the family 

Signs and Symptoms 

Date of Onset of Fever: 

Date of Admission: 

Course of Fever: Continuous/Intermittent/Remittent 

Presenting Symptoms: 

Haemorrhagic Manifestations: Yes/No 

Petechiae, Purpura, Ecchymosis 

Epistaxis, Gum bleeding 

Haematemesis, Malena 

Enlarged Liver: Yes/No 

Tourniquet Test: Positive/Negative/Not done 

Rash: Yes/No 

Shock: Yes/No 

Condition of Patient: Stable/Critical 

Any Platelet or Blood Transfusion given: 

Laboratory Findings: 

<table>
<thead>
<tr>
<th>Test</th>
<th>Serial Readings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematocrit (Percentage)</td>
<td>1</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>2</td>
</tr>
<tr>
<td>Differential Leucocyte Count</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Acute Sera Collected on Date</td>
<td>Sent on date</td>
</tr>
<tr>
<td>Convalescent Sera Collected on Date</td>
<td>Sent on date</td>
</tr>
<tr>
<td>Outcome of the Patient</td>
<td>Recovered/Expired/Discharged on</td>
</tr>
</tbody>
</table>

(Signature) 
Medical Officer
PREVENTION AND CONTROL OF DENGUE FEVER

The strategy for Dengue prevention and control

There is no curative medicine or effective vaccine available for Dengue/DHF the only effective method of choice is vector control through intersectoral collaboration and active community involvement. The mortality due to dengue/DHF can be minimized by early diagnosis and prompt management of the cases.

- Surveillance for clustering and/or increase of fever cases and entomological parameters.
- Early detection, proper and prompt management of Dengue/DHF cases to minimize mortality.
- Vector control through source reduction, observation of a weekly dry day and personal protection
- Implementation of civic building bylaws to prevent mosquitogenic conditions in all municipal and corporation areas.
- IEC activities and awareness generation for community involvement for source reduction, clearing domestic and peri domestic areas of unused containers, tyres, coconut shells, broken glassware etc. which can collect water and personal protection.

Entomological surveillance of Dengue/DHF

Introduction

Dengue virus causing Dengue/DHF is widely distributed in the tropical and subtropical regions of the world. It has been reported that over 100 countries with
approximately 200 million population is at risk. Annually there are millions of cases and at times tens of thousands of deaths. Dengue/DHF has become the most important mosquito-borne viral disease in the world. It affects young and old, rich and poor alike, especially those living under unsanitary conditions in densely populated urban areas throughout the tropics and sub-tropics in the world.

Dengue fever and in particular life threatening DHF, often occurs in large epidemics. The disease spreads rapidly, affecting a large population during the outbreak situations, resulting in reduced work productivity and most importantly loss of lives. It has been reported that upto 20% of people with severe Dengue/DHF may die, if not properly diagnosed and treated.

**Entomological surveillance**

In order to prevent the occurrence of Dengue/DHF, entomological surveillance should be undertaken in the urban, semi-urban and rural areas. An in-depth integrated entomological and epidemiological surveillance is needed to help in the forecasting of Dengue/DHF outbreaks. Due to change in ecology, cultural and social behaviour of rural population, changed life style, availability of civic amenities like electricity and tap water supply, the disease has also invaded and spreading fast in rural areas of the country. Thus entomological surveillance is necessary in rural areas also to prevent transmission of the disease.

**Selection of survey area:** Areas in congested locality is cities, towns, and in rural areas may be taken up for carrying out entomological surveillance. The entomological surveillance should be carried out in those areas which have reported Dengue cases previously. Central congested areas of the town should be selected for the initial survey.

**Time and frequency of the survey:** The survey will be carried out during pre-monsoon, monsoon and post-monsoon seasons. Monthly survey during pre-monsoon and weekly or fortnightly survey may be undertaken during the monsoon and post-monsoon periods. During outbreak situation, the surveillance should be undertaken on daily basis in the affected and in the surrounding areas till the outbreak is over.
Important entomological indices for survey: The most common survey methodologies employed are larval sampling rather than egg or adult surveys. The basic sampling unit is the house or premises which is systematically searched for water holding containers and examined for the presence of mosquito larvae and pupae. The following indices are used for entomological surveillance of *Aedes* larvae:

**House Index:** No. of houses found positive for *Aedes* larvae  × 100
No. of houses searched

**Container index:** No. of containers found positive for larvae  × 100
No. of wet containers searched

**Breteau index:** No. of positive containers  × 100
No. of houses checked

**Adult landing/biting rate:** *Aedes* mosquito can be collected on a human bait and landing rate /bait /hour is calculated. The mosquitoes thus collected can be used for the virus isolations.

**Epidemiological interpretation of entomological indices**

<table>
<thead>
<tr>
<th>Entomological indices</th>
<th>High risk of transmission</th>
<th>Low risk of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) Larval House index</td>
<td>&gt;10%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>ii) Breteau Index</td>
<td>&gt;50</td>
<td>&lt;5</td>
</tr>
<tr>
<td>iii) Landing/Biting rate</td>
<td>&gt; 2 per man hour</td>
<td>&lt;0.2 per man hour</td>
</tr>
</tbody>
</table>

The survey are undertaken to detect the change in the population density and prevalence of vectors. Various indices like House index, Container index, Breateau index of *Aedes* mosquitoes should be monitored for each locality surveyed.

**Sample size:** A minimum of 50-100 houses should be searched for *Aedes* mosquitoes in a given locality. If 25 houses are continuously found negative for *Aedes* breeding then a change of the locality (at least 100 mtrs.) away from the previous one is required.
Methodology of surveillance: The survey should be carried out in a given locality by searching breeding of *Aedes* mosquitoes in different water containers lying in domestic or peri-domestic situations in an area. All types of water container should be thoroughly checked for *Aedes* breeding with the help of torch light and if found positive some of the larvae may be picked up for the confirmation of *Aedes* larvae.

Adult *Aedes* mosquitoes can be collected on human bait. The worker can collect mosquitoes from his own body (exposed legs) or that of his helper. The collection should be carried out between 9.00 and 11.00 hrs. The males should not be counted while calculating the landing/Biting rate.

Material/equipments required for surveillance: Following material equipments required for entomological surveillance of Dengue vectors:

Guidelines for Prevention and Control of Dengue