

# **Clinical and Laboratory Guidelines for Dengue Fever and Dengue Haemorrhagic Fever/Dengue Shock Syndrome for Health Care Providers**

Produced by the

Caribbean Epidemiology Center (CAREC)  
Pan American Health Organization  
World Health Organization

## **General aspects**

Dengue is an endemic tropical viral disease in many areas in the World including the Caribbean and the Americas. Although cases may be detected all year-round, the number of cases is clearly related to cyclic changes in weather: an increase in the number of cases usually follows the onset of the rainy season. Occasionally this gives rise to major outbreaks that may involve one or more Caribbean islands.

The agent of Dengue is the Dengue virus, a member of the Flavivirus group. There are four acknowledged types of Dengue Viruses designated as types 1, 2, 3 and 4. Although these four types share common antigens, antibodies against each of these types are only able to neutralize the same type that elicited the response. Periodic epidemics are associated with the emergence or re-emergence of different serotypes. On average in the Caribbean major outbreaks by a given type tend to re-occur in the same country every decade. Also the reinfection of an individual by a different type (heterotypic reinfection) may trigger complex immunopathologic mechanisms leading to the two most severe manifestations of the disease: Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).

Dengue Viruses are transmitted by mosquitoes of the *Aedes* group. These are relatively small mosquitoes that feed exclusively in humans. Predominantly on humans and less so on other animals. They tend to bite during the day and are usually found resting in dark places inside human housing. They breed in small deposits of relatively clean water in or around human housing (flower pots, saucers under plant pots, old tires, etc.)

Currently, Dengue virus types 1, 2, 3 and 4 are circulating in the Caribbean. Dengue type 3 re-emerged in CAREC Member Countries (CMCs) in 1998 after several decades of absence from the region. Type 3 isolates have been detected in several CMCs to date, including, Jamaica, Belize, Barbados, British Virgin Islands, Antigua, Dominica and St. Kitts and Nevis.

## **Description**

### ***Dengue Fever***

#### Clinical Description

Sudden onset

High fever (> 100° F)

Headache / myalgias

Retro-orbital pain

Lymphadenopathies (cervical/occipital)

Maculo-papular rash

Other associated elements may include upper/lower respiratory involvement, pharyngitis, vomiting and diarrhea

#### Haematology laboratory results

Leukopenia

#### Differential diagnosis

Influenza

Acute viral exanthems (Measles, Rubella)

Leptospirosis

### ***Dengue Haemorrhagic Fever (DHF)***

#### Clinical description

Possibly more frequent in children and young adults

Similar onset as Dengue Fever

Complications usually start when fever subsides

Facial flush

Epigastric and abdominal pain

Hepatomegaly

Haemorrhagic tendencies

Petechiae, Bruises, Hematuria, Hematemesis, Epistaxis, Melena/Blood in stools, Gingival bleeding

Positive tourniquet test

#### Haematology laboratory tests

Platelet count < 100,000/mm<sup>3</sup>

Elevated haematocrit (hemoconcentration) > 20% the average value for the age

#### Differential diagnosis

Leptospirosis

Acute abdomen

Other forms of purpura or viral hemorrhagic diseases

### ***Dengue Shock Syndrome (DSS)***

#### Clinical description

Similar onset as Dengue Fever

Patient deteriorates after a few days of fever

Cold, clammy skin, cyanosis

Tachycardia, low pulse pressure or hypotension

Abdominal tenderness

Recurrent lipothymias (repeated fainting)

- Altered mental status (restlessness)
- Oliguria
- Hypotension
- Encephalopathy
- Coma
- Laboratory results
  - Increased hematocrit (hemoconcentration)
  - Metabolic acidosis
- Differential diagnosis
  - Acute abdomen
  - Septicemia
  - Acute Meningococemia

### **Case definitions**

*(When met, the cases are reportable to Public Health authorities)*

#### ***Dengue Fever (DF)***

##### **Probable**

An acute febrile illness with two or more of the following manifestations:

- Headache
- Retro-orbital pain
- Myalgias
- Arthralgias
- Rash
- Haemorrhagic manifestations
- Occurring at the same location and time as other confirmed cases of dengue.

##### **Confirmed**

A case with at least one of the following:

- An Haemagglutination In hibition titer equal or higher than 1280
- Sero-conversion (four-fold change in titer) by haemagglutination inhibition
- Positive for IgM anti-Flavivirus antibodies during a time consistent with the occurrence of the disease
- Isolation and typing of a dengue virus from an early blood specimen.

#### ***Dengue Hemorrhagic Fever (DHF)***

##### **Probable**

- Recent history of fever
- Haemorrhagic tendencies (see above)
- Thrombocytopenia at or below 100.000/ mm<sup>3</sup>
- Haematocrit 20% above average for that age, sex and population

##### **Confirmed**

Add the same criteria as for confirmed cases of DF.

## Dengue Shock Syndrome (DSS)

### Probable

All four criteria for DHF plus elements of shock:

Cold, clammy skin

Rapid weak pulse

Narrow pulse pressure or hypotension

### Confirmed

Add the same criteria as for confirmed cases of DF

## Laboratory Diagnosis

### General laboratory

One clinical manoeuvre (*tourniquet test*) and two laboratory studies performed in a general laboratory (*platelet counts* and *hematocrit*) are crucial to the diagnosis of dengue hemorrhagic fever: These studies should be requested as soon as DHF/DSS is suspected and should be repeated as appropriate during the follow-up of the patient. If hematocrit is not available, haemoglobin determination can be used to detect hemoconcentration, however, it is usually less sensitive.

### Specialized laboratory

CAREC offers the following virological tests for confirmation of DF/DHF/DSS:

#### Virus isolation (serum taken within 3 days after onset)

This study requires serum taken from the patient within three days of onset (hence the importance of indicating the date of onset and the date of collection in the submitting form). The serum should be separated from the clot and should never be frozen but should be kept refrigerated (4° C) or on ice until its reception in CAREC. This test may take several weeks as it requires serial passages in tissue cultures (in mosquito cell lines).

#### IgM ELISA (serum taken after 1 week of onset)

IgM antibodies rise quickly and fade down several weeks after the infection. Although IgM antibodies can appear very fast after infection, they can be consistently detected in most patients only after the first week. CAREC recommends the use of sera taken at least five days after onset. In earlier sera the result is diagnostic only if positive. A positive result indicates that the patient has been exposed to the virus in the recent past. The test is unable to identify the viral type. Strictly speaking, the test is not specific to Dengue as there can be cross-reaction with other Flavivirus as (Yellow Fever, Yellow Fever Vaccination and other Flavivirus or more rare occurrence in the Caribbean). However, in conjunction with the clinical presentation and the epidemiological knowledge it can strongly support the diagnosis of Dengue virus infection.

#### Hemagglutination Inhibition Assay (HAI) (acute serum if DHF/DSS is suspected)

This test detects IgG antibodies. If paired sera are provided it can be used to determine HAI titers in both the acute and convalescent sera and a four-fold increase in titer is diagnostic of primary dengue infection. Often individuals that were previously infected by a different Dengue virus type will have very high titers in their acute serum (equal or higher than 1280). This test is provided for cases suspected of DHF/DSS, but results are delayed in comparison

with Dengue IgM.

### ***Information required by the laboratory***

***! The submission form to accompany a sample should be completed thoroughly. In particular the laboratory requires data to identify the patient and the treating physician, the clinical diagnosis, a summary of the signs and symptoms, a clear indication if DHF/DSS is suspected, the date of onset, the date of collection and the indication whether Yellow Fever vaccine has recently been received.***

### **Dengue Laboratory Surveillance**

The laboratory in CAREC is committed to provide the best support available to the region to predict, detect, investigate, monitor and evaluate Dengue outbreaks. However, the resources of the laboratory are limited and need to be strategically assigned. In practical terms this translates into a strategy for Public Health diagnosis aimed at maximizing utilization of resources and the impact of laboratory results while avoiding the overwhelming of laboratory resources. This strategy consists in the successive application of four modes of laboratory participation in support of the epidemiological study of Dengue outbreaks.

1. **Baseline mode** / No outbreak

Between outbreaks or in the absence of any recognizable outbreak samples from all cases suspected of dengue and samples from isolated cases should be sent to the laboratory for virological confirmation. These samples are valuable to determine the most prevalent types in each community. Also, most shifts in the most prevalent type(s) occur during this phase.

2. **Investigative mode** / Pre-diagnostic mode / Outbreak suspected

When the number of probable cases with clinical diagnosis of Dengue are increasing the onset of an outbreak should be suspected. In this scenario the laboratory requires the fast submission of a sample of patient sera (<100 sera) drawn within 3 days after the onset. These sera are required to isolate and identify the viral type causing the outbreak.

3. **Monitoring mode** / Post-diagnostic mode / Outbreak already confirmed

After the virus causing the outbreak has been identified there is little need to continue to confirm all cases of dengue fever. In these circumstances the clinical diagnosis of dengue fever reinforced by the epidemiological diagnosis should provide the basis for firm diagnosis and patient management. In these circumstances PAHO recommends the following:

***During an epidemic when the clinical syndrome and the disease diagnosis are established and the infecting serotype(s) has/have been identified, it is a misuse of resources to attempt serologic or virologic confirmation of every suspected dengue case. Laboratory resources should be focussed on identifying new areas where the disease might be spreading, detecting new serotypes coming into already infected areas, and monitoring severe and fatal cases attributed to dengue.≈***

PAHO Scientific Publication No. 548, pp 26, PAHO, 1994.

During this period CAREC suggests the submission of reasonable numbers of two kinds of specimens:

- a. Sera drawn within 3 days of the onset of symptoms for viral isolation with the purpose of monitoring the prevalence of different viral types, and
- b. Sera drawn on the second week after the onset of symptoms with the purpose of using single-sera IgM ELISA results to monitor the size of the outbreak.

**4. Evaluation mode / Post-outbreak mode / Surveying mode**

Immediately following the outbreak, from an epidemiology point of view the Public Health service of the country may wish to conduct population-based surveys with the aim, for example, of estimating the true incidence of the disease. In this case the numbers and type of the samples being requested will be part of the design of the epidemiological study, to be determined in consultation with CAREC.

**5. Post-outbreak switch / return to baseline mode**

After the outbreak has subsided there is a need to explicitly indicate to Public Services and physicians the return to the baseline mode, and thus resume the normal submission of specimens for viral diagnosis.

**N.B.: Complicated cases (DHF/DSS)**

**The modes outlined above only apply to Dengue Fever cases. Complicated cases of suspected DHF and DSS should continue to be admissible in the laboratory throughout the outbreak cycle. In these cases sera should be submitted to CAREC without delay accompanied by appropriate clinical data. Virus isolation, IgM Elisa and Haemagglutination inhibition will be performed as appropriate depending on the date of onset and the date in which the serum sample was taken.**

**Note: CAREC does not offer platelet counts and hematocrit. However, it is essential that these studies be performed in these patients both for diagnosis and to monitor their recuperation and convalescence.**

**Children:**

**Samples from children will be accepted at all times once appropriate information is provided to facilitate testing.**

**Post-mortem cases**

Non-fixed tissue samples in saline solution or PBS should be submitted to the laboratory as soon as possible after death. The samples to consider include heart blood, liver, kidney and spleen).

**Patient Management**

Note: In all cases: the patient is infectious to *Aedes* mosquitos which should be prevented from feeding on the patient, e.g. through use of an insect repellent and through use of an insecticide spray inside the house and bedroom.

Probable diagnosis of **Dengue Fever**

Home or ambulatory treatment

Analgesics and antipyretics (no aspirin! Due to risk of bleeding)

Paracetamol

1 yr - 60 mg/dose

1-3 yr - 20-120 mg/dose

3-6 yr - 120 mg/dose

6-12 yr - 240 mg/dose

Watch for signs of DHF/DSS (see below)

Probable diagnosis of **Dengue Hemorrhagic Fever**

Hospitalized (outpatient observation room or rehydration ward)

Analgesics and antipyretics as for the preceding group

Serial hematocrit and platelet counts (daily)

Fluids ingested by mouth

WHO Oral rehydration solution

Fruit juices

Monitor for signs of shock (shock usually occurs after the third day during transition from febrile to afebrile phases)

I/V rehydration therapy

the fluid and its volume should be determined according to the degree of dehydration and electrolyte loss.

Probable diagnosis of **Dengue Shock Syndrome**

Hospitalized (Intermediate treatment room)

Analgesics and antipyretics as for the preceding group

Serial hematocrit and platelet counts (daily) to monitor treatment and recovery

I/V resuscitation therapy

Ringer's acetate or 5% glucose in PSS at a rate of 10-20 ml/kg of body weight per hour (or as fast as possible).

If shock persists after 20-30 ml/kg of body weight add a plasma expander at the rate of 10-20 ml/kg per hour.

If shock persists significant internal bleeding should be suspected

Continuation of intravenous therapy should be adjusted according to hematocrit and the rate should be reduced to 10 ml/kg per hour. In general there is no need to continue the therapy beyond 48 hours.

**Table 1**  
**Algorithm for the virological diagnosis of Dengue**

Outbreak mode	Clinical Diagnosis	Specimen	Time of collection	Storage	Shipmen t	Test of choice	Delay for report(*)
Baseline, investigative and evaluation modes	DF	Serum	Less than 4 days after onset	Let blood clot at room temperature then store at 4°C.	Ship on ice (at 4°C)	Virus isolation	2-4 weeks
			RT-PCR			2-7 days	
		Serum	After 1 week after onset			IgM ELISA	1-7 days
Monitoring mode	DF	Call the laboratory before taking and submitting the sample as laboratory resources may be overwhelmed					
Always, regardless of the mode/phase of the outbreak	DHF/DSS	Serum	Less than 4 days after onset	Let blood clot at room temperature then store at 4°C.	Ship on ice (at 4°C)	Virus isolation	2-4 weeks
						RT-PCR	2-7 days
						HAI - 1st serum	Result will be reported in 1- 7 days only if titer is high. Otherwise the laboratory will wait for 2nd serum
		IgM Elisa	1-7 days				
		HAI - 2nd serum	1-7 days				
	Post- mortem	Heart blood, liver, kidney or spleen	As soon as possible after death	In sterile container with saline or PBS.	Virus isolation	2-4 weeks	
					RT-PCR	2-7 days	

(\*) These are normal delays after the sample is received in CAREC



**Table 2**  
**Interpretation of test results**

Type of test	Specimen	Positive result	Negative result
Virus isolation and typing	Serum taken less than 4 days after onset	Confirms etiology and identifies the Dengue virus type	Does not rule out Dengue infection. The rate of false negatives depends mainly on the conditions of shipment
RT-PCR (reverse transcriptase-polymerase chain reaction)			Under investigation. The general impression is that under routine conditions it will yield less false negatives than virus isolation
IgM ELISA	Serum taken after the first week of onset	Confirms that the etiology is a Flavivirus (*)	False negatives are common in sera taken very close to the onset of the disease (**)
Hemagglutination Inhibition Assay (HAI)	Single serum taken within the first week after onset	Single serum: Titer $\geq 1280$ Suggestive of secondary infection	Single serum: titer $< 1280$ Not informative
	Paired sera: 1st serum taken during the first week after onset and the 2nd one taken 10 to 15 days after the 1st.	Paired sera: 4-fold increase in titer Confirms that the etiology is a Flavivirus (*)	Paired sera: less than 4-fold increase in titer Rules out that the etiology is a Flavivirus

(\*) Together with Clinical presentation and epidemiological knowledge it confirms a recent exposure to a Dengue virus.

(\*\*) This is the reason to prefer sera taken after the first week.

**Representation of Temporal Occurrence of Virus, IgM & IgG Antibodies  
in Persons infected with the Dengue Virus**



