ABSTRACT

Dengue fever, a mosquito-borne tropical disease and is also known as breakbone fever, caused by the dengue virus. Symptoms include fever, headache and joint pains and a characteristic skin rash that is similar to measles. In a minor proportion of cases, the disease develops into the life-threatening dengue hemorrhagic fever, resulting in bleeding, thrombocytopenia and plasma leakage, or into dengue shock syndrome, where dangerously low blood pressure occurs. There are five strains of the virus, called serotypes, of which the first four are referred to as DENV-1, DENV-2, DENV-3 and DENV-4 (DENV-Dengue Virus). The fifth type (not named) was announced in 2013. The distinctions between the serotypes are based on their antigenicity. Dengue is one of the most rapidly spreading mosquito-borne viral diseases in the world. In the last 50 years, the incidence of dengue infection has increased almost 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural settings. This increasing rate of incidence has led to the development of various diagnostic and treatment modalities for the different serotypes of dengue virus. Numerous direct methods like Virus isolation, Genome detection, NS (Nonstructural protein) 1 detection, etc. and certain direct methods like Serology IgM, Serology IgG are routinely used for in the laboratories for detection of dengue infection. The time for detection ranges from 30 minutes as in IgM rapid test to 1-2 weeks as in Viral isolation and Serotype identification. Clinical management of dengue depends on the condition of the patient, and it ranges from management at home to immediate admission to
the hospital. This review highlights the current and future trends in the diagnosis along with the various treatment options available for dengue.

**KEYWORDS:** Dengue Fever, ELISA, IgM/IgG, Antigen NS1.

**INTRODUCTION**

Dengue is a mosquito-borne arboviral infection that places a significant socioeconomic and disease burden on many tropical and subtropical areas of the world.\(^1,2\) It is currently regarded as one of the most important arboviral disease internationally as more than 50% of the world’s population live in areas where the risk of the disease is very high, and approximately 50% live in countries known to be endemic for dengue.\(^2-6\)


Dengue is found in various regions especially the tropical and subtropical regions all around the world, especially in urban and semi-urban areas. Disease is caused by a virus belonging to the family Flaviviridae and is spread by the Aedes aegypti mosquitoes. An estimated 50-60 million dengue infections occur worldwide annually. Dengue and DHF i.e. Dengue Haemorrhagic Fever is endemic in around 100 countries in the WHO regions of Africa, the Americas, South-East Asia and the Western Pacific regions. The South-East Asia along with the Western Pacific regions is the most seriously affected. The contour lines indicated in
figure 2 indicated that in northern and southern hemisphere survival of Aedes aegypti, is round the year.

(Source: http://gamapserver.who.int/mapLibrary/Files/Maps/Global_DengueTransmission_IT HRiskMap.png).

Figure 2: Country wise risk of dengue transmission.

Laboratory Diagnosis and Diagnostic Tests
Efficient and accurate diagnosis of dengue is of primary importance for clinical care (i.e. early detection, case confirmation and appropriate management), surveillance activities, outbreak control, pathogenesis, vaccine development and clinical trials.

Laboratory diagnostic methods for confirming dengue virus infection may include detection of the virus, viral nucleic acid, antigens or IgM/IgG antibodies, or a combination of them. After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells. During the early stages of the infection, virus isolation, nucleic acid or antigen detection can be used to diagnose dengue. At the end of the acute phase of infection, serology is the best method for diagnosis.

Antibody response to infection varies according to the immune status of the host.\(^{7,8}\) IgM antibodies are the first immunoglobulin (Ig) isotype to appear. These antibodies are detectable in serum/plasma of 50\% of patients by 3\(^{\text{rd}}\) to 5\(^{\text{th}}\) day after onset of illness, increasing to 80\% by day 5 and 99\% by the end of day 10.
Figure 3: Approximate time-line of primary and secondary dengue virus infections and the diagnostic methods that can be used to detect infection.

Serum IgG is generally detectable at low titres at the end of the first week of illness, increasing slowly, with serum IgG still detectable after several months, and probably even for life.[9,10,11,1]

During a secondary dengue infection, the predominant immunoglobulin isotype is IgG which is detectable at high levels, even in the acute phase of the disease and persists for periods which may last from 10 months to almost the whole life. IgM levels in the early convalescent stage are significantly higher in primary infections than in secondary ones.

(Source: WHO Dengue: Guidelines for Diagnosis and Prevention, 2009).

Figure 4: Comparison of diagnostic tests according to their accessibility and confidence.

(Source: https://asiagendenguetestkit.wordpress.com/principles-asigen-dengue-combo-rapid-test-kit/).
Considerations in the Choice of Diagnostic Methods
Prior to day 5 of illness, during the period of febrile illness, dengue infections may be diagnosed by virus isolation in cell culture, or even by detection of viral RNA by Nucleic Acid Amplification Tests (NAAT), or by detection of viral antigens by ELISA (Enzyme Linked Immunosorbent Assay) or some rapid tests. Virus isolation in cell culture is generally performed only in laboratories with the necessary infrastructure and technical expertise. Several days are needed for the isolation and identification of dengue viruses in cell cultures. Nucleic acid detection assays with great performance characteristics may identify dengue viral RNA within 24–48 hours. Kits for NS1 antigen detection are now becoming commercially available and can be used in laboratories even with limited equipment and yield results within a short span of a time like a few hours.

Table 1: Summary of operating characteristics of dengue diagnostic methods[9-16]

<table>
<thead>
<tr>
<th>Diagnostic Methods/Techniques</th>
<th>Diagnosis of Acute Infection</th>
<th>Detection period</th>
<th>Specimen</th>
<th>Peak duration for assay sensitivity after onset of symptoms</th>
<th>Technology availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral Isolation and Serotype Identification</td>
<td>Confirmed</td>
<td>1-2 weeks</td>
<td>Whole blood, Serum, tissues</td>
<td>1-5 days</td>
<td>Mosquito or cell culture facilities, fluorescence microscope</td>
</tr>
<tr>
<td>Nucleic acid detection</td>
<td>Confirmed</td>
<td>1 or 2 days</td>
<td>Tissues, whole blood, serum, plasma</td>
<td>1–5 days</td>
<td>Equipment for molecular biology</td>
</tr>
<tr>
<td>Antigen detection</td>
<td>Not yet determined/ Confirmed</td>
<td>1 day</td>
<td>Tissues, whole blood, serum, plasma</td>
<td>1-6 days</td>
<td>ELISA facilities</td>
</tr>
<tr>
<td>IgM ELISA</td>
<td>Intermittent</td>
<td>1-2 days</td>
<td>Serum, plasma, whole blood</td>
<td>After 5 days</td>
<td>ELISA facilities</td>
</tr>
<tr>
<td>IgM rapid test</td>
<td></td>
<td>30 minutes</td>
<td>Serum, plasma, whole blood</td>
<td></td>
<td>Rapid Card (ICT) method</td>
</tr>
<tr>
<td>IgG ELISA, HAI or neutralization test</td>
<td>Confirmed</td>
<td>7 days or more</td>
<td>Serum, plasma, whole blood</td>
<td>Acute sera, 1–5 days; Convalescent after 15 days</td>
<td>ELISA facilities</td>
</tr>
</tbody>
</table>

Current Dengue Diagnostic Methods
A) Virus isolation
Specimens for virus isolation should be collected early in the period of the infection, during the period of viraemia which is generally before day 5. Virus may be recovered from serum, plasma and peripheral mononuclear cells in the blood and it may be attempted made from tissues collected at autopsy (e.g. liver, lung, and bone marrow). Cell culture is the most widely used method for dengue virus isolation. Virus isolation which is usually followed by
an immunofluorescence assay for confirmation of the infection generally requires 1–2 weeks.\[17,18\]

**B) Nucleic acid detection**

RNA is heat-labile and therefore nucleic acid detection specimens must be handled and stored according to the procedures described for virus isolation.\[18,19\]

**C) RT-PCR**

Reverse transcriptase-polymerase chain reaction (RT-PCR) assays offer better sensitivity compared to virus isolation with a much faster turnaround time. In situ RT-PCR provides the ability to detect dengue RNA. Compared to virus isolation, sensitivity of RT-PCR methods varies from 80% to 100%.\[19,20\]

**D) Real-time RT-PCR**

The real-time RT-PCR assay is a one-step assay method which can be used to quantitate viral RNA and using primer pairs and certain assay probes that are specific to each and every dengue serotype. The use of a fluorescent probe enables the identification of the reaction products in real time, in a specialized PCR machine, without needing electrophoresis. An advantage of this method is the ability to estimate viral titre in a sample, which may be used to study the pathogenesis of dengue disease.\[19,20\]

**E) Isothermal amplification methods**

The NASBA (nucleic acid sequence based amplification) assay is an isothermal RNA specific amplification assay that works without thermal cycling instrumentation. NASBA has been adapted to dengue virus detection with sensitivity almost near to that of the virus isolation in cell cultures and may be a useful diagnostic method for studying dengue infections in field studies.\[21\]

**F) Detection of antigens**

New developments in ELISA (Enzyme Linked Immunosorbent Assay) and dot blot assays directed to the envelop/membrane (E/M) antigen and the non-structural (NS) protein 1 demonstrated that very high concentrations of these antigens as immune complexes could be detected in patients with both primary and secondary infections up to a maximum of nine days after the onset of illness.\[22\]
Serological tests

A) MAC-ELISA
For the IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) total IgM in patients’ sera is trapped by anti-µ chain specific antibodies coated onto a microtitre plate. Dengue-specific antigens, the four serotypes (DEN-1, -2, -3 and -4), are bound to the captured IgM antibodies and are detected by polyclonal or monoclonal dengue antibodies directly or indirectly conjugated with an enzyme that transforms a non-coloured substrate into coloured products. The measurement of optical density is done by spectrophotometer.[20,21]

B) IgG ELISA
This method is generally used for the diagnosis 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. This method can be used to detect IgG antibodies in serum or plasma and permits identification of a case as a primary or secondary dengue infection.[20,21]

Hematological tests
Blood platelets and haematocrit values are generally measured during the acute stages of dengue infection. A decrease in the platelet count below 1, 00,000 per µL may be observed in dengue fever but it is a constant feature of dengue haemorrhagic fever. Between day 3 and day 8 following the onset of illness a fall in the platelet count is usually observed.

Future Test Developments
Microsphere-based immunoassays (MIAs) are becoming increasingly popular as a serological option for the laboratory diagnosis of various diseases. This technique is based on the covalent bonding of antigen or antibody to microspheres or beads. Microarray technology makes it possible to screen a sample for many different nucleic acid fragments corresponding to various different viruses.

Table 2: Advantages and limitations of diagnostic methods of Acute dengue fever[21,22]

<table>
<thead>
<tr>
<th>Diagnostic Tests</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleic acid detection</td>
<td>-Most sensitive and specific</td>
<td>-Possible false positive due to contamination</td>
</tr>
<tr>
<td></td>
<td>-Possible to identify serotype</td>
<td>-Expensive</td>
</tr>
<tr>
<td>Isolation in cell culture</td>
<td>-Specific</td>
<td>-Needs expertise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Not possible to differentiate between primary and secondary dengue infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Need expertise and facility for cell culture</td>
</tr>
</tbody>
</table>
and identification using immuno-fluorescence

- Possible to identify serotype by using specific antibodies
- and fluorescent microscopy
  - Takes more than 1 week
  - Not possible to differentiate between primary and secondary dengue 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

- Useful for confirmation of acute infection
- Cheapest
- Technically, easy to perform
- Can distinguish between primary and secondary infection
- May miss cases because IgM levels may be low or sometimes undetectable in some secondary infections
- Need two samples
- Delay in confirming diagnosis

**Clinical Management of Dengue**

Reducing dengue mortality requires an organized process that guarantees early diagnosis of the disease and its management and referral as and when required. The main component of the process is the delivery of good clinical services at all levels of health care, from primary to secondary and tertiary levels. Most dengue patients recover without requiring hospital admission while some may progress to and manifest with severe disease.

**Disease notification**

In dengue-endemic countries, cases of suspected, probable and confirmed dengue infection should be notified as soon as possible so that appropriate public health care measures can be initiated. Laboratory confirmation is not generally required before notification, but should be obtained. In countries which are not endemic for dengue, usually only confirmed cases will be notified. Suggested criteria for early notification of suspected cases are that the patient resides at or has travelled to a dengue-endemic area, has fever for three days or more, has low or decreasing white blood cell (WBC) counts, and/or has thrombocytopenia ± positive tourniquet test.

**Management decisions**

Depending on the clinical manifestations and other certain other circumstances, patients may\[^{23}\] be sent home (Group A), or may be referred for in-hospital management (Group B), or require emergency treatment and urgent referral (Group C).

**Treatment according to groups A–C**

**Group A** – patients who may be sent home.\[^{24}\]
These are patients who are able to tolerate adequate volumes of fluids orally and are able to pass urine at least once every six hours and don’t show any of the warning signs, particularly when fever subsides.

Ambulatory patients should be monitored and reviewed daily for disease progression (decreasing white blood cell count and warning signs) until they are out of the critical period. Those who have stable haematocrit levels can be discharged from the hospital and sent home after being advised to return to the hospital urgently if they develop any of the warning signs and to adhere to the following action plan.

- Encourage intake of oral rehydration solution (ORS), fruit juice and other fluids containing electrolytes and glucose to replace losses from fever and vomiting. Adequate oral fluid intake may be able to reduce the number of hospitalizations.\[^{25,26,27}\]

- Give paracetamol for high fever if the patient is uncomfortable. The interval of paracetamol dosing should be at least for six hours. Tepid sponge if the patient still has high fever. Do not give aspirin, ibuprofen or other non-steroidal anti-inflammatory drugs (NSAIDs) as these drugs may aggravate gastritis or bleeding in the gastrointestinal tract. Also, acetylsalicylic acid (aspirin) may be associated with Reye’s syndrome.

**Group B** – patients who should be referred for in-hospital management.\[^{28,29}\]

Patients may need to be admitted to a secondary health care centre for close observation, especially if they reach a critical phase. These include patients with certain warning signs, and those with co-existing situations that may make dengue or its management more complicated (such as pregnancy, infancy, old age, diabetes, renal failure and chronic haemolytic diseases).

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

a) Obtain a base-line haematocrit before fluid therapy. Give only isotonic solutions such as 0.9% saline, Ringer’s lactate, or Hartmann’s solution. Start with 5–6 ml/kg/hour for 1–2 hours, then reduce to 3–5 ml/kg/hr for 2–4 hours, and then reduce to 2–4 ml/kg/hr or less according to the clinical response.

b) Reassessment of the clinical status should be done and the haematocrit measurement should be repeated. If the haematocrit remains the same or rises only minimally, continue with the same rate of infusion of the solution i.e. 2–3 ml/kg/hr for another 2–3 hours. If
the vital signs are worsening and haematocrit is rising very fast, increase the rate to 5–8 ml/kg/hour for 1–2 hours. Reassess the clinical status, repeat the haematocrit measurement and reassess the fluid infusion rates accordingly.

c) Give the minimum required volume of intravenous fluid to maintain optimal perfusion of vital organs and urine output of about 0.5-1 ml/kg/hr. Intravenous fluids are generally needed for only 24–48 hours. Reduce intravenous fluids slowly when the rate of plasma leakage declines towards the end of critical phase. This is indicated by urine output that is adequate, or haematocrit values which decrease below the baseline value in a stable patient.

**Group C** – patients who require emergency management and urgent referral when they have severe dengue.\(^{30,31,32}\)

Some patients require emergency treatment and should be referred urgently when they are in the critical phase of disease, i.e. when they are suffering with one or multiple condition/s mentioned below:

a) Severe plasma leakage which can lead to dengue shock and/or fluid accumulation with respiratory distress.

b) Severe petechial haemorrhages.

c) Organ impairment (hepatic impairment, renal impairment, cardiomyopathy, encephalopathy or encephalitis).

All patients with severe dengue should on an urgent basis be admitted to a hospital with access to intensive care facilities and blood transfusion. Adequate fluid resuscitation intravenously (i.v.) is the essential and usually single intervention required. The crystalloid solution should be more appropriately, isotonic and the volume just sufficient to maintain an effective circulation during the period of plasma leakage. Immediate replacement of plasma losses should be done with isotonic crystalloid solution or, in the case of hypotensive shock, colloid solutions.\(^{33,34,35}\)

**Treatment of dengue haemorrhagic shock**

The action plan for treating patients with compensated shock is as mentioned below.

a) Start intravenous fluid resuscitation preferably with isotonic crystalloid solutions at 5–8 ml/kg/hour 1-2 hours. Then reassess the patient’s condition (vital signs capillary refill time, haematocrit, urine output).
b) Follow-up closely with the patient and if the patient’s condition improves, intravenous (i.v.) fluids should be gradually reduced to 6–7 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 management with fluids depending upon the haemodynamic status of the patient, which can be maintained for up to the next 24–48 hours.

Management of Dengue by vaccine and antiviral drugs

a. **CYD (Chimeric Yellow Fever-Dengue vaccine)**

CYD-TDV is a live attenuated tetravalent chimeric vaccine which is made by using recombinant DNA technology by replacing the PrM (pre membrane) and E (envelope) structural genes of the yellow fever live attenuated 17D strain vaccine with those from each of the four dengue serotypes.\(^{[36,37]}\)

b. **DENVax (Dengue Vaccine)**

DEN-Vax is a recombinant chimeric vaccine with DENV1, DENV3 and DENV4 components on a dengue virus type 2 (DENV2) backbone developed at Mahidol University in Bangkok.\(^{[38]}\) There are approximately six dengue vaccine candidates under evaluation in various clinical trials. The vaccine candidate currently at the most advanced clinical development stage is a live attenuated recombinant tetravalent dengue vaccine. This has now been evaluated as a 3-dose series on a 0/6/12 month schedule in Phase III clinical trials and has been submitted for registration in several countries which are endemic for dengue infection. A recent detailed report provides an efficacy analysis and interim long-term follow up on CYD-14,15,57.

c. **Dengue Antiviral Drugs**

RNAi (RNA interference) is a sequence specific RNA degradation process that initiates with dsRNA. This pathway has led to the use of siRNAs against several viral diseases, especially ssRNA (single stranded RNA) genome containing viruses. Several researches have been conducted to use siRNA against DENV and many of them have given results of effectively using siRNA against DENV replication. Hopefully, in future siRNA approach may provide a much better and probably a cost-effective treatment that will lead to eradication of dengue infection.\(^{[39-42]}\)
Management of Dengue by herbs and goat/sheep milk

a. Carica papaya leaves extracts
Several studies were conducted to investigate the potential benefits of *Carica papaya* leaves extracts against Dengue fever due to their high complex protein content and their ability to help the bone marrow increase the production of platelets. Blood samples examined after administration of *C.* papaya leaves showed an increase in platelet count along with an increase in White Blood Cells (WBC) and Neutrophils in the blood after a twice daily administration (for a period of 5 days).[43]

b. Goat or sheep milk
Taking raw goat or sheep milk may have shown benefits in improving platelet count for many patients. But in some rare cases, this natural cure could cause bacterial infection that is deadlier than the dengue virus. This is because the milk from goat or sheep can contain harmful bacteria (e.g. Brucellosis) which when consumed causes severe infection. But medical professionals say that there has been no medical evidence to prove that it cures dengue.

Though Carica papaya leaves extract and Goat/Sheep milk are helpful for production of platelets, clinical and scientific statement on its authenticity is unclear. So it is necessary to know which component of papaya leaves and goat/sheep milk is responsible for production of platelet production. The scope of this traditional treatment of dengue fever should be studied at large scale to attempt a confirmatory statement and scientific evidence[43].

CONCLUSION
Dengue being a global health problem, proper diagnosis with the available state-of-the-art diagnostic modalities and instituting appropriate and timely treatment constitutes the basis of reducing the morbidity and preventing the transmission of the disease. Though nucleic acid detection is currently the most sensitive and specific test for detection of a specific serotype of dengue, it is expensive, so antigen detection and serological tests are gold standard methods. Dengue fever is usually a self-limited illness. There is no specific antiviral treatment presently available for dengue fever. Supportive care with analgesics, fluid replacement and bed rest are instituted for better health care delivery. Papaya leaves and goat/sheep milk is responsible for production of platelet production, so more focus is emphasized to know scientific validation.
REFERENCES


