CHAPTER 4

LABORATORY DIAGNOSIS

In endemic areas, early symptoms of dengue fever mimic many other prevalent diseases such as chikungunya, malaria, viral infection, urinary tract infection, typhoid, leptospirosis, etc. For proper management exclusion of these conditions is hence very crucial.

4.1 Laboratory diagnosis tests

Laboratory diagnosis can be carried out by one or more of the following tests.

4.1.1 ELISA-based NS1 antigen tests

Dengue NS1 antigen, a highly conserved glycoprotein which is produced in both membrane-associated and secretion forms, is abundant in the serum of patients during the early stages of DENV infection. It has been found to be useful as a tool for the diagnosis of acute dengue infections. It is a simple test that is more specific and shows high sensitivity.

NS1 enables detection of the cases early, i.e. in the viremic stage, which has epidemiological significance for containing the transmission.The NS1 ELISA-based antigen assay is commercially available for DENV and many investigators have evaluated this assay for sensitivity and specificity. The NS1 assay may also be useful for differential diagnostics between flaviviruses because of the specificity of the assay.

4.1.2 IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA)

MAC-ELISA has been widely used in the past few years. It is a simple test that requires very little sophisticated equipment. MAC-ELISA is based on detecting the dengue-specific IgM antibodies in the test serum by capturing them using anti-human IgM that was previously bound to the solid phase. This is followed by addition of dengue antigen if the IgM antibody from the patient's serum is anti-dengue, it will bind to the dengue antigen. An enzyme-substrate is added to give a colour reaction for easy detection.

The anti-dengue IgM antibody develops a little faster than IgG and is usually detectable by day 5 of the illness. However, the rapidity with which IgM develops varies considerably among patients. Some patients have detectable IgM on days 2 to 4 after the onset of illness, while others may not develop IgM for seven to eight days after the onset. In some primary infections, detectable IgM may persist for more than 90 days, but in most patients it wanes to an undetectable level by 60 days. It is reasonably certain, however, that the person had a dengue infection sometime in the past two to three months. MAC-ELISA has become an invaluable tool for surveillance of DF/DHF. In areas where dengue is not endemic, it can be used in clinical surveillance for viral illness or for random, population-based serosurveys, with the certainty that any positives detected are recent infections. It is especially useful for hospitalized patients, who are generally admitted late in the illness after detectable IgM is already present in the blood.

4.1.3 Isolation of dengue virus

Isolation of most strains of dengue virus from clinical specimens can be accomplished in the majority of cases, provided that the sample is taken in the first five days of illness and processed without delay. Specimens that may be suitable for virus isolation include acute phase serum, plasma or washed buffy coat from the patient, autopsy tissues from fatal cases, especially liver, spleen, lymph nodes and thymus and mosquitoes collected in nature. Isolation of the virus takes 7–10 days, hence it may not be very useful for starting the management of patients with DF/DHF.

4.1.4 Polymerase chain reaction (PCR)

Molecular diagnosis based on reverse transcription polymerase chain reaction (RT-PCR), such as one-step or nested RT-PCR, nucleic acid sequence-based amplification (NASBA) or real-time RT-PCR has gradually replaced the virus isolation method as the new standard for the detection of dengue virus in acute-phase serum samples.

4.1.5 IgG-ELISA

An IgG-ELISA has been developed that compares well to the hemagglutination-inhibition (HI) test. This test can also be used to differentiate primary and secondary dengue infections. The test is simple and easy to perform but not considered as a diagnostic test as it indicates past infections only.

4.1.6 Serological tests

Besides MAC-ELISA and IgG-ELISA, there are a few serological tests available for the diagnosis of dengue infection such as HI, complement fixation (CF) and neutralization test (NT). These are not commonly used due to various technical problems.

4.1.7 RDTs

A number of commercial RDT kits for anti-dengue IgM/IgG antibodies and NS1 antigen are commercially available, which give the results within 15 to 25 minutes. However, the accuracy of most of these tests is not known since they have not yet been properly validated. Some of the RDTs have been independently evaluated. The results showed a high rate of false positives compared to standard tests, while some others have agreed closely with standard tests. The sensitivity and specificity of some RDTs are also found to vary from batch to batch. According to WHO guidelines, these kits should not be used in clinical settings to guide management of DF/DHF cases because many serum samples taken in the first five days after the onset of illness will not have detectable IgM antibodies. The tests would thus give a false negative result. Reliance on such tests to guide clinical management could, therefore, result in an increase in the case–fatality ratio. Hence, use of RDT is not recommended under the programme.

4.2 Collection of samples

Laboratory diagnosis of dengue depends on proper collection, processing, storage and shipment of the specimens. While collecting blood for serological studies from suspected DF/DHF cases, all universal precautions should taken.

While sending the samples for lab confirmation, the day of onset of fever and day of sample collection should be mentioned to guide the laboratory for the type of test to be performed (NS1 for samples collected from day 1 to day 5 and IgM after day 5).

4.3 NVBDCP-recommended tests for laboratory diagnosis

– For confirmation of dengue infection, Government of India (GoI) recommends use of ELISA-based antigen detection test (NS1) for diagnosing the cases from the first day onwards and antibody detection test IgM capture ELISA (MAC-ELISA) for diagnosing the cases after the fifth day of onset of disease.[19]

– Directorate of National Vector Borne Disease Control Programme (NVBDCP), GoI has identified a network of laboratories (sentinel surveillance hospitals and apex referral laboratories) for surveillance of dengue fever cases across the country since 2007. These laboratories are also meant to augment the diagnostic facilities in all endemic areas. They are linked with Apex Referral Laboratories (ARLs) with advanced diagnostic facilities for backup support and serotyping of dengue samples. For details, please refer to NVBDCP website www.nvbdcp.gov.in.

– These laboratories receive the samples, diagnose and send the report (line list) regularly to districts/municipal health authorities for implementation of preventive measures to interrupt the transmission.

– NS1 antigen tests – GoI introduced ELISA-based NS1 antigen test in 2010 in addition to MAC-ELISA tests which can detect the case during day 1 to day 5 of illness.

4.4 Supply of kits

– IgM ELISA test kits (1 kit = 96 tests) are being provided to the identified laboratories through the National Institute of Virology (NIV), Pune since 2007. The cost is borne by GoI. Buffer stock is also maintained at NIV, Pune.

– For procurement of dengue NS1 antigen test kits, fund has been provided to the states. States are suppose to procure it as per GoI guidelines and provide the same to sentinel surveillance hospitals (SSHs) every year as per their technical requirement.