PAHO Pan American Health Organization World Health Organization Americas

Technical note
Algorithm for laboratory confirmation of dengue cases

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Dengue is transmitted through the bite of a mosquito infected with one of the four serotypes of the dengue virus (DENV-1, DENV-2, DENV-3, and DENV-4). DENV infection can affect people of all ages, occurring asymptomatically or producing various clinical manifestations that range from a mild fever to a disabling fever, accompanied by severe headache, eye, muscle, and joint pain, erythema, and even progress to severe forms, characterized mainly by shock due to significant plasma leakage. There is neither specific medicine to treat dengue, nor a recommended vaccine in the Region to be incorporated into national immunization programs.

The main vector responsible for transmitting dengue in the Americas is the *Aedes aegypti* mosquito, and currently nearly 500 million people in the Region live at risk of contracting dengue. The number of dengue cases in the Americas has increased in the last four decades, going from 1.5 million cumulative cases in 1980-1989 to 16.2 million in 2010-2019. The four DENV serotypes circulate throughout the Americas and in some countries they circulate simultaneously. Infection with one serotype followed by another infection with a different serotype increases a person's risk of developing severe dengue and even dying *(1)*.

The initial diagnosis of DENV infection is clinical, and adequate suspicion can guide the confirmation protocol. However, laboratory results should always be analyzed in conjunction with demographic, clinical, and epidemiological information, for surveillance purposes and not for making clinical decisions in the treatment of the patient.

Laboratory confirmation of dengue infection is based on virological tests (RNA detection by RT-PCR, NS1 antigen detection by ELISA[1], and in some cases viral isolation) and serological tests (IgM and/or IgG detection by ELISA) *(2)*. However, to confirm cases, virological tests that demonstrate the presence of the complete virus, its genetic material, or its proteins should be prioritized. In general, virological assays for dengue are performed on serum samples collected during the first 5 days after the onset of symptoms (acute phase), although highly sensitive molecular methodologies can detect viral RNA for up to 7 days depending on the viremia. Virus isolation is carried out mainly in cell culture or by inoculation of suckling mice and other rodents. However, viral isolation is not used for routine diagnosis nor is it a requirement for diagnostic confirmation and is primarily useful for additional characterization or reagent production.

On the other hand, serological assays based on the detection of IgM (or IgG) must be analyzed carefully, considering the time that antibodies circulate in the blood after an infection (which can be several months for IgM antibodies and years for IgG antibodies), as well as the possibility of cross-reaction with other flaviviruses (including Zika, yellow fever, and others) and nonspecific detection. Thus, a single IgM result in a patient only indicates possible recent contact with the virus, but this may have occurred up to 6 months before. A second paired sample, collected at least one week later, processed in parallel with the first using a quantitative serological assay (PRNT, for example) that shows a seroconversion or an increase in antibody titer may be useful to clarify the diagnosis. Because IgG antibodies are long-lived, the diagnostic value of IgG measurements in a single sample is limited. For the confirmation of an infection, it is necessary to detect an

[1] The detection of the NS1 protein using the ELISA technique is not considered a rapid test. The detection of NS1 by rapid (immunochromatographic) test is not confirmatory and is described below.

IgG seroconversion or a four-fold or greater increase in IgG titers between the acute sample and the convalescent sample.

It is important to have a clear laboratory algorithm that allows for early detection (Figure 1). Although multiplex molecular methodologies are useful when there is no clear clinical suspicion, in the case of a dengue case that meets the established definitions of a suspected case (2) and where the clinical symptoms are compatible, it is suggested to prioritize protocols for the specific detection (*singleplex*) of the virus.

In cases of neurological disease (e.g., encephalitis or other encephalopathies) with suspected dengue, detection of viral RNA (by RT-PCR) and IgM (by ELISA) can also be performed in cerebrospinal fluid (CSF) samples. For the IgM ELISA, it is recommended to process serum and CSF samples in parallel. The CSF sample is tested pure or slightly diluted (maximum dilution of 1:5). The presence of IgM in the CSF confirms a recent infection of the central nervous system, always considering the potential persistence of IgM antibodies and the probability of cross-reactivity between viruses of the same genus. The CSF sample should be only collected by clinical indication and not for the sole purpose of identifying the etiological agent.

In fatal cases, tissue samples (liver, kidney, lung, lymph node, thymus, bone marrow, and brain) can be considered for the detection of genetic material (RT-PCR) and for histopathological and immunohistochemical analyses. Collecting biopsies on a patient with suspected dengue for the sole purpose of identifying the etiological agent is completely contraindicated.

On the other hand, the use of rapid tests (NS1 and/or antibodies) is not recommended since their low sensitivity can lead to false negative results. If molecular or ELISA platforms are not available (e.g., in remote or difficult-to-access areas), it is important to keep in mind that, although a positive result for the detection of the NS1 antigen allows confirmation of the infection, a negative result does not rule it out. The detection of antibodies by rapid tests is not confirmatory and is subject to the same considerations previously stated. Furthermore, it is recommended that the rapid tests used have external validation (different from that offered by the manufacturer), or at least a performance evaluation. Where possible and available, molecular diagnosis and antigen detection by ELISA should be prioritized.

Since laboratory services are a key component of dengue epidemiological and virological surveillance, timely detection and characterization of appropriate samples must be maintained. As possible and according to the capacity of each laboratory, it is recommended to collect and process all cases of severe dengue and dengue with warning signs, while only a proportion (10-30% or a limited number of samples depending on capacity) of those dengue cases without alarm signs are necessary for surveillance purposes.

References

1. Pan American Health Organization. Topics: Dengue. Available at: https://www.paho.org/en/topics/dengue
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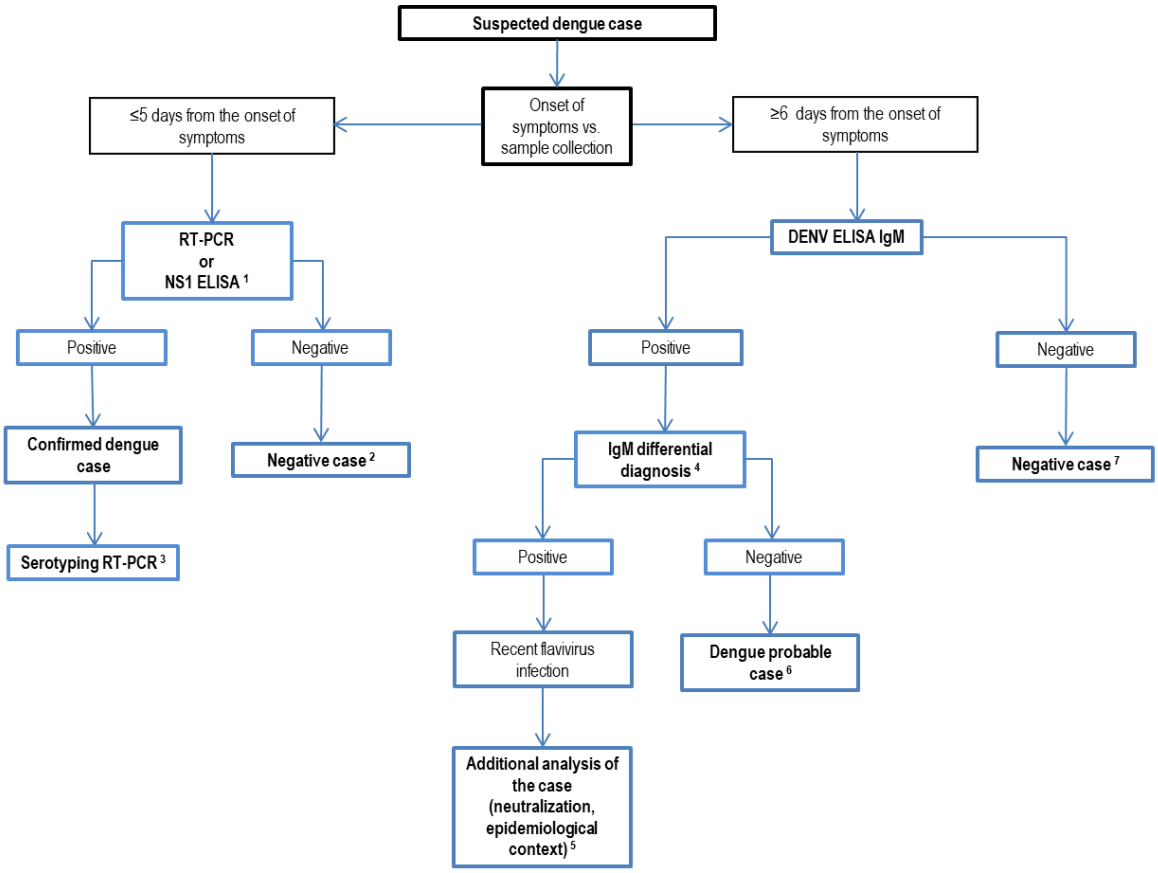

[1] RT-PCR is the recommended technique during the acute phase of the disease and its sensitivity allows the detection of viral RNA even for more than 5 days from the onset of symptoms. If RT-PCR is unavailable, detection of the NS1 antigen by ELISA can be used, considering it has a lower sensitivity than RT-PCR.
[2] In general, from the onset of symptoms on, a decrease in viremia with time is observed. This may affect the sensitivity of molecular (RT-PCR) and antigenic (NS1 ELISA) detection, particularly in samples collected after the fifth day from the onset of symptoms. In these cases, serological detection can be considered.
[3] This step is required only for cases confirmed with NS1 ELISA or an RT-PCR assay that does not differentiate viral serotypes.
[4] Consider the Zika virus, recent vaccination for yellow fever, as well as other flaviviruses depending on the epidemiological situation of the area/country.
[5] In cases of cross-reactivity, IgM ELISA results do not allow for the confirmation of the etiological agent. However, this result does not rule out a dengue virus infection. Additional clinical and epidemiological criteria should be used for the final case interpretation. Performing PRNT in a reference laboratory to analyze cross-reactive samples (ideally, in paired acute and convalescent samples) can also be considered.
[6] A positive IgM result in a single sample is not confirmatory and may be due to a dengue virus infection in recent months. Seroconversion in paired samples collected at least one week apart allows for dengue virus infection to be inferred, as long as no cross-reactivity with other flavivirus(es) is observed.
[7] IgM levels may be below the limits of detection in some secondary infections. Investigate the cases and perform the differential diagnosis.

Figure 1. Algorithm for laboratory confirmation of dengue cases.