Serologic Diagnosis of Dengue/
Dengue Haemorrhagic Fever

by

Duane J. Gubler
Division of Vector-Borne Infectious Diseases
National Center for Infectious Diseases
Centers for Disease Control and Prevention
PO Box 2087, Fort Collins, CO 80522, USA

Five basic serological tests are routinely used for the diagnosis of dengue infection, haemagglutination-inhibition (HI), complement fixation (CF), neutralization test (NT), IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA), and indirect IgG ELISA. Regardless of the test used, unequivocal serological diagnosis depends upon a significant (4-fold or greater) rise in specific antibodies between acute and convalescent-phase serum samples.

The antigen battery for most of these serological tests should include all four dengue serotypes, another flavivirus, such as yellow fever, Japanese encephalitis, or St Louis encephalitis, a non-flavivirus, such as chikungunya or eastern equine encephalitis, and an uninfected tissue control antigen.

Of the above tests, HI has been most frequently used for routine serologic diagnosis of dengue infections. It is sensitive, easy to perform, requires only minimal equipment, and is very reliable if properly done. Because HI antibodies persist for long periods (up to 60 years or longer), the test is ideal for seroepidemiologic studies. The HI test is based on the fact that the dengue viruses, under controlled conditions of pH and temperature, can agglutinate goose red blood cells, and this effect can be inhibited by specific antibodies. The antigens employed are prepared from infected suckling mouse brains by extraction with sucrose and acetone to remove the lipids, or from infected mosquito cell cultures that have been concentrated or purified. Serum specimens must be treated to remove non-specific inhibitors and agglutinins.
HI antibody usually begins to appear at detectable levels (titer of 10) by day 5 or 6 of illness and antibody titers in convalescent-phase serum specimens are generally at or below 640 in primary infections, although there are exceptions. By contrast, there is an immediate anaemic response in secondary and tertiary dengue infections, and antibody titers increase rapidly during the first few days of illness, often reaching 5120 to 10240 or more. Thus, a titer of 1280 or greater in an acute-phase serum is considered a presumptive diagnosis of a current dengue infection. Such high levels of HI antibody may persist for 2-3 months in some patients, but, in most, antibody titers generally begin to wane by 30-40 days and fall below the 1280 level.

The major disadvantage of the HI test is lack of specificity, which generally makes this test unreliable for identifying the infecting virus serotype. However, some primary infections may show a relatively monotypic HI response that generally correlates with the virus isolated.

The CF test is not widely used for routine dengue diagnostic serology. It is more difficult to perform and requires highly trained personnel. The CF test is based on the principle that complement is consumed during antigen-antibody reactions. Two such reactions are involved, the test system and an indicator system. Antigens for the CF test are prepared in the same manner as those for the HI test.

CF antibodies generally appear later than HI antibodies, are more specific in primary infections, and usually persist for short periods, although low level antibodies may persist in some persons. Because of the late appearance of CF antibodies, some patients may show a diagnostic rise by CF, but have only stable antibody titers by HI. The greater specificity of the CF test in primary infections is demonstrated by the monotypic CF responses when HI responses are broadly heterotypic; it is not specific in secondary infections. The CF is a useful test for patients with current infections, but is of limited value for seroepidemiological studies where detection of persistent antibodies is important.

The NT is the most specific and sensitive serological test for dengue viruses. The most common protocol used in most dengue laboratories is the serum dilution plaque reduction neutralization test (PRNT). It is based on the fact that dengue viruses produce cytopathic effects (CPE) which can be observed as plaques in susceptible cell cultures. This CPE is neutralized by the presence of specific antibody. In general, neutralizing antibodies rise at about the same time or at a slightly slower rate than HI antibodies, but more quickly than CF, and persist for at least 50 years or longer. Because NT is more sensitive, neutralizing antibodies may be detectable in the absence of detectable HI antibodies in some persons with past dengue infection.

The NT can be used to identify the infecting virus in primary dengue infections, provided the serum samples are properly timed. Thus, relatively monotypical responses are observed in the convalescent-phase serum. As noted above, the HI and CF
tests may also give monotypical responses to dengue infection that generally agree with NT results. In those cases where the responses are monotypical, the interpretation is generally reliable. In secondary and tertiary infections, it is not possible to reliably determine the infecting virus serotype by NT. Because of the long persistence of neutralizing antibodies, the test may also be used for seroepidemiological studies. Major disadvantages are the expense, time required to perform the test, and technical difficulty. It is, therefore, not employed routinely by most laboratories.

The MAC-ELISA has become widely used in the past few years. It is a simple, rapid test that requires very little sophisticated equipment. MAC-ELISA is based on detecting dengue-specific IgM antibodies in the test serum by capturing them out of solution, using anti-human IgM that was previously bound to the solid phase. If the IgM antibody from the patient's serum is anti-dengue antibody, it will bind the dengue antigen that is added in the next step and can be detected by subsequent addition of an enzyme labelled anti-dengue antibody, which may be human or monoclonal antibody. An enzyme substrate is added to give a colour reaction.

Anti-dengue IgM antibody develops a little faster than IgG, and by day 5 of illness most cases in Puerto Rico (80%) that were subsequently confirmed by HI on paired serum samples, or by virus isolation, had detectable IgM antibody. Nearly all patients (93%) developed detectable IgM antibody 6 to 10 days after onset, and 99% of patients tested between 10 and 20 days had detectable IgM. The rapidity with which IgM develops varies considerably among patients. Although dates of onset are not always recorded accurately, it appears that some patients have detectable IgM on days 2 to 4 after the onset of illness, while others may not develop IgM for 7 to 8 days after the onset. This variation is also reflected in the amount of IgM produced, and the length of time detectable IgM persists after infection. The IgM antibody is produced by patients with both primary and secondary dengue infections and probably most tertiary infections are low level and transient. IgM antibody titers in primary infections are significantly higher than in secondary infections, although it is not uncommon to obtain IgM titers of 320 in the latter cases. In some primary infections, detectable IgM may persist for over 90 days, but in most patients, it wanes to an undetectable level by 60 days.

The MAC-ELISA is slightly less sensitive than the HI test for diagnosing dengue infection. It has the advantage, however, of frequently requiring only a single, properly-timed blood sample. In one series of 288 patients during the 1986 epidemic in Puerto Rico, paired blood samples were tested by HI and the single acute-phase sample from the pair was tested by MAC-ELISA. By the HI test, 228 (79%) were considered positive, while 203 (70%) were positive by MAC-ELISA on the acute-phase serum alone. Five cases (1.7%) showed a false positive response, and 30 cases (10%) showed a false negative response by MAC-ELISA. Considering the difficulty in obtaining
second blood samples and the long delay in obtaining conclusive results from the HI test, this low error rate would be acceptable in most surveillance systems. It must be emphasized, however, that because of the persistence of IgM antibody, MAC-ELISA positive results on single serum samples are only provisional and do not necessarily mean that the dengue infection was current. It is reasonably certain, however, that the person had a dengue infection some time in the previous 2 to 3 months.

The specificity of MAC-ELISA is similar to that of HI. In both primary and secondary dengue infections, some monotypical responses may be observed, but, in general, the response is broadly reactive among both dengue and other flavivirus antigens. With serum samples from other confirmed flavivirus infections, such as Japanese encephalitis, St. Louis encephalitis, and yellow fever, however, the response is generally more specific. While there may be some crossing with dengue antigens, most specimens show relatively monotypical IgM responses to the infecting flavivirus. In dengue infections, monotypical IgM responses frequently do not correlate with the virus serotype isolated from a patient. MAC-ELISA, therefore, should not be used to identify the infecting dengue serotype. However, it has a slightly higher sensitivity than the HI test. It is expected that as more data are accumulated on the IgG ELISA, it will replace the HI test as the most commonly used IgG test in dengue laboratories.

A number of commercial test kits for anti-dengue IgM and IgG antibodies have become available in the past few years. Unfortunately, the accuracy of most of these tests is unknown because evaluation results have not been published. Those kits that have been independently evaluated at CDC have had a high rate of false positive results compared to standard tests. It is anticipated that these test kits can be reformulated to make them more accurate, thus making global laboratory-based surveillance for dengue/dengue haemorrhagic fever an obtainable goal in the near future.