EARLY LABORATORY DIAGNOSIS OF
DENGUE INFECTIONS

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Dengue virus is of increasing concern to health authorities the world over. Aided by the upsurge in human mobility and the rapid spread of the mosquito vector, the virus has snowballed in recent decades, resulting in millions of new infections and thousands of deaths. More than 2.5 billion individuals worldwide, almost half of the world's population, are now at risk of infection.

Since the febrile infection is difficult to diagnose clinically, laboratory tests play an essential role in identifying dengue infections. In particular, analysis of the viral antigen NS1 allows cost-effective, early diagnosis already at symptom onset, while antibody analysis provides confirmation of acute and past infections. The combined determination of NS1 and specific antibodies enables reliable diagnosis of dengue virus infections in all phases.

DENGUE VIRUS

Dengue virus is a member of the Flaviviridae family, along with yellow fever, West Nile, Japanese encephalitis and tick-borne encephalitis (TBE) viruses. There are four characterised serotypes of dengue virus, designated DENV1 to 4. Reservoir hosts for the virus are monkeys and above all, humans.

The virus is transmitted by mosquitoes of the genus Aedes, especially A. aegypti and A. albopictus. A. aegypti is an extremely efficient transmission vector. It shows a strong preference for human blood, feeding during the daytime and biting more than one person during a feed. It is adapted to human habitats and breeds in water stored for drinking or washing purposes and in rainwater collected in artificial containers. The human population boom over recent decades and the subsequent migration from rural to urban areas, often with poor living conditions, has resulted in the rapid spread of the mosquito in almost all tropical countries.

DISEASE SYMPTOMS

Dengue virus causes a wide spectrum of diseases. Dengue or dengue fever is a self-limiting illness of short duration. Known popularly as break-bone fever, it manifests with incapacitating fever of more than 40°C, severe headache, severe muscle and joint pain, exanthema and lymph node swelling. The symptoms typically last for two to seven days. First infections are usually mild. Following an infection, life-long immunity exists against the respective serotype. Cross-protective immunity against other serotypes is, however, limited and transient.

Second infections with a different serotype carry an increased risk of severe dengue, also known as dengue haemorrhagic fever. This disease manifests with varying intensities of haemorrhagic symptomologies such as petechiae, melena, nosebleeds and skin bleeding. In critical cases patients may also experience shock from plasma leakage (dengue shock syndrome) and organ impairment.

There is no specific treatment for dengue in the form of antiviral drugs, and therapy focuses on the symptoms. With adequate treatment the fatality rate of uncomplicated dengue is less than 1%. However, severe dengue carries a mortality of 26%.

Since the symptoms of dengue virus infection are often unspecific, diagnosis can be difficult, especially in non-epidemic situations. Dengue must be differentially diagnosed from other tropical diseases such as malaria, yellow fever, typhus abdominalis, West Nile virus infection and chikungunya fever. Laboratory methods based on detection of antibodies, antigens, nucleic acids or infectious virus support the clinical procedure and secure an accurate diagnosis.

EARLY DIAGNOSIS

The most important laboratory analyses for early diagnosis of dengue are detection of the viral NS1 antigen (nonstructural protein 1), viral RNA or the virus itself. The highly conserved NS1 glycoprotein can be detected in patient serum at the onset of clinical symptoms in both primary and secondary infections (see figure 2). Along with the virus and viral RNA, it is detectable before the appearance of IgM and IgG antibodies in first infections and also before IgM in subsequent infections.

NS1 offers a longer detection window than viral RNA or whole virus. Detection of viral RNA by reverse transcription polymerase chain reaction (RT-PCR) or detection of infectious virus by in vitro cultivation is only effective during the viraemic phase in the initial two to seven days of illness. The NS1 antigen can be detected in the early stage of infection and is still detectable when RT-PCR analysis of viral RNA is already negative. NS1 analysis thus minimises the diagnostic gap prior to antibody appearance, when infections might otherwise be overlooked. NS1 analysis by ELISA offers the additional advantage of being technically simpler and less expensive than viral RNA analysis by RT-PCR or virus cultivation.

The NS1 antigen can be measured by ELISA using microplates coated with monoclonal anti-NS1 antibodies. The antibodies specifically detect the NS1 antigen from all dengue virus serotypes. Clinical studies have affirmed the high sensitivity and specificity of the Dengue Virus NS1 ELISA. In quality assessment schemes this ELISA yielded 100% agreement with the target results.
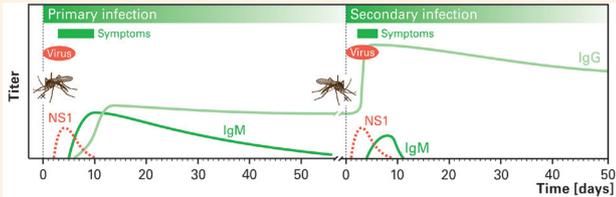
LATER-STAGE DIAGNOSIS

Serology is the method of choice for identifying late acute phase and past infections. Dengue virus antibodies become detectable after the appearance of symptoms (see figure 2). Antibodies of class IgM are detectable from the fifth day of illness and remain detectable for two to three months following a first infection. They are often not detected after a second infection with another serotype. Antibodies of class IgG arise several days later than IgM in a primary infection and reach their maximum concentration two to three weeks after infection. They are presumed to ➔

EPIDEMIOLOGY

Dengue is the most common and most rapidly spreading vector-borne viral infection in humans. Its incidence has increased 30-fold in the last 50 years. Around 50-100 million new infections are estimated to occur annually, with half a million hospitalisations and 22,000 deaths, many of them in children. Due to the recent surge in infections, the World Health Organization classifies dengue as a re-emerging disease. Dengue is endemic in more than 100 countries (see figure 1). The main distribution areas are Latin America, Central Africa, India, South East Asia and parts of the Pacific Islands. Recently, dengue has extended into the Eastern Mediterranean region, affecting especially the coastal lines of the Red Sea and Arabian Sea and Pakistan. It is also brought into normally unaffected regions by travellers, causing small, local epidemics. In Germany, for example, dengue is one of the most frequently imported viral infections, with 879 reported cases in 2013.

▼**FIGURE 1:** Dengue endemic regions

▼**FIGURE 2:** Timeline for NS1 and specific antibodies

▼**FIGURE 3:** BIOCHIP slide: Flavivirus Profile 2

Upper row

| TBE virus (TBEV) | | Yellow fever virus (YFV) |
| West Nile virus (WNV) | | Japanese encephalitis virus (JEV) |

Lower row

| Dengue virus type 1 (DENV 1) | | Dengue virus type 4 (DENV 4) |
| Dengue virus type 2 (DENV 2) | | Dengue virus type 3 (DENV 3) |

▼**FIGURE 4:** Anti-dengue virus IIFT mosaic serotypes 1 to 4

DENV 1 | DENV 4

DENV 2 | DENV 3

▼**FIGURE 5:** Anti-flavivirus IIFT mosaic: exclusion of cross reactions

TBEV | YFV

WNV | JEV

persist life-long. After a second infection with another serotype, an increase in IgG concentration of more than 10-fold is often observed already in the first week after infection. The analysis of paired sera is useful for confirming seroconversion or demonstrating a secondary titer increase. If a follow-up sample is not yet available, the additional measurement of specific IgA can aid diagnosis of acute cases. ELISA and indirect immunofluorescence test (IIFT) can detect antibodies against dengue virus.

Highly purified viral particles from DENV2-infected cultured cells are used as the antigenic substrate in the Anti-Dengue Virus ELISA. Due to high structural similarities, use of one serotype is sufficient to detect antibodies against all other serotypes. In clinical characterised samples this ELISA demonstrated an excellent sensitivity of 100% for IgG and 100% for IgM. 69% of patients with an acute infection and 27% of those with a past infection exhibited in addition IgA antibodies. The ELISA also showed a high specificity (IgG 100%, IgM 98%, IgA 99%).
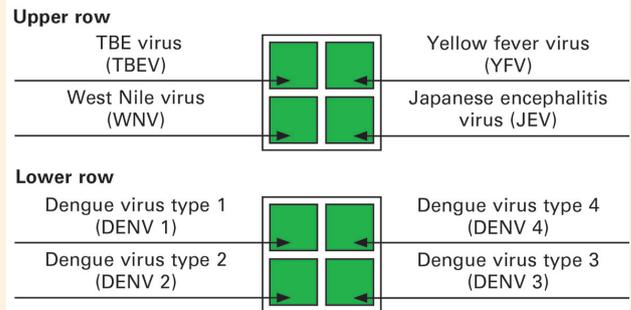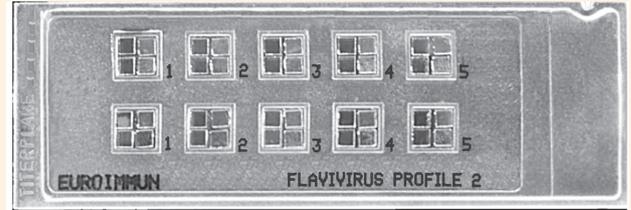
In the Anti-Dengue Virus IIFT Mosaic, developed in collaboration with the Robert-Koch Institute (Berlin, Germany), four BIOCHIPS containing cells infected with serotypes 1, 2, 3 and 4, respectively, are fixed into each field of a microscope slide (see *figure 3*) and analysed in parallel (see *figure 4*). In clinical studies this IIFT mosaic yielded a high overall sensitivity (IgG 97%; IgM 99%) and specificity (IgG 96%; IgM 96%).

Since there are structural similarities between flaviviruses, cross-reactions, especially regarding IgG detection, cannot be excluded. This could occur, for example, after recent infection or vaccination (e.g. TBE, yellow fever, Japanese encephalitis). Cross reactivity can be efficiently investigated using a further BIOCHIP Mosaic containing cells infected with relevant flaviviruses (see *figures 3 and 5*). Here, differentiation of the flaviviral antibodies may be achieved by comparing the reaction titers in parallel, with the highest titer indicating the serotype causing the current infection.
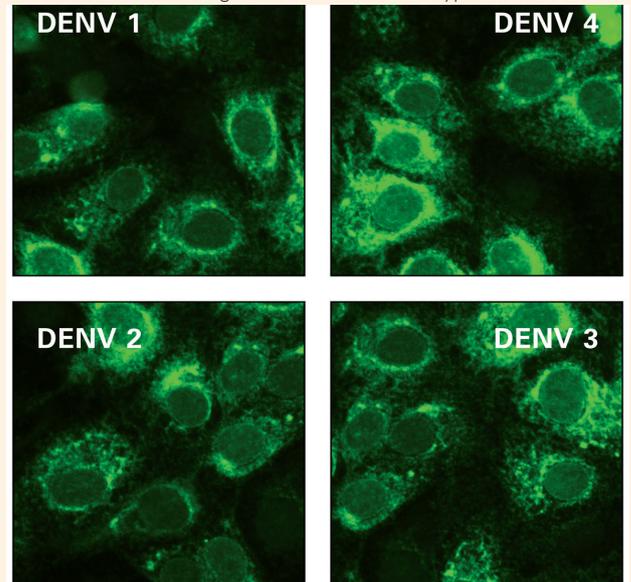
PERSPECTIVES

The dramatic increase in the incidence, mortality and geographic reach of dengue in recent decades has propelled the disease into the limelight as a major global health threat. In the absence of a vaccine, current strategies to reduce the disease burden are focused on vector control, surveillance and careful clinical care of patients to reduce complications and increase survival rates. Early diagnosis is of primary importance as some patients deteriorate rapidly to severe disease and death. Laboratory tests play a key role in dengue management, allowing prompt and effective diagnosis, discrimination from other infectious diseases, case confirmation and outbreak monitoring. In particular, a combination of NS1 antigen detection and antibody analysis reliably covers all stages of infection. The cost-effectiveness and simplicity of these tests are added benefits given the magnitude of the dengue crisis.