

ECDC TECHNICAL DOCUMENT

Zika virus disease epidemic

Interim guidance for healthcare providers and Zika virus laboratory diagnosis

Background information

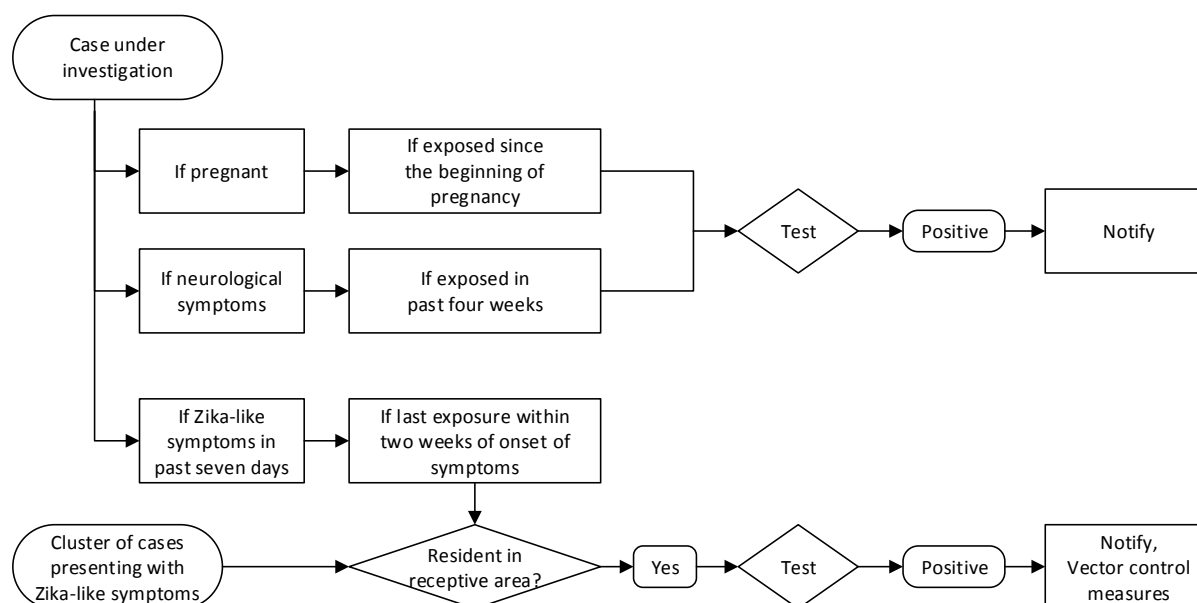
As of 29 February 2016, the spread of the Zika virus epidemic in the Americas and Caribbean is continuing. In the light of the current disease trend and the association with severe complications (such as adverse pregnancy outcomes or neurological complications), ECDC is proposing an algorithm for public health management of cases under investigation for Zika virus infection and an outline of the strategy for the laboratories performing Zika virus infection diagnostic tests.

This document aims to present an algorithm for deciding whom to test and provide guidance on the laboratory tests for Zika virus infection diagnosis in order to support clinical diagnostic and case reporting through surveillance among EU Member States.

The information is provisional and subject to revision when new information becomes available.

Algorithm for public health management of cases under investigation for Zika virus infection

The aim of this algorithm is to determine when a person who has been exposed to Zika virus needs to be tested and notified, and when vector control measures should be considered around a case. The algorithm is **not** intended for clinical management of patients with suspected Zika virus infection.

Figure 1. Algorithm for investigating cases suspected of Zika virus infection

Definitions

Exposure:

- History of exposure in an area with transmission of Zika virus; or
- Sexual contact with a male confirmed case of Zika virus infection; or
- Sexual contact with a male who had been in an area with Zika virus transmission in the past four weeks.

A list of Zika-affected areas is kept updated on the ECDC website ([link](#)).

Neurological symptoms: symptoms consistent with Guillain–Barré Syndrome (GBS) or other neurological syndromes such as acute flaccid paralysis, myelitis, meningitis and meningoencephalitis.

Zika-compatible symptoms: rash with or without fever and at least one of the following: arthralgia, myalgia or non-purulent conjunctivitis/hyperaemia.

Positive test: laboratory confirmation by PCR or by serology in a reference laboratory.

Notify: notification to the public health authority in accordance with the EU case definition for surveillance.

Receptive area: between 1 May and 31 October, a geographical area with presence of *Aedes albopictus* or *Aedes aegypti* mosquitoes, as determined by national authorities.

Vector control measures: measures designed to interrupt vector-borne transmission of Zika virus in the vicinity of an imported case of Zika virus infection, as per national or relevant protocols.

Prioritisation of testing

If capacity for testing is limited, priority should be considered in the following order:

1. Exposed pregnant women
 - 1.1 Pregnant women with suspicion of congenital malformation of the foetus
 - 1.2 Pregnant women with history of Zika-like infection during pregnancy
 - 1.3 Other pregnant women
2. Exposed symptomatic patient in a receptive area
3. Exposed patient presenting with neurological symptoms
4. Exposed symptomatic patient in a non-receptive area

Information to healthcare providers

Healthcare providers should ensure that between May and October, Zika virus-infected patients in areas with *Aedes albopictus* or *Aedes aegypti* mosquitoes avoid getting bitten during the first week of illness. The measures include the use of bed nets and screened doors and windows, as recommended by WHO-PAHO.

Healthcare providers who provide prenatal care should be made aware of the association between Zika virus infection during pregnancy and microcephaly so that they can provide prenatal care in accordance with the mother's history of exposure to the virus.

In addition, due to the unprecedented size of the Zika virus epidemic, health services and practitioners should be alert to the possible occurrence of neurological syndromes (GBS and other neurological syndromes such as acute flaccid paralysis, myelitis, meningitis, and meningoencephalitis) and other potential complications of Zika virus infections that have not yet been described in the scientific literature, as well as atypical clinical presentation among specific populations (i.e. children, the elderly, immunocompromised patients and people with sickle cell disease).

Zika virus laboratory diagnosis

Biosafety and transport of specimens

Zika virus is a risk group 2 pathogen and is handled in Bio Safety Level 2 (BSL2) containment in Europe* (with exception of UK where the virus is a risk group 3 pathogen), USA and Canada [1,2]. For virus isolation through culture, the sample may be handled under BSL 3 containment conditions as precautionary measures due to the possibility of presence of other pathogens that belong to risk group 3 in Europe (e.g. dengue virus). Handling of biological samples for Zika virus diagnostic should be conducted following national guidelines on laboratory biosafety [3].

International transport of specimens for Zika virus diagnosis may be shipped as biological substances category B, UN3373 [4].

Zika virus laboratory tests

Laboratory evidence of Zika virus infection is generally accomplished by viral RNA detection and/or serology.

Direct viral diagnosis

Zika virus diagnosis is primarily based on detection of viral RNA by reverse transcription polymerase chain reaction (PCR) from clinical samples. Specific assays have been published for Asian and African Zika viral strains targeting the envelope gene or NS5 region [5-7]. There are several commercial assays available for Zika virus genome detection. A pan-flavivirus by PCR assay and subsequent sequencing analysis can be used as an alternative screening or confirmatory test for possible ZIKV infection.

The viraemic period is considered to be short, allowing for molecular virus detection in blood samples during the first 5 days after onset of symptoms (Figure 2). The time needed for the detection of viral RNA in blood may also depend of the viral load during the acute phase of the disease. The duration and level of viraemia in asymptomatic patients are still unknown. Because viraemia decreases over time, a negative PCR test in blood collected 5–7 days after symptom onset does not exclude flavivirus infection and therefore serologic testing should be considered. Information about the duration of viraemia may change when more sensitive assays become available.

The detection period of Zika viral RNA in saliva (for up to 6 to 8 days after onset of symptoms) is not significantly longer than the presence of RNA in blood and although testing of saliva has the advantage of being non-invasive it does not extend the period during which an acute infection can be diagnosed with PCR. However, one study showed that it increases sensitivity in testing [8].

The use of urine as a specimen for Zika viral RNA detection seems to be a diagnostic method to consider as reported for several other flaviviruses [9,10]. On several occasions Zika viral RNA has been detected in urine more than 10 days after onset of disease [11-16]. However, there are no validated data at this point to suggest replacing blood with urine testing for PCR detection after onset of disease [11-13].

One study demonstrated Zika viral RNA in semen up to 62 days after onset of symptoms [17] and one study found infectious Zika virus in semen more than three weeks after onset of Zika symptoms [18].

Detection of Zika virus can also be performed in other specimens such as amniotic fluid, cerebrospinal fluid (CSF), placenta, or biopsy by PCR and histoimmunochemistry. Long-term persistence of the virus in foetal fluid and tissues has been suspected [19,20].

Genome sequencing analysis would be needed for further epidemiological studies and research investigations. Isolation of Zika virus on cell culture is conducted mainly for research purposes.

* Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC).

Serological diagnosis

Clinical signs and symptoms of Zika virus infection are non-specific and therefore require laboratory investigations for differential diagnostic. Specific Zika IgM antibodies can be detected by enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assays (IFA) from day 4 to 5 after onset of symptoms. Specific IgM for flaviviruses can be detected usually for 2 to 3 months but sometimes for a much longer period of time. Specific IgG antibodies appear later, usually from day 8 to 10 and remain detectable for months. There are currently no validated commercial assays for Zika serological diagnosis. Therefore, serological confirmation should be performed in a laboratory with experience in discriminating flaviviruses sero-diagnostic.

Serological investigation requires collection of at least a pair of blood samples taken at a 2 to 3 week interval. Recent infection diagnosis can be supported by a sero-conversion or four-fold increase of specific Zika virus antibody titres in a pair of serum samples. Depending on the context and the specificity of the initial serological assays, confirmation by virus neutralisation test (NT), sometimes referred to as plaque reduction neutralisation test (PRNT), is required. Virus neutralisation tests are the most specific tests for flavivirus serology.

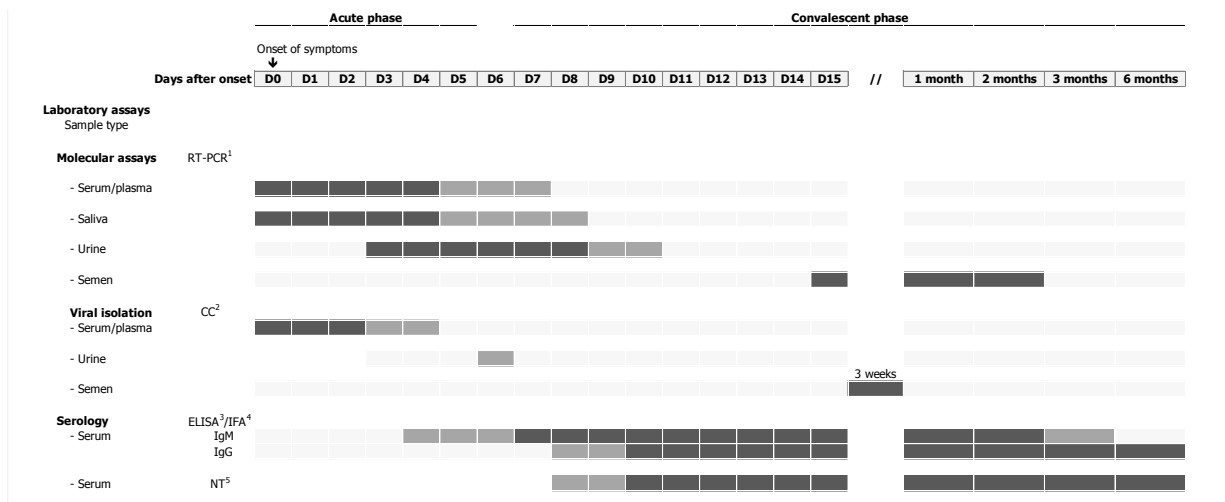
In most currently affected countries, other arboviruses with close clinical presentations are co-circulating, particularly dengue (4 serotypes), as well as chikungunya (alphavirus). As a significant cross reactivity of IgM/IgG antibodies among flaviviruses exists, such as for dengue and West Nile viruses, additional testing for laboratory confirmation is required.

Interpretation of serological results must systematically take into account the status of the individual (pregnant women, newborns, immune deficiency), previous/concomitant possible flaviviral infections, immunisation status against flaviviruses (e.g. vaccination for yellow fever) as well as flavivirus endemicity in region of exposure. This also applies for neutralisation assays that may require additional testing.

Information for conducting Zika virus diagnostic

Laboratories should receive clinical and epidemiological information for establishing their investigation strategy, including date of onset of illness, travel history (date and locations), past flaviviral immunisation records and pregnancy status.

Figure 2. Provisional overview of laboratory tests for Zika virus diagnostic



Notes:

- █ Optimal period of use per current knowledge.
- █ Sub-optimal period for detection per current knowledge

(1) RT-PCR, reverse transcription polymerase chain reaction; (2) CC, Cell culture (mammalian, mosquito cells); (3) ELISA, enzyme-linked immunosorbent assay; (4) IFA, Immunofluorescent assay; (5) NT, neutralisation test.

Detailed investigations on other samples such as cerebrospinal fluid, amniotic fluid, placenta, nasopharyngeal swabs, and other biopsies are not included.

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