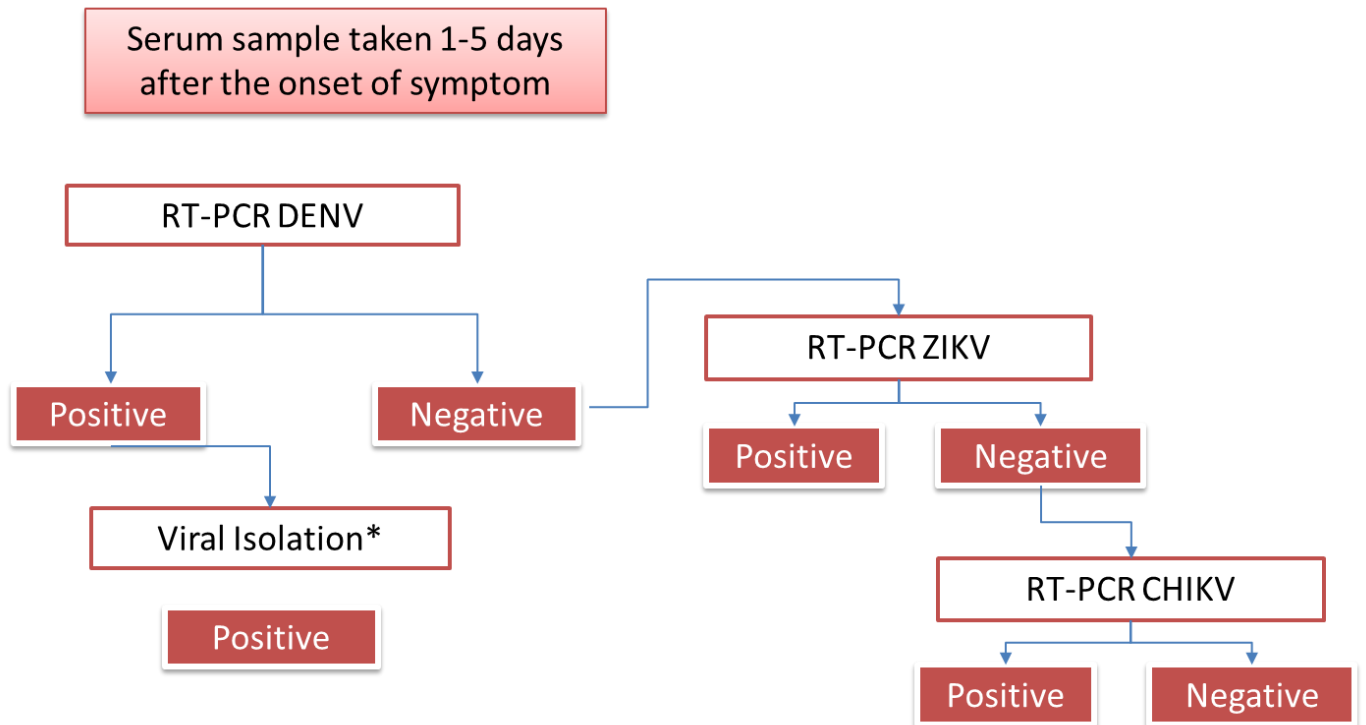


## Algorithm for detecting Zika virus (ZIKV) <sup>1</sup>

This algorithm is addressed to laboratories with established capacity (molecular, antigenic and/or serological) to detect dengue (DENV), Zika (ZIKV)<sup>2</sup>, and chikungunya (CHIKV) as part of the differential diagnosis for arboviruses. A BSL2 containment level is required to handle suspected samples.

### Suspected case of infection due to arbovirus: Acute phase

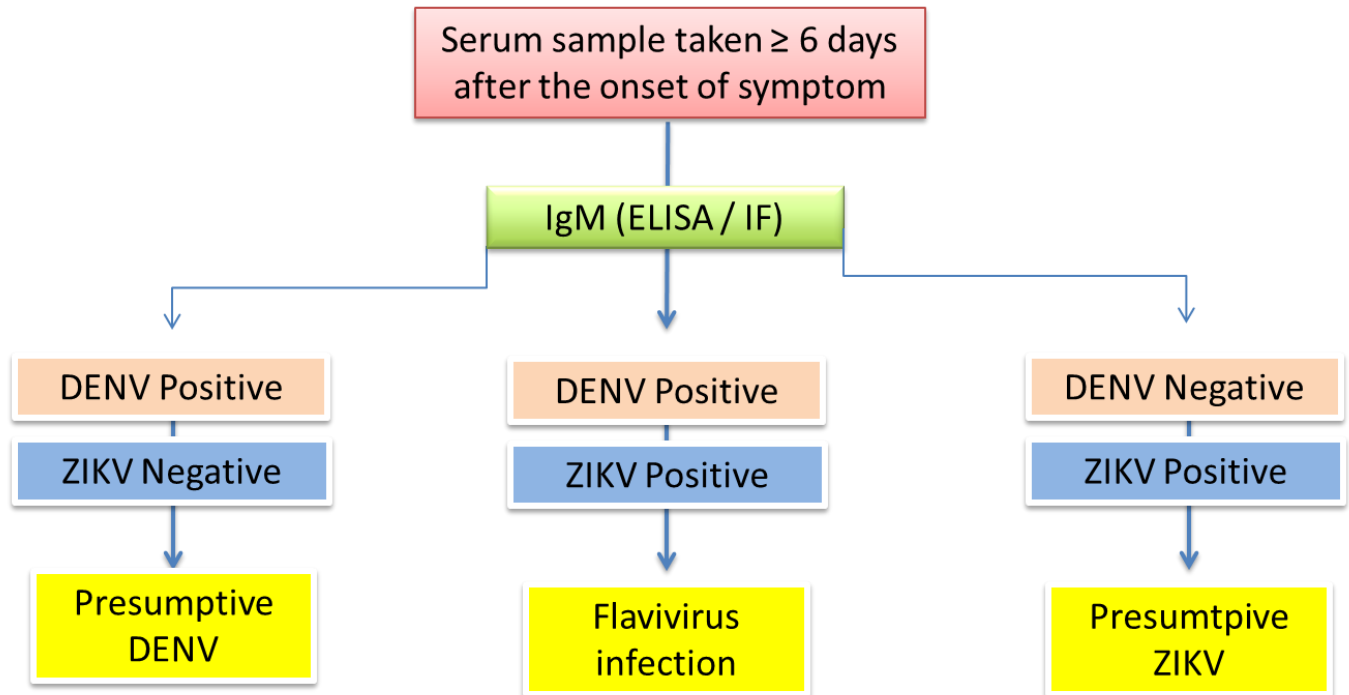


\* Percentage depending on the criteria of each laboratory for typification

<sup>1</sup> According to the epidemiological profile of each country and taking into account the clinical manifestations of the infection, other Arboviruses should be included in the differential algorithm for the Zika virus.

<sup>2</sup> These recommendations are subject to later modifications that take into account advances in knowledge of the disease and the etiologic agent.

**Suspected case of infection due to arbovirus: Convalescent phase**



**Sample collection and shipment**

- **Virological diagnosis (acute phase)**

Type of sample: serum (collected on dry tube, 5 to 7 cc whenever possible) or urine

Symptoms due to ZIKV infections are usually mild tends to be mild, the initial symptoms can escape notice, lessening the opportunity to take a sample. Although the viremic period still has not been fully established, viral RNA has been detected in serum up to day 10 after the onset of symptoms. ZIKV RNA also has been detected in urine over an extended period in the acute phase, which means that could be an alternative sample to be considered. However, and since more studies are needed, **it is recommended that the serum sample be taken during the first 5 days after the onset of symptoms.**

- **Serological Diagnosis**

Type of sample: serum (collected on dry tube)

ZIKV-specific IgM antibodies can be detected by ELISA or immunofluorescence assays in serum specimens from day 5 after the onset of symptoms. Since a single serum in the acute phase is presumptive, it is recommended that a second sample be taken 1–2 weeks after the first sample to demonstrate seroconversion (negative to positive) or a fourfold increase on the antibody titer (with a quantitative test).

The interpretation of the serological tests is especially important for the diagnosis of ZIKV. In primary infections (first infection with a flavivirus) it has been demonstrated that antibodies cross-reaction is minimal with other genetically related viruses. However, it has been demonstrated that sera of individuals with a previous history of infection from other flaviviruses (especially dengue, yellow fever and West Nile) can cross-react in these tests. Although neutralization by plaque reduction (PRNT) offers a greater specificity in the detection of neutralizing antibodies (IgG), cross-reactions have also been documented; in fact, some patients with a previous history of infection by other flaviviruses have shown up to a fourfold increase in neutralizing antibody titers when infected with ZIKV.

- **Sample preservation**

- Keep refrigerated (2-8 °C) if it is to be processed (or sent to a reference laboratory) within 48 hours.
- Keep frozen (-10 to -20 °C) if it is to be processed after the first 48 hours or within 7 days.
- Keep frozen (-70 °C) if it is to be processed after a week. The sample can be preserved for extended periods.

- **Shipping the sample by air to the reference laboratory**

- Ship (insofar as possible) with dry ice; at the very least, maintain the cold chain with cooling gels. Always use triple packaging.
- Ship within 48 hours.
- The original samples should be packed, labeled and marked (if dry ice is used), and documented as **Category B**.
- Always include the completely filled out clinical and epidemiological record.

## **General recommendations for the interpretation of serology results:**

- Serology confirmation of ZIKV infection is a major concern showing both, biological and technical limitations. The cross reactivity in secondary infection for flaviviruses is a challenge in areas where dengue and Zika circulation has been established.
- In cases where ZIKV is the first flavivirus infection, detection of IgM (ELISA) or neutralizing antibodies (PRNT) is specific and titers for heterologous flaviviruses are negative (or shows very low background).
- Secondary flavivirus-infected patients may show a high degree of serologic cross reactivity with other flaviviruses in both ELISA IgM detection and neutralizing antibodies detection by PRNT.
- On the other hand, PRNT assay is complex and time consuming. There are not commercial test for ZIKV PRNT (so far), so the availability of reagents will be limited.
- In this context, serology results should be carefully interpreted and reagents optimized only for selected cases, and not for routinely surveillance or seroprevalence surveys.
- Nevertheless, the relevance of the serology results could be very different under especial circumstances. In those cases where birth defects (including microcephaly) are identified in a newborn (low chances of previous infection for flavivirus), IgM detection in sera or in cerebral spinal fluid (CSF), is highly suggestive of a previous-intrauterine infection. Because of the immature immune system, a negative result does not necessarily rule out the ZIKV infection.

## **Comments and additional recommendations**

- There are different protocols (primers and probes) for detecting ZIKV by RT-PCR (both conventional and real time). Because of their sensitivity, the tools used by the U.S. Centers for Disease Control and Prevention (CDC) are recommended. These tools should be standardized for diagnostic use at the local level. Additional recommendations will be issued once the first cases are described.
- IgM can be determined using different tests (ELISA or IF). However, to date there are not commercial kits (approved or validated) for the serological determination of ZIKV. In any case, the most sensitive tests are in-house tools that use the complete virus as the antigen rather than recombinant proteins (or peptides) as used in other tools.

- Viral isolation is not regarded as a diagnostic tool and is recommended only for supplemental research studies in public health surveillance.
- Laboratories that do not have the capacity to viral confirmation (RT-PCR, viral isolation, sequencing) or serology (PRNT) should send the samples to a reference laboratory or World Health Organization (WHO) Collaborating Center. Before making any shipment, please communicate with the contact people at each center and with the Pan American Health Organization (PAHO) office in Washington, D.C.

## Contacts

### WHO Collaborating Center:

**National Center for Emerging and Zoonotic Infectious Diseases, Division of Vector-Borne Diseases, Arboviral Diseases Branch, Centers for Disease Control and Prevention (CDC)**

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### Pan American Health Organization, Regional Office of the WHO (PAHO/WHO)

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Washington D.C.  
United States of America

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