

Background

The `autochthonous` transmission of the chikungunya virus (CHIKV) has recently been demonstrated in the Region of the Americas, with more than 100 cases confirmed to date in some Caribbean islands (Saint Martin, Martinique, Guadeloupe, Saint Barthelemy). Given the eco-epidemiological conditions and distribution of the vectors, the dissemination of the virus to other countries will be a matter of time. Fortunately, in recent years and in collaboration with our members (CDC and RELDA, among others), the Pan American Health Organization (PAHO) has made efforts to prepare laboratories of the region before the eventual (and awaited) introduction of the virus through the preparation of guides, trainings and distribution of strategic reagents.

In this context, the following algorithm is proposed for detection of the CHIKV in order to allow the national laboratories to assume partial or total roles in the diagnosis and surveillance of the virus. The algorithm is based on the platform of dengue surveillance (DENV) because its clinical manifestations and broad distribution in our countries constitutes the principal differential diagnosis of the infection (without putting aside leptospira infections, malaria, and viral meningitis).

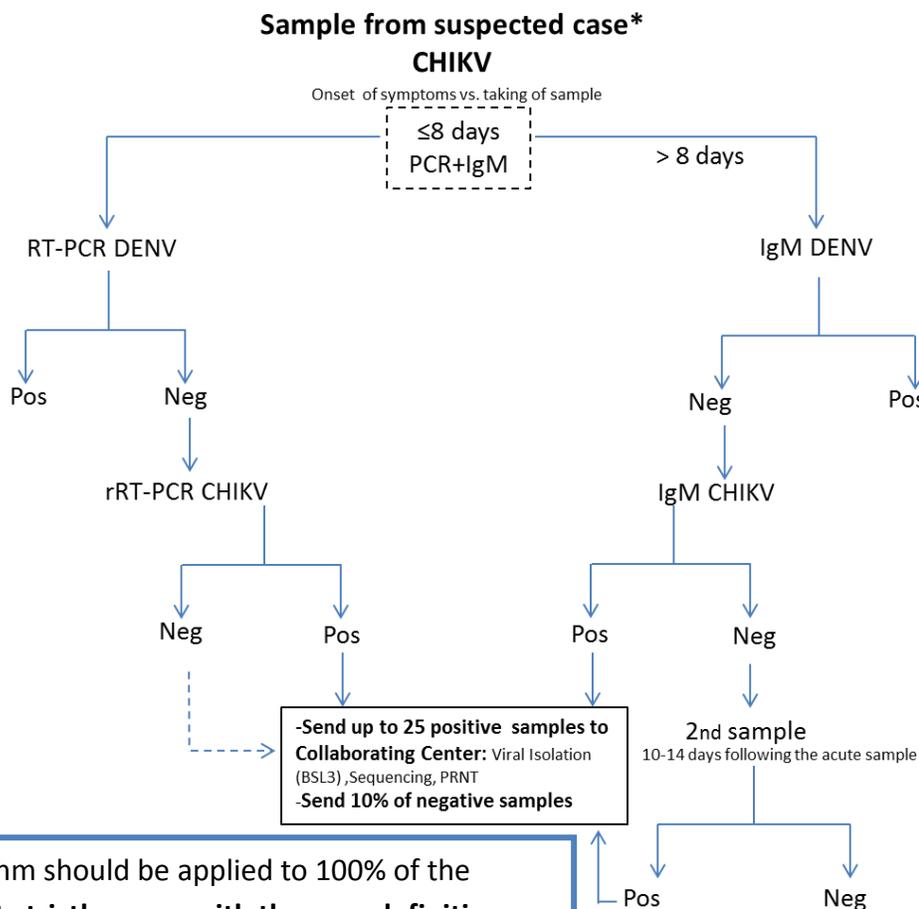
In this first stage where the goal is to establish the `autochthonous` transmission of the virus, it is recommended that samples from suspected cases of CHIKV be shipped to the PAHO/WHO reference centers and collaborating centers for final confirmation.

Algorithm for detection of CHIKV

Epidemiological Scenario: suspicion of introduction of the virus in a specific area ¹

This algorithm is intended to be followed by those reference laboratories with established capacity for the detection of both dengue (DENV) and Chikungunya (CHIKV) virus.

The samples should be processed according to the day of sampling with respect to symptom onset. If the sample is taken between the 1st and the 8th day after symptom onset (acute phase), the sample will be processed both by RT-PCR and for detection of IgM. The serological tests to rule out the diagnosis of DENV in areas of co-circulation should be carefully interpreted, taking into account the limitations of the techniques (sensitivity versus specificity; ELISA vs. rapid tests). The detection of IgM for DENV does not necessarily rule out an infection by CHIKV, which means that the clinical description of the case should be analyzed in detail.



¹As of December 2013, this applies to Caribbean countries and territories where the CHIKV has not yet been detected, as well as Latin American Countries.

Selection, collection and shipment of samples

The samples should be taken from **suspected cases**, defined as:

"Patient with onset of acute fever $>38.5^{\circ}\text{C}$ and severe arthralgia or arthritis not explained by another medical condition, who resides in or has visited epidemic or endemic areas in the two weeks prior to symptom onset " ²

A **confirmed case** is defined as²:

Any suspected case with a positive result by any of the following tests:

- Detection of nucleic acids (RT-PCR).
- Viral isolation (in BSL3).
- IgM detection (in acute sample), followed by a positive neutralization test.
- Seroconversion (ELISA IgM/IgG) or increase in the antibody titer by neutralization in coupled samples.

Sample selection (areas where there has not been demonstrated circulation):

- Negative samples for dengue, in patients with severe arthralgia or arthritis not explained by another medical condition.
- Samples of patients who meet the suspected case definition, from areas without activity of dengue.
- Clusters of patients with fever and severe arthralgia.

Type of sample: Serum

- Acute phase: Until 8 days after symptom onset
- Convalescent phase: 10–15 days after symptom onset

Conservation of the sample:

- Keep refrigerated ($2-8^{\circ}\text{C}$) if sample will be processed (or sent to a reference laboratory) within 48 hours.
- Keep frozen (-10 to -20°C) if sample will be processed after the first 48 hours.
- Maintain frozen (-70°C) if sample will be processed after one week. The sample is conserved adequately during prolonged time periods.

Shipment of the sample to the reference laboratory:

- Send (if possible) with dry ice; at a minimum, with cooling gels to ensure the cold chain.
- Send during the first 48 hours.
- The original samples can be packed and sent as **category B**.
- It is suggested that **viral isolations** be packed and sent as **category A**.
- Always send the completely filled out clinical-epidemiological form.

² CDC/PAHO. Preparation and response before the eventual introduction of the virus Chikungunya in the Americas. Washington DC, 2011.

Comments and additional recommendations

- Different protocols exist (primers and probes) for the detection of CHIKV by RT-PCR (both conventional and real time). Taking into account the sensitivity, it is recommended that the protocols from the Centers for Disease Control and Prevention (CDC) and the Institute Pasteur be used (sequences available in <http://wwwnc.cdc.gov/eid/article/13/5/pdfs/07-0015.pdf> and <http://wwwnc.cdc.gov/eid/article/14/3/pdfs/07-0906.pdf>). These protocols should be standardized and validated for diagnostic use at local level.
- The determination of IgM can be made by different commercially available techniques (ELISA or IFA). However, it should be taken into account that the best sensitivity is from techniques that use the complete virus as antigen compared to those that use recombinant proteins (or peptides). It is recommended that *in house* techniques for IgM/IgG ELISA be implemented using the purified viral antigen and following the CDC protocols, since the commercially available kits have not been sufficiently evaluated. The use of rapid tests is not recommended.
 - o The second sample for serological determination should be taken between 1 and 2 weeks after the first sample. The confirmation will be given by seroconversion (IgM/IgG) or 4-fold or more increase of the neutralizing antibody titer in the coupled samples.
 - o In accordance with the eco-epidemiological conditions, each country should consider the need to include in their algorithm the detection of other arboviruses that could complement the differential diagnosis (for example, Mayaro).
- Taking into account that the CHIKV is emerging in the Region of the Americas (in addition to its infectious potential), the attempt of viral isolation should be carried out in conditions of biosafety BSL-3.
- Laboratories that do not have the capacity for virologic (RT-PCR, viral isolation, sequencing) or serologic (PRNT) confirmation, should send the samples to a reference laboratory or PAHO/WHO collaborating center. Before making any shipment, please contact the center and PAHO/WHO office in Washington DC.



CHIKV Surveillance in The Americas: Detection and laboratory diagnosis

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