Guidelines for the Detection and Surveillance of Emerging Arboviruses in the Context of the Circulation of Other Arboviruses

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Context and general considerations

Arthropod-**bo**rne **viruses** (arboviruses) infect humans primarily through the bite of hematophagous arthropods (e.g., mosquitoes, ticks, sandflies, and biting midges). Arboviruses constitute a polyphyletic group of viruses from several families and genera, including orthoflaviviruses, alphaviruses, orthobunyaviruses, phleboviruses, and coltiviruses (*1*). The most relevant arboviruses in the Americas are the dengue (DENV) and Zika (ZIKV) orthoflaviviruses, and the chikungunya (CHIKV) alphavirus, all transmitted by the *Aedes aegypti* mosquito that is widely distributed in the Region. Other arboviruses have been detected in more restricted geographic areas, such as: Yellow fever (YFV), West Nile (WNV), and St. Louis encephalitis (SLEV) orthoflaviviruses; and equine encephalitis alphaviruses. In addition, some arboviruses have caused outbreaks in a limited number of countries in the Region but are considered to have the potential to re-emerge, notably, Oropouche virus (OROV) and Mayaro virus (MAYV) (*1*). This guideline focuses on these two arboviruses that cause Oropouche and Mayaro fevers, respectively. However, surveillance recommendations could be applied to other emerging or re-emerging arboviruses.

OROV belongs to the species *Orthobunyavirus oropoucheense*, family *Peribunyaviridae*. It was first detected in a febrile forestry worker in Trinidad and Tobago in 1955. Since then, in the Americas, numerous outbreaks of Oropouche fever have been registered in rural and urban communities in Brazil, Ecuador, French Guiana, Panama, Peru, and Trinidad and Tobago. In recent months, an increase in the detection of cases of Oropouche fever has been observed in some areas of the Americas, mainly in the Amazon region of Brazil (*2*). The OROV virus has a segmented genome consisting of the S (*small*), M (*medium*) and L (*large)* segments and is subject to genomic reassortment. Thus, several recombinants have been described within the species *Orthobunyavirus oropoucheense*, such as the Iquitos, Madre de Dios and Perdões viruses. Of these, only the Iquitos virus has been associated with human infections detected in Peru and causes a disease similar to that caused by OROV.

MAYV belongs to the genus *Alphavirus*, family *Togaviridae* and was first isolated in Trinidad and Tobago in 1954 from the serum of febrile patients. After the Trinidad and Tobago cases, cases of the disease have been reported in Bolivia, Brazil, Colombia, Ecuador, French Guiana, Haiti, Mexico, Panama, Peru, Suriname, and Venezuela, as well as imported cases from Bolivia and Peru into the United States of America (*3*). The MAYV virus is divided into three genotypes: genotype D circulating in South America and the Caribbean, and genotypes L and N that have been detected in Brazil and Peru, respectively.

[1] The recommendations presented in this document may be subject to subsequent modifications based on advances in knowledge about the disease and the etiological agent.

Hosts, vectors, and life cycle

OROV is transmitted in a sylvatic cycle that probably involves primates, sloths, and birds as reservoirs. The vector of this cycle is unknown, although there is evidence of the involvement of mosquitoes such as *Aedes serratus* and *Coquillettidia venezuelensis*. OROV can also be transmitted in an urban cycle, in which the human is the amplifier host. The main vector of this cycle is the biting midge *Culicoides paraensis*. The *Culex quinquefasciatus* mosquito can also be a vector (2).

In the studied outbreaks of **Mayaro fever**, the vector involved was the mosquito of the genus *Haemagogus*, which has a sylvatic habit. MAYV reservoirs have not been described, but some studies have reported virus isolation or high levels of antibodies in vertebrate hosts such as non-human primates (*3*).

Clinical Presentation

Oropouche fever has an incubation period of 4 to 8 days (range: 3-12 days). The onset is sudden, usually with fever, headache (often severe), arthralgia, myalgia, rash, chills, and sometimes persistent nausea and vomiting for up to 5 to 7 days. Aseptic meningitis and encephalitis are rare complications. Most cases recover within 7 days; however, in some patients, convalescence can take weeks (*2*). In some cases, a brief recurrence of symptoms may occur.

Mayaro fever has an incubation period of 1 to 12 days. The course of the disease is self-limiting, lasting 3 to 5 days. In the first few days, the patient presents a non-specific clinical picture: fever, headache, myalgia, retroocular pain, chills, severe arthralgia, dizziness, nausea, photophobia, anorexia, joint edema that is often disabling, rash mainly on the chest, legs, back, arms and less frequently on the face, abdominal pain, leukopenia, and thrombocytopenia. Arthralgias can last for weeks or months. Hemorrhagic manifestations have been described in some cases, and one case with encephalopathy has been documented (*3*).

International notification

Since these are emerging and relatively under-detected arboviruses in the Americas, an infection event in humans should be assessed using the "Decision instrument for the assessment and notification of events that may constitute a public health emergency of international concern", Annex 2 of the International Health Regulations (2005) (*4*) for reporting through the mechanisms of the Regulations.

Laboratory Diagnostics

Given the clinical presentation of Oropouche and Mayaro fevers and considering the current situation of dengue and other arboviruses in the Region of the Americas, laboratories are essential to confirm viral circulation, to characterize outbreaks, and to monitor diseases trends. Laboratory methods include virological (direct) diagnostic methods by amplification of the virus genome or virus isolation, and serological (indirect) methods to detect antibodies produced against the virus. In general, the sample for diagnosis is serum (*1*).

Biosafety

Fresh biological samples, of any type, should be considered potentially infectious. Samples should be processed and handled exclusively by trained professionals after a local risk assessment, considering all biosafety indications and appropriate personal protective equipment. Any procedure involving sample manipulation should be conducted in certified Class II biosafety cabinets. The manipulation of extracted RNA does not require biosafety cabinets. Additionally, all necessary precautions should be taken to prevent percutaneous exposure. The manipulation of materials or cultures with high viral load and/or high volume should be considered only after a local risk assessment considering the necessary containment measures is conducted (*1*).

Virological methods

During the acute phase of the disease, the genetic material (RNA) of these viruses can be detected by molecular methods (real-time or endpoint **RT-PCR**) using specific primers (and probes) for OROV or MAYV. Generic protocols followed by specific RT-PCR or nucleotide sequencing may also be used.

Detection is usually performed on serum samples, although it is also possible to detect OROV RNA in cerebrospinal fluid (CSF) in those cases associated with meningitis or encephalitis. The CSF sample should only be collected by clinical indication.

Most molecular methods for OROV are based on the detection of the conserved genetic segment S and do not differentiate OROV from other viruses of the O. oropoucheense species (e.g., Iquitos virus).

On the other hand, OROV or MAYV **viral isolation** can be done with the same samples used for RT-PCR by intracerebral inoculation in suckling mice or in Vero or C6/36 cell cultures. However, viral isolation is not considered a diagnostic method, but rather a tool for further characterization and investigation, and therefore is not routinely used nor a requirement for confirmation of diagnosis.

Serological methods

IgM antibodies against OROV or MAYV are generally detectable in serum from day 5 after symptom onset. A variety of in-house serological tests have been used to detect these antibodies, including ELISA, immunofluorescence, hemagglutination inhibition, complement fixation, or neutralization tests. In cases of OROV-associated meningitis or encephalitis, antibodies can also be detected in available CSF samples collected by clinical indication.

The detection of antibodies in a single serum sample is not considered confirmatory. Confirmation by serology generally requires paired specimens (acute and convalescent specimens collected more than 7-10 days apart, convalescent specimen collected more than 14 days after symptom onset) to establish seroconversion or increased antibody titers.

Cross-reactive serological testing has been described between alphaviruses of the Semliki forest virus complex, which includes MAYV and CHIKV. Although cross-reactive reactivity may also occur between the orthobunyaviruses of the Simbu serogroup to which OROV belongs, the only members that have been associated with human infections are OROV and the Iquitos virus. Cases of cross-reactivity can be evaluated by neutralization assays such as **plaque reduction neutralization test (PRNT)** or **microneutralization**, ideally making use of paired samples.

Given the technical limitations and availability of reagents for serological methods for surveillance, **it is recommended to prioritize and use molecular methods (RT-PCR) in acute samples for confirmation of OROV or MAYV infection** (Figure 1).

Laboratory Reagents

There are no validated commercial kits for the molecular or serological detection of OROV or MAYV infection. The use of protocols validated by reference laboratories is recommended. For more information, contact the PAHO Regional Office (e-mail: laboratoryresponse@paho.org, ricoj@paho.org).

Epidemiological Surveillance and Laboratory Algorithm

Given the clinical presentation of Oropouche and Mayaro fevers, it is recommended to monitor these diseases based on the systems already established for dengue surveillance. It is suggested to process representative samples of this surveillance, which meet the suspected case definitions of infection by endemic arboviruses with high circulation (mainly DENV, but also consider CHIKV and ZIKV according to the epidemiological context) (*5*), taking into account in particular, the clinical characterization and the epidemiological context, including age, sex, geographic location, and incidence of suspected endemic arboviroses. As indicated above, it is recommended to prioritize the detection of OROV and MAYV by molecular methods (RT-PCR) in acute samples (up to a maximum of 7 days after the onset of symptoms) that have tested negative for the molecular detection of DENV and, eventually, of other endemic arboviruses that are routinely processed in the laboratory (CHIKV and ZIKV). Depending on laboratory capacity and epidemiological context, a percentage of acute negative samples (which could range from 10% to 30%) or a limited number of samples may be selected for the molecular detection of OROV and MAYV (Figure 1). To optimize laboratory resources, it is suggested to use molecular methods that simultaneously detect OROV and MAYV viruses (*6*). Finally, it is recommended to regularly monitor the positivity of laboratory tests for dengue virus and other endemic arboviruses under surveillance. For example, an unexplained decrease in positivity, in a context of increased incidence of suspected cases, should lead to a situational analysis to identify possible causes, including the emergence or re-emergence of other arboviruses.

Genomic surveillance

The number of available OROV and MAYV sequences is relatively limited, particularly if only whole genomes are considered.

As for other pathogens, the objectives of genomic surveillance of OROV and MAYV are multiple and include studying and describing viral evolution, characterizing viruses into lineages and variants, and determining chains of transmission and sources of infection. In addition, genomic surveillance data from OROV and MAYV can be used for the development and evaluation of diagnostic tests, and, eventually, vaccines and therapeutic options (7).

Moreover, due to the nature of its genome, the OROV virus is subject to genetic reassortment, an important phenomenon that generates viral diversity within the species O. oropoucheense. For example, the Iquitos, Madre de Dios, and Perdões viruses contain the same L and S segments as OROV but specific M segments. Similarly, the OROV characterized in Brazil in the current outbreak is the result of a reassortment between segments of two OROV viruses (8). Therefore, genomic surveillance may be useful in identifying reassortment events that could have an impact on the viral phenotype.

For these reasons and to expand knowledge about these viruses, genomic surveillance can also be implemented where there is capacity and without neglecting the priority of diagnosis and timely detection.

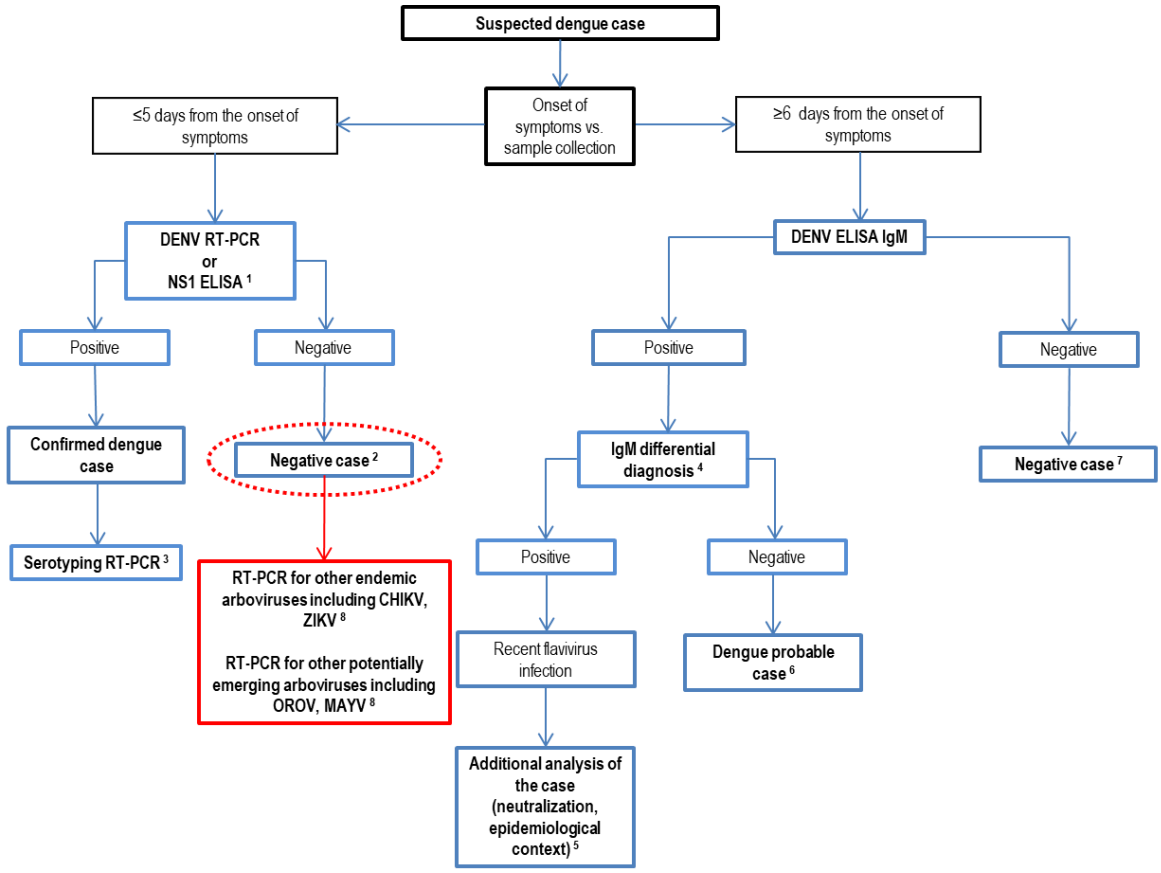
Sample storage

- Serum and CSF samples:
    - Keep refrigerated (2 - 8 ° C) if processed (or sent to a reference laboratory) within 48 hours.
    - Keep frozen (-10 to -20° C) if processed after 48 hours or within 7 days.
    - Keep frozen (-70° C or less) if processed more than one week after collection. The sample is adequately preserved at -70° C for extended periods of time.
- Tissue samples: freeze and ship on dry ice.
- Avoid multiple freeze-thaw cycles.

Shipping of samples to the reference laboratory

- Ensure the cold chain preferably with dry ice (tissues), or with refrigerant gels. Always use triple packaging (*9*).
- Ship samples preferably within the first 48 hours.
- The original samples should be packaged, marked, appropriately labeled, and documented as **category B.**
- The shipment must be accompanied by a complete clinical and epidemiological record, correctly identifying the sample type, the date of symptom onset, and the date of sample collection.

Laboratory algorithm



[1] RT-PCR is the recommended technique during the acute phase of the disease and its sensitivity allows the detection of viral RNA even for more than 5 days from the onset of symptoms. If RT-PCR is unavailable, detection of the NS1 antigen by ELISA can be used, considering it has a lower sensitivity than RT-PCR.
[2] In general, from the onset of symptoms on, a decrease in viremia with time is observed. This may affect the sensitivity of molecular (RT-PCR) and antigenic (NS1 ELISA) detection, particularly in samples collected after the fifth day from the onset of symptoms. In these cases, serological detection can be considered.
[3] This step is required only for cases confirmed with NS1 ELISA or an RT-PCR assay that does not differentiate viral serotypes.
[4] Consider the Zika virus, recent vaccination for yellow fever, as well as other flaviviruses depending on the epidemiological situation of the area/country.
[5] In cases of cross-reactivity, IgM ELISA results do not allow for the confirmation of the etiological agent. However, this result does not rule out a dengue virus infection. Additional clinical and epidemiological criteria should be used for the final case interpretation. Performing PRNT in a reference laboratory to analyze cross-reactive samples (ideally, in paired acute and convalescent samples) can also be considered.
[6] A positive IgM result in a single sample is not confirmatory and may be due to a dengue virus infection in recent months. Seroconversion in paired samples collected at least one week apart allows for dengue virus infection to be inferred, as long as no cross-reactivity with other flavivirus(es) is observed.
[7] IgM levels may be below the limits of detection in some secondary infections. Investigate the cases and perform the differential diagnosis.

[8] The list of endemic arboviruses may vary depending on the epidemiological context. Some laboratories use multiplex assays for dengue/chikungunya/Zika viruses.
[9] Depending on laboratory capacity and epidemiological context, a percentage (which may range from 10% to 30%) or a limited number of representative DENV RT-PCR-negative samples may be processed.

Figure 1. Algorithm for laboratory confirmation of dengue virus infection and for detection of infections by other arboviruses. *The algorithm for dengue has been previously published (10). The steps and additional notes related to other arboviruses are highlighted in red. DENV: dengue virus, CHIKV: chikungunya virus, ZIKV: Zika virus, OROV: Oropouche virus, MAYV: Mayaro virus.*

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