Laboratory Diagnosis of Mayaro Virus infection

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Mayaro virus (MAYV) is a mosquito-borne *Alphavirus* in the family *Togaviridae*. MAYV causes a self-limited illness that lasts 3-5 days and is characterized by the presence fever, headache, myalgia, rash, arthralgia and, occasionally, arthritis, which is similar to chikungunya virus (CHIKV) and other members of the Semliki Forest antigenic complex (1). Joint involvement after MAYV infection may persist for several months and in some cases precede the fever.

MAYV was originally isolated in Trinidad and Tobago in 1954 from the serum of febrile patients (2). It is likely maintained through sylvatic cycle that involves non-human primates and *Haemagogus* mosquitoes. MAYVs are divided in three distinct genotypes: D, L and N (3; 4). Genotype D has been reported in the Central Plateau and the Amazon region of Brazil, as well as in Trinidad and Tobago, Peru, Bolivia, Venezuela, Suriname and French Guyana. Genotype L has only been detected in the Amazon region of the Brazilian state of Pará, and genotype N in Peru.

MAYV infection misdiagnosis and underdiagnosis are likely to occur because of limited surveillance and laboratory diagnostic capacity, and of serological cross-reactivity with other alphavirues (5). In fact, recent MAYV infections occurring in Brazil between December 2014 and January 2016 were misreported as CHIKV infections (6).

In the light of chikungunya routine surveillance, Mayaro laboratory testing should be considered depending on the epidemiologic context and when clusters of chikungunya positive results are obtained only by serologic methods.

Laboratory testing

The diagnosis of Mayaro fever is performed through virologic (namely, detection of the viral genome,or virus isolation) and/or serological methods (ELISA, PRNT). As for any other laboratory testing, results need to be considered in the epidemiologic and clinical context.

Mayaro laboratory testing should be considered as a differential diagnosis for CHIKV and be performed after a sample of chikungunya suspected case has been negative for CHIKV infection (especially by PCR in acute face). Dengue and Zika should also be considered depending on the clinical and epidemiological background.

Biosafety considerations

All biological samples (whole blood, serum or fresh tissue) should be considered as potentially infectious (7). It is recommended to carry out all procedures in certified class II biosafety cabinets and to take all necessary precautions to avoid percutaneous exposure. Procedures for handling non-human primate and other animal samples should be carefully assessed according to national regulations and the biosafety manual of each laboratory, and the use of class III biosafety cabinets should be considered.

Virological diagnosis

- ***Molecular diagnostics:*** Viral RNA can be detected in serum samples up to 5 days since the onset of symptoms (viremic phase), by molecular methods such as conventional or real-time reverse transcription polymerase chain reaction (RT-PCR). A positive result by molecular testing (when using the appropriate controls and interpretation) confirms the diagnosis of MAYV infection.

  Because of the low viremia, high Ct values with well-defined amplification curves can be observed in real time RT-PCR assays. Results should be carefully evaluated in the light of the clinical and epidemiological background.

- ***Viral isolation:*** Viral isolation can be performed in cell culture (using Vero or C6/36 cells). Because of its complexity, this methodology is rarely used as a first-line diagnostic tool. However, as MAYV viremia may be very low, viral isolation might be able to increase viral concentration for subsequent detection by molecular assays

Serological diagnosis

- ***IgM detection:*** Anti-MAYV IgM antibodies can be detected by enzyme-linked immunosorbent assay (ELISA) (mainly IgM antibody-capture, MAC-ELISA) or any other immunoassay (e.g., indirect immunofluorescence). To date, there are not commercially available, validated serology kits for Mayaro. Therefore, in-house protocols using purified antigens are used (5; 8; 9; 10). **Cross-reactivity of Mayaro IgM assays with other alphaviruses has been described** (5)**. Thus, in areas where other alphaviruses co-circulate (especially CHIKV), the probability of cross-reactivity is high.** As with any IgM test, a positive result in a single sample is only **presumptive** of a recent infection. Laboratory confirmation requires demonstration of seroconversion in paired serum samples (acute and convalescent with at least one week of difference) and no seroconversion to other relevant alphavirus.

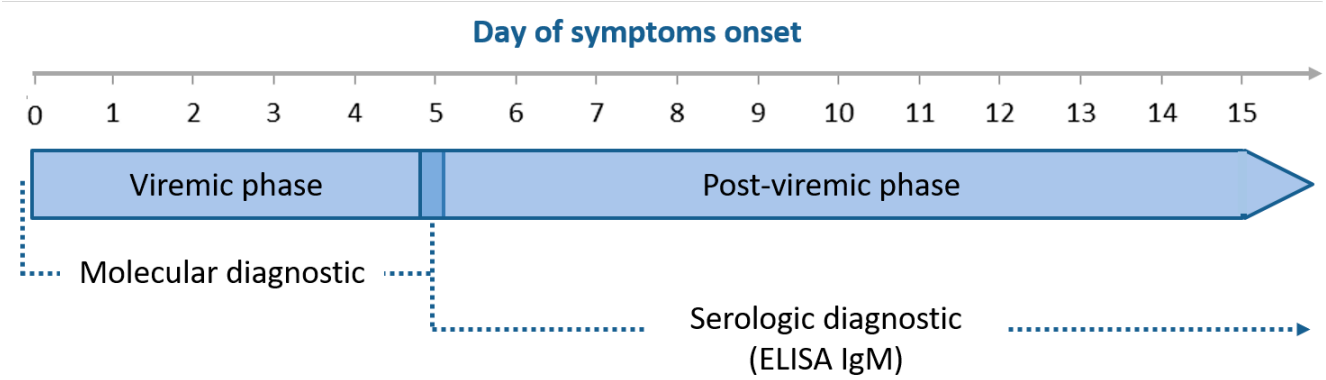
- ***Other serological techniques:*** These methods include the detection of IgG antibodies by ELISA and of neutralizing antibodies by plaque reduction neutralization test (PRNT) (11). In general, the PRNT offers better specificity than the detection of total IgM and IgG antibodies. However, **cross-reactivity among alphaviruses may also occur in neutralization assays**, and the utility of PRNT might be limited in areas where multiple alphaviruses have recently circulated or are endemic. Thus, it is recommended that this technique be performed with a panel of alphaviruses, especially CHIKV. Laboratory confirmation requires a Mayaro-specific seroconversion or more than 4-fold increase in antibody titers in paired acute and convalescent samples.

Testing algorithms and differential diagnosis

The replication dynamics of MAYV is not well defined yet. In contrast to CHIKV high and relatively long viremia, MAYV seems to present with low levels of viremia lasting up to 5 days after the symptoms onset. Thus, samples collected during this period should be tested by molecular methods or virus isolation (Figure 1).

On the other hand, IgM for both CHIKV and MAYV viruses can be detected from day 6 after the symptoms onset, so these samples should be tested by serological diagnostic (Figure 1). Serological techniques often cross-react among alphavirus infections. Thus, **the use of RT-PCR is preferred for Mayaro detection and diagnosis.**

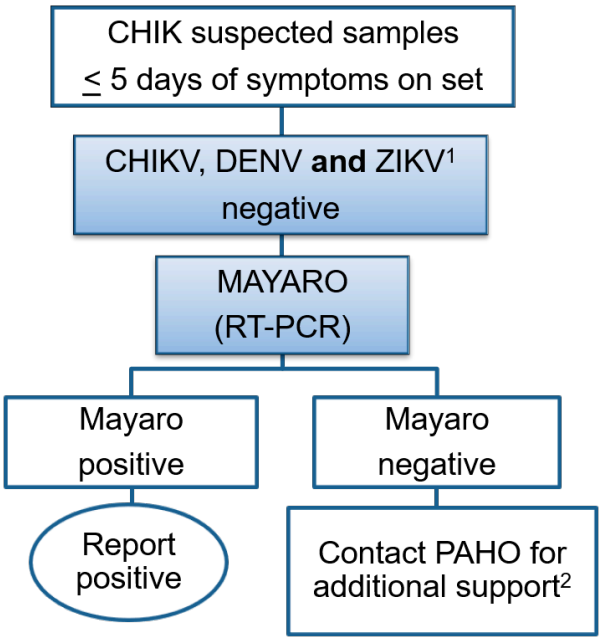
Figure 1. Indications for Mayaro diagnosis according to the number of days since the onset of symptoms



Samples collected up to 5 days of symptoms onset

For chikungunya suspected cases in the context of possible MAYV circulation, samples collected up to 5 days of symptoms onset, initial testing includes parallel screening for chikungunya, dengue (DENV) and Zika (ZIKV) viruses, followed by MAYV testing for negative samples (Figure 2).

Figure 2. Algorithm for Mayaro laboratory testing by molecular diagnostic for samples collected until 5 days of symptoms onset.

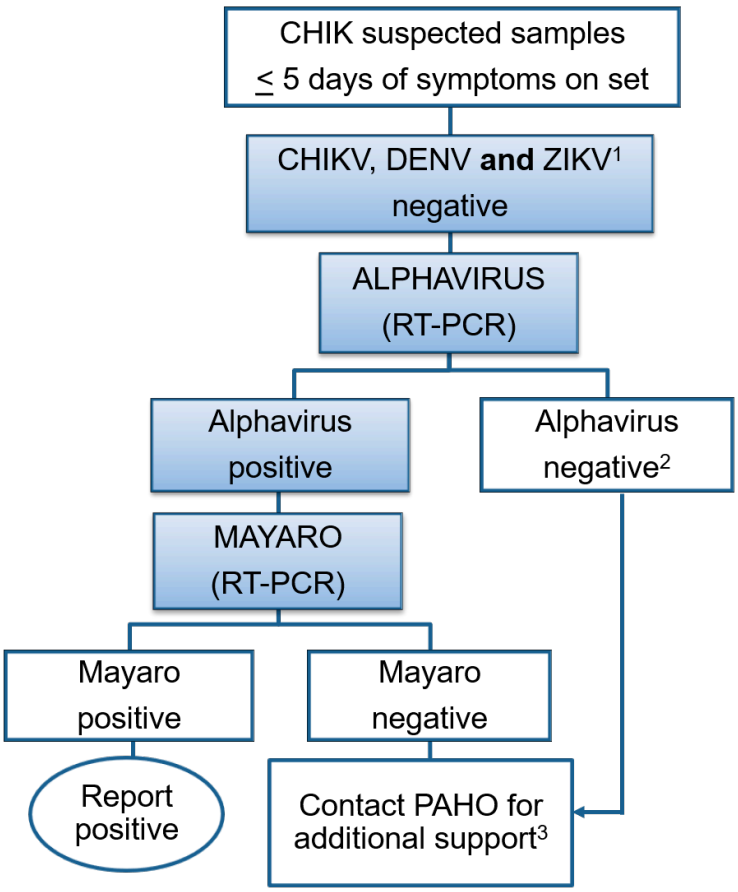


1 – Depending on clinical presentation, epidemiological context, and availability of multiplexed RT-PCR assays, the detection of CHIKV, DENV and ZIKV might be attempted sequentially or in parallel prior to MAYV testing.

2 – For laboratories with virus isolation capacity, virus isolation attempt should be held in samples from highly suspected patients or when high Ct values with well defined amplification curves are observed in real time RT-PCR. Shipping samples to a reference laboratory with PAHO's support should also be considered.

For laboratories with generic alphavirus molecular testing (pan-alphavirus RT-PCR) capacity, testing should be considered prior to specific MAYV testing (Figure 3). The sensitivity of the detection using generic testing should also be taken into account,

Figure 3. Algorithm for Mayaro laboratory testing by molecular diagnostic for samples collected until 5 days of symptoms onset for laboratories with generic alphavirus molecular testing capacity.



1 – Depending on clinical presentation, epidemiological context, and availability of multiplexed RT-PCR assays, the detection of CHIKV, DENV and ZIKV might be attempted sequentially or in parallel prior to MAYV testing.

2 – For a cluster negative samples tested for alphavirus by RT-PCR, with CHIKV IgM positive results from the same cluster, PAHO should be contacted for additional support.

3 – For laboratories with virus isolation capacity, virus isolation should be attempted in samples from highly suspected patients or when high Ct values with well defined amplification curves are observed in real time RT-PCR. Shipping samples to a reference laboratory with PAHO's support should also be considered.

Samples collected after 5 days of symptoms on set

Samples collected after 5 days of symptoms onset should be tested serologically for Mayaro and chikungunya IgM. For samples with positive results for both IgMs, considering using PRNT with a set of alphavirus antigens if available.

Sample storage

- Whole blood (EDTA tube) or serum (red-top tube) should be kept refrigerated (2 - 8 °C) if processed (or sent to a reference laboratory) within 48 hours.
- Serum should be kept frozen (-10 to -20 °C) if processed after 48 hours but in a period of no more than 7 days.
- Serum should be kept frozen (-70 °C) if processed more than a week after collection. Serum samples can be stored at -70 °C C for extended periods of time. Serum samples should be aliquoted in at least two vials.
- Freeze-thaw cycles should be avoided.
- Fresh tissue samples (approximately 1 cm^3) can be used for molecular diagnosis. Freeze at -70 °C and send to a reference laboratory on dry ice. If not possible, ship fresh tissues dry with refrigerant gels. Alternatively, fresh tissue samples can be stored in an RNA stabilization solution and shipped at room temperature.
- For histopathological and immunohistochemistry analyses, tissue samples (approximately 1 cm^3) must be fixed in 10% buffered formalin and sent to a pathology laboratory at room temperature. Liver and kidney are the tissues of choice for histopathological and immunohistochemistry analyses. Spleen, brain, lung, heart and lymph node samples may also be useful.

Shipping of samples to the reference laboratory by air

The following are some aspects to consider for shipping samples by air (15):

- The cold chain should be maintained preferably with dry ice or with refrigerant gels. Triple packaging should always be used.
- Samples should be shipped preferably within the first 48 hours.
- The original samples must be packaged, marked, appropriately labeled and registered as **category B**.
- The shipment must be accompanied by the complete clinical and epidemiological record.
- Formalin fixed tissues must be packed separately from fresh tissue and blood samples as formalin is volatile.

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