Laboratory Diagnosis of Yellow Fever Virus infection

September 2018

The yellow fever virus (YFV) belongs to the genus *Flavivirus* and is related to other viruses of the same genus such as dengue, Zika, Japanese encephalitis and West Nile viruses. The virus is transmitted to humans by sylvatic mosquito vectors of the genera *Haemagogus* and *Sabethes* as well as by the *Aedes aegypti* mosquito (1-3). The clinical spectrum of yellow fever ranges from asymptomatic or mild infection to potentially fatal severe conditions with hemorrhage and jaundice (1-4). Suspicion of yellow fever is based on the patient's clinical features, places and dates of travel (if the patient is from a non-endemic country or area), activities, and epidemiologic history of the location where the presumed infection occurred. However, specific diagnosis and case confirmation requires laboratory analysis.

The most important measure for the prevention of yellow fever is vaccination. In clinical trials, 80%–100% of vaccine recipients develop protective immunity within 10 days and 99% do so within 30 days. Although the yellow fever vaccine is safe and adverse events are uncommon, contraindications and safe immunization practices must be followed (1-3).

Laboratory testing

The diagnosis of yellow fever is performed through virological (namely, detection of the viral genome, detection of viral antigens, or virus isolation) and/or serological methods (ELISA, PRNT) (5-7). As for any other laboratory testing, results need to be considered in the epidemiologic and clinical context

Biosafety considerations

All biological samples (whole blood, serum or fresh tissue) should be considered as potentially infectious (8). All laboratory personnel handling these samples must be vaccinated against yellow fever and use appropriate personal protective equipment. Furthermore, 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 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

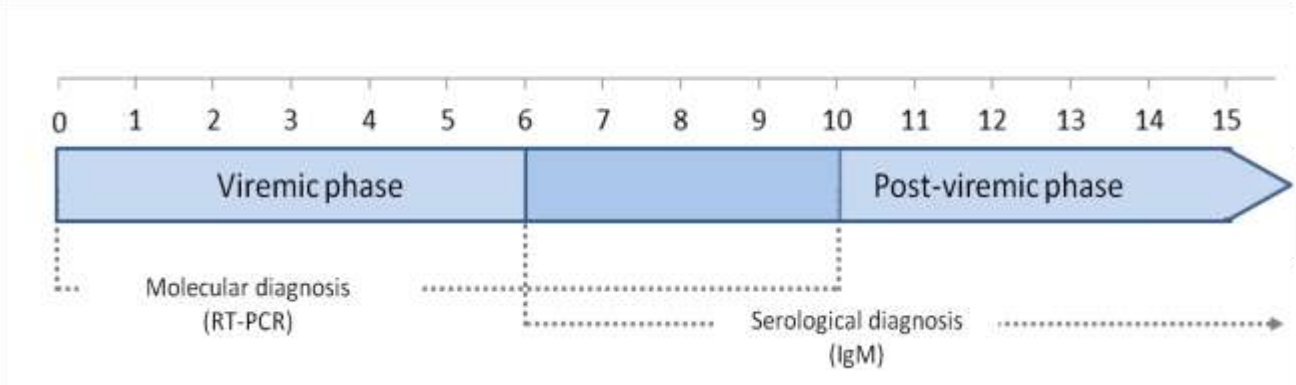
- ***Molecular diagnostics:*** Viral RNA can be detected in serum samples during the first 10 days since the onset of symptoms (viremic phase) or even longer than 10 days in severe cases, by molecular methods such as conventional (end-point) or real-time reverse transcription polymerase chain reaction (RT-PCR) (Figure 1). A positive result by molecular testing (when using the appropriate controls and interpretation) confirms the diagnosis of YFV infection.

- ***Viral isolation:*** Viral isolation can be performed through intracerebral inoculation in mice or in cell culture (using Vero or C6/36 cells; may be performed under BSL2 containment). Because of its complexity, this methodology is rarely used as a first-line diagnostic tool.

However. virus isolation capacity is important for the characterization of circulating strains, to produce diagnostic reagents and for research studies.

- ***Immunohistochemistry:*** Histopathological analysis with immunohistochemistry performed on liver sections (and other tissues) is considered the "gold standard" assay for the diagnosis of yellow fever in fatal cases. Additionally, molecular detection can also be performed in fresh or formalin fixed (paraffin-embedded) tissue samples to confirm fatal cases.

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



Serological diagnosis

- ***IgM detection:*** Anti-YFV 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 IgM ELISA kits for yellow fever. Therefore, in-house protocols using purified antigens are widely used (7, 9). **Significant cross-reactivity of yellow fever IgM assays with other flaviviruses has been described, in particular in secondary flavivirus infections. Thus, in areas where other flaviviruses co-circulate (especially dengue and Zika viruses), the probability of cross-reactivity is high.** Moreover, 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 1 week of difference) and no seroconversion to other relevant flavivirus. Finally, in areas where active yellow fever vaccination campaigns are ongoing, detection of vaccine-induced antibodies may occur, and laboratory tests should be carefully interpreted (see below the section *Post-vaccination immune response*).

- ***Other serological techniques:*** These methods include the detection of IgG antibodies by ELISA and of neutralizing antibodies by plaque reduction neutralization test (PRNT). In general, the PRNT offers better specificity than the detection of total IgM and IgG antibodies. However, **cross-reactivity among flaviviruses has also been documented for neutralization assays**, and the utility of PRNT might be limited in areas where multiple flavivirus have recently circulated or are endemic (10). Thus, it is recommended that this technique be performed with a panel of flaviviruses. Laboratory confirmation requires a yellow fever-specific seroconversion or more than 4-fold increase in antibody titers in paired samples (see below).

Detection of vaccine-induced antibodies may also occur, and laboratory tests should be carefully interpreted (see below the section *Post-vaccination immune response*).

Testing algorithm, interpretation of serological results and differential diagnosis

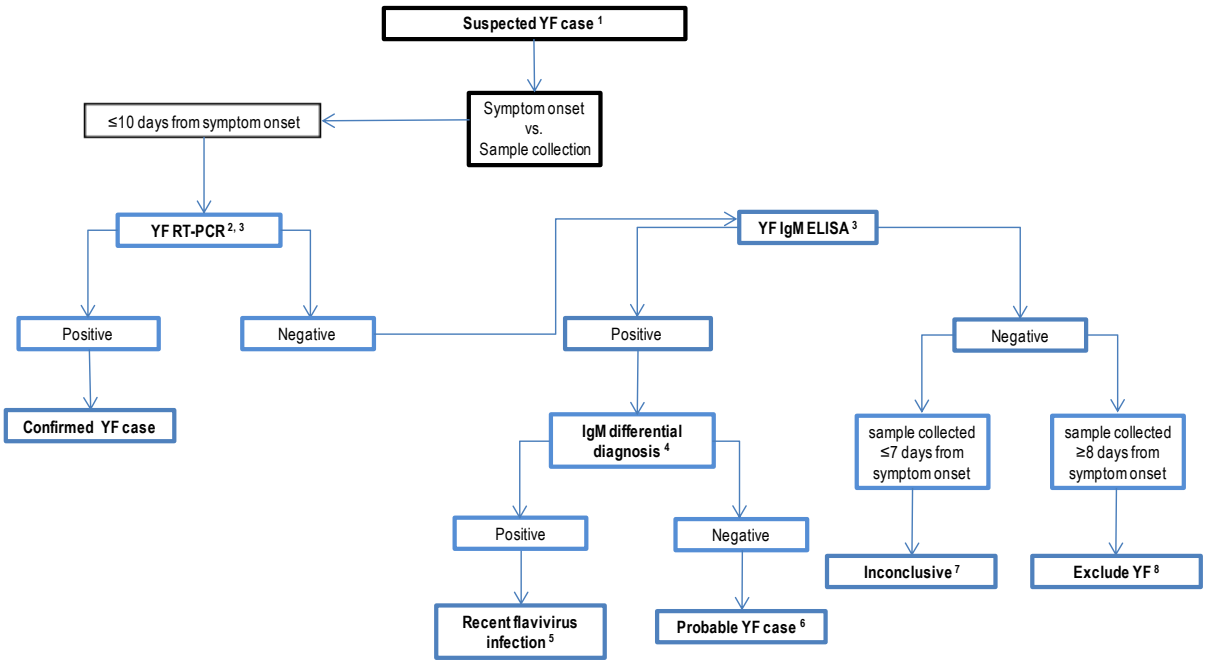
Serological techniques often cross-react among flavivirus infections (particularly during secondary flavivirus infections). Thus, **the use of RT-PCR should be prioritized** (Figure 2). Nevertheless, a negative result by RT-PCR does not discard the infection, so the sample should subsequently be tested by serology.

A positive IgM result should be followed up by differential IgM testing, particularly in areas where the co-circulation of YFV with other flaviviruses (e.g., dengue, Zika, St. Louis encephalitis, and Japanese encephalitis complex viruses) is documented and the population is likely to have been previously infected with one or more of these viruses.

A positive differential test does not confirm or rule out a YFV infection. Thus, in non-endemic areas or in areas where no recent YFV circulation has been described, this should prompt further investigation. A positive YF IgM test with a negative differential test should be interpreted as a probable YF case. Laboratory confirmation of these cases by serology can be achieved using paired samples. A negative YF IgM test is inconclusive for samples collected up to day 7 post-symptom onset. YF can be ruled out if the IgM test is negative in a sample collected from day 8 post-symptom onset.

Moreover, the differential diagnosis of yellow fever should include other febrile and febrile icteric syndromes – such as dengue, leptospirosis, malaria, typhoid fever, rickettsial infections, toxic or viral hepatitis, Bolivian, Brazilian, Argentinean, and Venezuelan hemorrhagic fevers, among others – depending on the epidemiological profile of the affected country or area (1).

Figure 2. Algorithm for laboratory confirmation of yellow fever (YF) cases



[1] No YF vaccination within 30 days or unknown YF vaccination history.
[2] Laboratories that only have the capacity to perform RT-PCR or IgM ELISA should test samples with the available technique. Results should be interpreted according to the algorithm.
[3] RT-PCR sensitivity is higher in the first 10 days from symptom onset. However, detection up to 14 days has been reported, in particular in severe (and fatal) cases.
[4] Must include dengue virus as well as other flaviviruses depending on the epidemiological situation of the area/country.
[5] Consider performing PRNT in a reference laboratory. This result does not rule out yellow fever. Thus, in areas where no YF circulation has been described recently, this should prompt an investigation.
[6] A positive IgM test in a single sample is not confirmatory. Additional clinical and epidemiological criteria must be used for the final interpretation of the case, in particular in areas where no YF circulation has been described recently.
[7] A second sample should be requested and tested according to the algorithm.
[8] Cases should be investigated and clinical differential diagnosis performed.

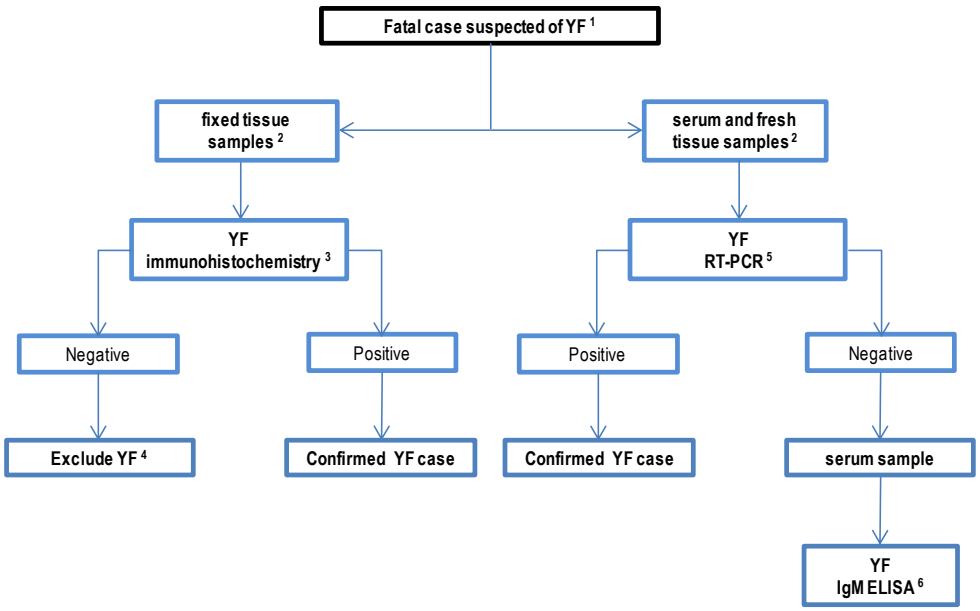
Fatal cases suspected of yellow fever

In fatal cases possibly due to yellow fever, the following samples should be collected regardless of the time since symptom onset:

- Serum for molecular and serological assays
- Fresh tissue for molecular assays (liver and kidney tissue should always be collected; additionally, spleen, lung, brain and heart tissue can be collected)
- Fixed tissue for immunohistochemistry (same tissues as above)

The algorithm for confirming fatal cases is shown in Figure 3.

Figure 3. Algorithm for laboratory confirmation of fatal yellow fever cases



[1] No YF vaccination within 30 days or unknown YF vaccination history.
[2] Fresh and fixed tissue samples should be collected (in particular, liver and kidney).
[3] Immunohistochemistry should be performed on liver and kidney sections and, if available, on other tissue samples.
[4] Cases should be investigated and clinical differential diagnosis performed.
[5] RT-PCR should be performed on RNA extracted from serum and fresh tissue samples.
[6] Follow the interpretation described in the algorithm for laboratory confirmation of yellow fever (YF) cases (Figure 2).

Post-vaccination immune response

Vaccination induces a relatively low viremia that decreases after 4 to 7 days. Concurrently, an IgM response develops. This response cannot be differentiated from the IgM response induced by a natural infection. Approximately 10 days after vaccination, the vaccinee is considered to be protected against a natural infection. The IgM response may be detected from around day 5 onwards with a peak occurring generally 2 weeks after vaccination. Subsequently, antibody levels tend to decrease. However, in a significant proportion of individuals, the IgM response can be detected for one month and in some cases up to 3-4 years post-vaccination (11). In addition, neutralizing antibodies induced by vaccination can be detected for several decades. Therefore, **the interpretation of serological results in vaccinated individuals is complex, particularly in individuals who have recently been vaccinated and results should be carefully assessed**.

In recent vaccinees (<30 days) who develop classical symptoms of YFV infection, surveillance should aim to differentiate between infections with wild-type YFV and the vaccine strain (12-14). Serious events supposedly attributable to vaccination or immunization (ESAVIs) associated with yellow fever vaccine are rare (~1.6 cases per 100,000 vaccine doses). The most common serious ESAVIs are viscerotropic disease (VTD), neurologic disease, and severe hypersensitivity reactions. In yellow fever endemic areas, differentiating between wild-type infections and ESAVIs require the confirmation of

the presence of the yellow fever vaccine virus (strain 17D) by nucleotide sequencing, which is usually available only in reference laboratories. Guidelines for the confirmation of yellow fever vaccine-associated serious events are available (12-14).

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.
- Multiple 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

预览已结束，完整报告链接和二维码如下：
https://www.yunbaogao.cn/report/index/report?reportId=5_25715