Epidemiological Update
Yellow Fever

16 February 2017

Situation summary in the Americas

Since epidemiological week (EW) 1 to EW 5 of 2017, **Brazil**, **Colombia**, **Peru,** and **the Plurinational State of Bolivia,** have reported yellow fever cases. Colombia and Peru have reported probable cases, one and three respectively; the Plurinational State of Bolivia reported a case under investigation while Brazil has reported confirmed and suspected cases under investigation.

The following is a situation summary in Bolivia and Brazil.

The **Bolivia** Ministry of Health reported a yellow fever case with a positive result for IgM by ELISA. The case is a 28-year-old unvaccinated male tourist who arrived in Bolivia on 8 January 2017 and on 9 January went to the municipality of Caranavi, where he likely acquired the infection. On 28 January, the patient received medical attention in a local hospital and later transferred to a private clinic in Chile, from which he was discharged on 13 February.[1] During the probable period of infection, the case did travel outside of Bolivia.

Yellow fever in Bolivia is endemic and occurred cyclically with outbreaks of varying magnitude up to 2012. Since 2013, only sporadic cases have been reported.

In **Brazil**, yellow fever is endemic and occurs with cyclic outbreaks of varying magnitude; however, as shown in **Figure 1**, the number of confirmed cases during the current outbreak exceeds the number of cases observed in the preceding decades.

[1] In both Bolivia and Chile, IgM results for yellow fever were positive.

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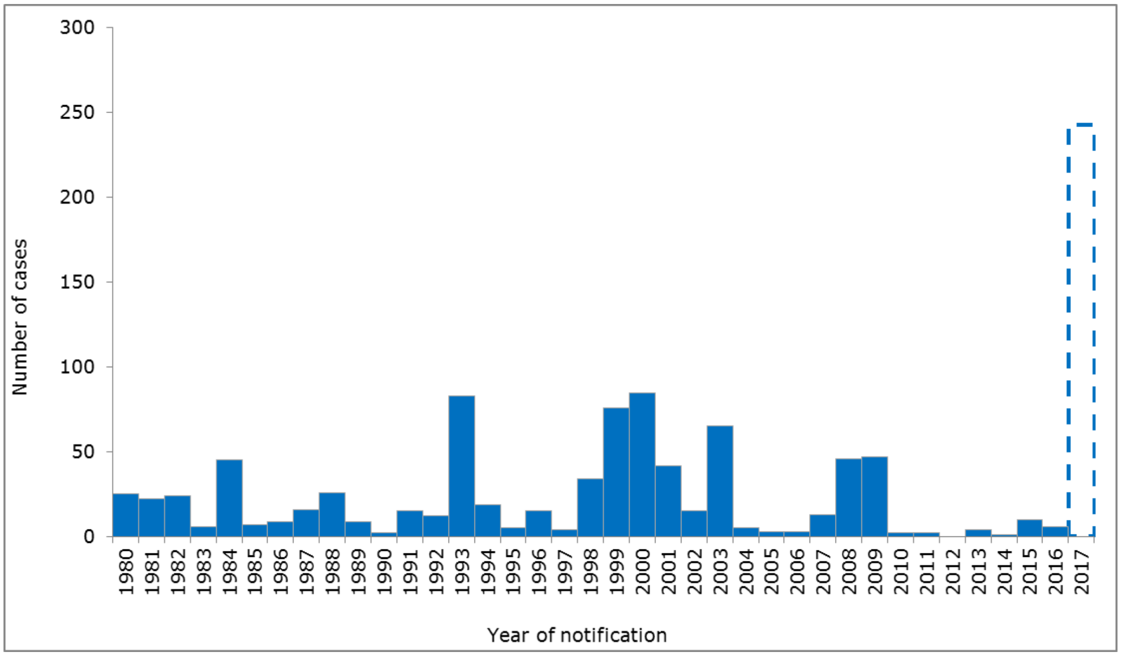
Figure 1. Distribution of confirmed human yellow fever cases, by year. Brazil, 1980 to 2017 (as of EW 5)



Source: Data published by the Brazil Ministry of Health[2] and reproduced by PAHO/WHO

Between 1 December 2016 and 15 February 2017, there were 1,236 cases of yellow fever reported (243 confirmed, 108 discarded, and 885 suspected cases remain under investigation), including 197 deaths (82 confirmed, 3 discarded, and 112 under investigation) in Brazil. The case fatality rate (CFR) is 34% among confirmed cases and 13% among suspected cases.

According to the probable site of infection, the suspected and confirmed cases are distributed in six states: Bahia (12), Espírito Santo (130), Minas Gerais (967), Rio Grande do Norte (1), São Paulo (10), and Tocantins (3).[3] While the confirmed cases are distributed in three states: Espírito Santo (31), Minas Gerais (208), and São Paulo (4) (**Figure 2**). Of the confirmed cases, 86% (n=209) are men, of which 80% are between 21 and 60 years of age (**Figure 3**).

With regard to the confirmed deaths, 70 occurred in the state of Minas Gerais, 3 in the state of São Paulo and 9 in the state of Espírito Santo. In decreasing order, the CFR among suspected and confirmed cases by state is 75% in São Paulo, 34% in Minas Gerais, and 29% in Espírito Santo.

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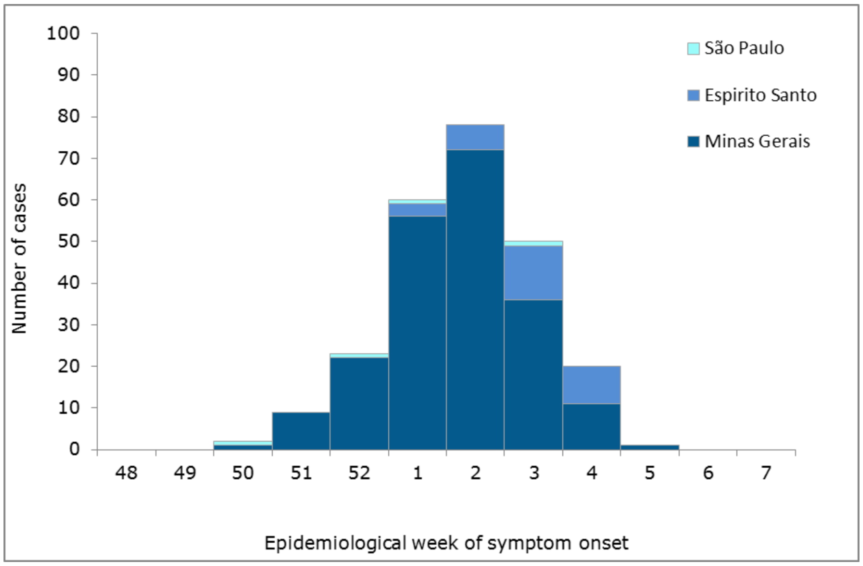
[2] The number of confirmed cases from 1980 to 2016 were taken from *Historical series of confirmed cases of yellow fever in Brazil, 1980 - 2016, figure 1* and confirmed cases of yellow fever during 2017 obtained from Report N ° 17 of *Monitoring cases and deaths of Yellow Fever in Brazil*. Available at: http://portalsaude.saude.gov.br/index.php/situacao-epidemiologica-dados-febreamarela

[3] There are also five suspected case for which the probable site of infection remains under investigation.

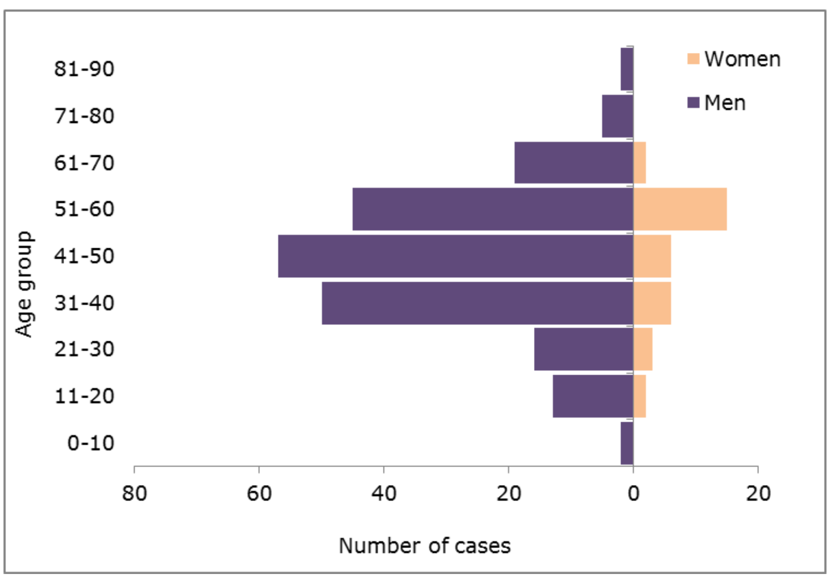
Following the distribution of cases by EW of symptom onset and by state of occurrence, a decreasing trend is observed. It will be necessary to continue to monitor the situation to determine whether this trend persists in the coming weeks.

Figure 2. Distribution of confirmed cases of yellow fever by EW of symptom onset and state of occurrence, Brazil, EW 48 of 2016 to EW 7 of 2017



Source: Data published by the Brazil Ministry of Health

Figure 3. Distribution of confirmed cases of yellow fever by age and sex, Brazil, EW 48 of 2016 to EW 5 of 2017 (N= 243)

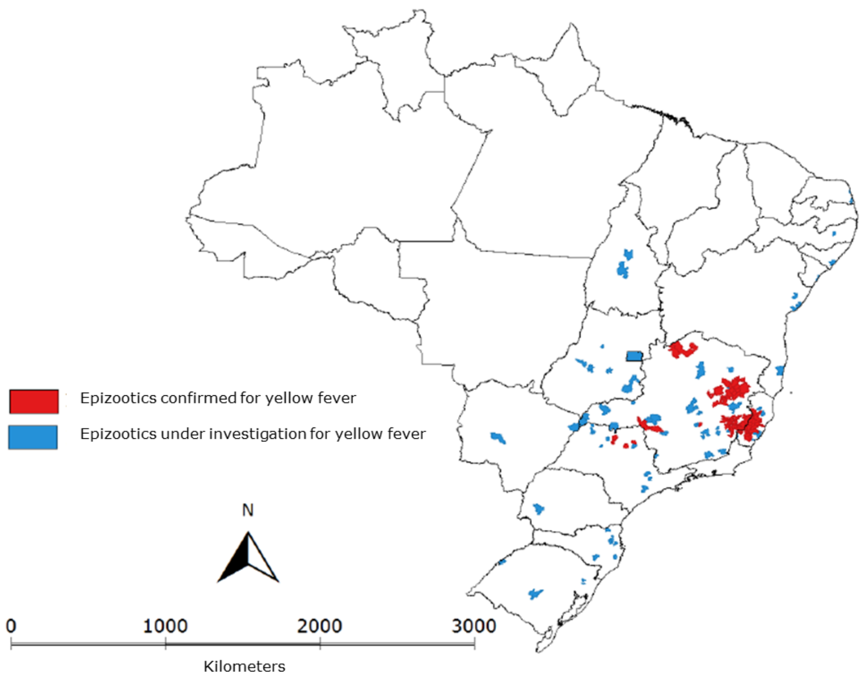


Source: Data published by Brazil Ministry of Health

In addition, 647 epizootics were reported in nonhuman primates (NHP), of which 342 were yellow fever confirmed.

Epizootics in NHP were reported in the Federal District and in the states of Alagoas, Bahia, Goiás, Espírito Santo, Mato Grosso do Sul, Minas Gerais, Paraná, Pernambuco, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina, São Paulo, Sergipe, and Tocantins (**Figure 4**). Although there have been no reports of yellow fever cases linked to the current outbreak in Brazil in other countries and/or territories in the Americas, reports of epizootics, currently under investigation, in states of Brazil bordering other countries—Mato Grosso do Sul (bordering Bolivia and Paraguay), Santa Catarina (bordering Argentina), Rio Grande do Sul (bordering Uruguay and Argentina), and Paraná (bordering Argentina and Paraguay)—represent a risk of spread of the virus to the bordering countries, especially in areas with similar ecosystems.

Figure 4. Distribution of yellow fever epizootics. Brazil, 1 December 2016 to 15 February 2017



Source: Published by Brazil Ministry of Health

In response to this situation, public health authorities at the federal, state and municipal levels are implementing various activities, including the distribution of approximately 12.5 million vaccines to the states of Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo.

While the possibility of a change in the yellow fever transmission cycle in this current outbreak remains, to date there is no evidence that *Aedes aegypti* plays a role in the transmission.

The Brazil situation report on the yellow fever outbreak is published daily by the Brazil Ministry of Health and is available at: http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/leia-mais-o-ministerio/619-secretaria-svs/l1-svs/27300-febre-amarela-informacao-e-orientacao.

Recommendations

The PAHO / WHO guidance on Laboratory Diagnosis of Yellow Fever Virus Infection, February 2017, is included below and the complete document is available at: http://www.paho.org/hq/index.php?option=com_topics&view=rdmore&cid=5514&Itemid=40784&lang=en.

Laboratory Diagnosis of yellow fever virus infection

The yellow fever virus 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 mainly by sylvatic mosquito vectors of the genera *Haemagogus* and *Sabethes* as well as by the *Aedes aegypti* mosquito. The clinical spectrum of yellow fever ranges from asymptomatic or mild infection to potentially fatal severe conditions with hemorrhage and jaundice. 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. Thus, confirmation by laboratory techniques should be addressed for characterization of cases and of the outbreak.

The most important measure of prevention of yellow fever is vaccination which provides protective immunity against the disease to 80-100% of those vaccinated after 10 days and 99% immunity after 30 days. Although the yellow fever vaccine is safe and adverse events are uncommon, contraindications and safe immunization practices must be respected.

Sample types and laboratory procedures

The diagnosis of yellow fever is made by virological methods (detection of the virus or of its genetic material in serum or tissue) using virus isolation or Reverse transcription polymerase chain reaction (RT-PCR), or by means of serological testing for the detection of antibodies.

Biosafety considerations

All biological samples (whole blood, serum or fresh tissue) should be considered as potentially infectious. All laboratory personnel handling these samples must be vaccinated against yellow fever and use appropriate personal protective equipment. Likewise, 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 of non-human samples should be carefully assessed according to the biosafety manual of the laboratory, and use of Class III biosafety cabinets should be considered.

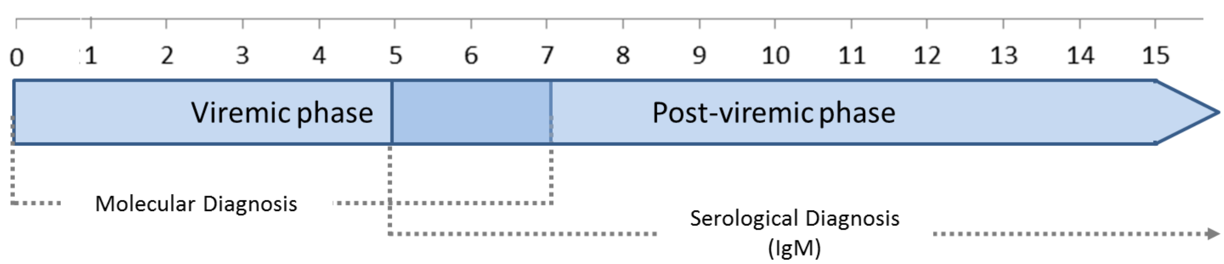
Virological diagnosis

- ***Molecular diagnostics:*** Viral RNA can be detected during the first 5 days from symptom onset (viremic phase) it using molecular techniques such as end-point of real-time RT-PCR. Occasionally, viral RNA can be detected up to 7 days from symptom onset. Thus, it is recommended to perform both RT-PCR and IgM ELISA for samples collected between days 5 and 7 from the onset of symptom (figure 1). A positive result (when using the appropriate controls) confirms the diagnosis.

- ***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). However, because of its complexity, this methodology is rarely used as a diagnostic tool and is recommended mainly for research studies complementary to public health surveillance.

- ***Postmortem diagnosis:*** Histopathological analysis with immunohistochemistry performed on liver sections is considered the "gold standard" for the diagnosis of yellow fever in fatal cases. Additionally, molecular detection can also be performed in fresh or paraffin-embedded tissue samples to confirm the cases. The procedure may be carried out under BSL2 containment (See above the section *Biosafety considerations* for non-human samples).

Figure 5. Indications for yellow fever diagnosis according to the number of days since the onset of symptoms



Serological diagnosis

Serology (the detection of specific antibodies) is useful for diagnosing yellow fever during the post-viremic phase of the disease (i.e., from the 5th day since the onset of symptoms).

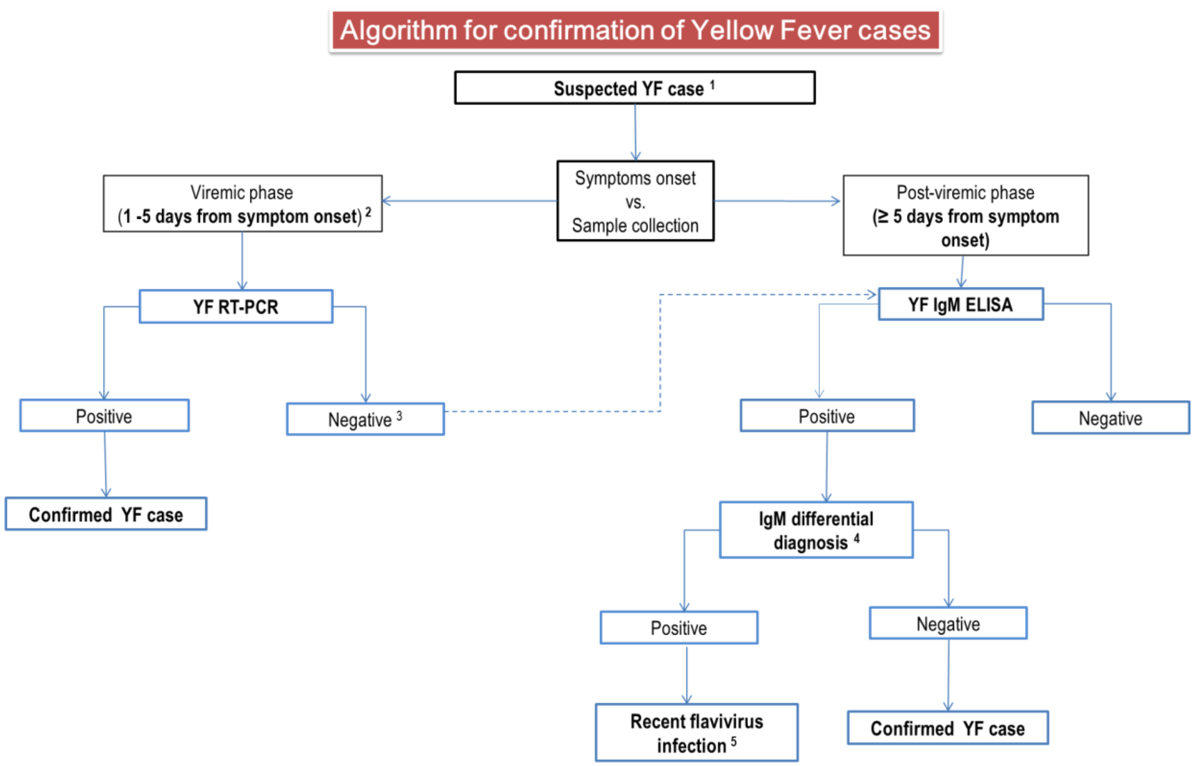
A positive IgM reaction by enzyme-linked immunosorbent assay (ELISA) (mainly IgM antibody-capture, MAC-ELISA) or any other immunoassay (indirect immunofluorescence) in a sample collected from the 5th day of symptom onset is presumptive of recent yellow fever virus infection. Currently, there are not commercially available, validated IgM ELISA kits. Therefore, in-house protocols using whole purified antigen may be standardized.

The confirmation of a case of yellow fever by IgM ELISA will depend on the epidemiological situation and the results of the laboratory differential diagnosis. In areas where other flaviviruses co-circulate (especially dengue and Zika), the probability of cross-reactivity is higher (Figure 6).

Other serological techniques include the detection of IgG antibodies by ELISA and of neutralizing antibodies by plaque reduction neutralization test (PRNT). IgG ELISA is useful with paired samples (collected at least 1 week apart), while PRNT90 may be useful with paired samples, or with a single post-viremic sample if the assay includes multiple flaviviruses.

A seroconversion (negative results in the first sample and positive result in the second sample), a more than 4-fold increase in antibody titers in paired samples, or a detectable antibody titer against yellow fever in a post-viremic sample by PRNT90 is presumptive of yellow fever virus infection. Confirmation of a yellow fever case using these techniques will depend on the epidemiological situation and the results of the laboratory differential diagnosis. In areas with co-circulation of other flaviviruses the probability of cross reactivity is higher (see figure 2). Additionally, in those areas where active vaccination campaigns are ongoing, detection of vaccine induced antibodies may occur and then diagnosis should be carefully interpreted (see below the section *Post-vaccination immune response*).

Figure 6. Algorithm for confirmation of yellow fever cases



[1] Suspected YF case (**unvaccinated or unknown vaccination history)**
[2] Occasionally, viral RNA can be detected up to 7 days from symptom onset
[3] If the PCR is negative, check sample quality, collection and transport conditions. Also, consider collecting a post-viremic sample for serological testing.
[4] Must include at least dengue (and other flaviviruses depending on the epidemiological situation of the region / country).
[5] If samples are available, consider performing PRNT on paired samples in a reference laboratory

Interpretation of serology results and differential diagnosis

Serological techniques are often cross-reactive among flavivirus infections (in particular, in secondary flavivirus infections). This should be considered in areas where the co-circulation of yellow fever virus with other flaviviruses (dengue, St. Louis encephalitis, Zika, and others of the Japanese encephalitis complex) is documented and the population is likely to have been previously infected with these viruses. Also, it should be noted that in individuals vaccinated against yellow fever, vaccine-induced IgM can be detected for several months or even years.

Therefore, it is recommended to carry out the parallel detection of antibodies to other flaviviruses and to carefully interpret the results taking into consideration the individual vaccination history as well as the available epidemiological information.

In general, the PRNT offers greater specificity than the detection of IgM and IgG. However, cross-reactivity has also been documented for the neutralization assays. Thus, it is also recommended that this technique be performed using antigens for several flaviviruses.

Moreover, the differential diagnosis of yellow fever should include other febrile and febrile icteric syndromes – such as dengue, leptospirosis, malaria, viral hepatitis, among others – depending on the epidemiological profile of the affected country or area.

A case of yellow fever will be confirmed by serological techniques only if the differential laboratory diagnosis, taking into consideration the epidemiological situation of the country, is negative for another flavivirus (Figure 6).

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 one month and in some cases (mainly travelers) up to 3-4 years. 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 those who have recently been vaccinated and results should be carefully assessed.

Sample conservation

- Whole blood (in 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. Serum samples can be stored at -70 °C C for extended periods of time.

- 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, store fresh tissue in sterile saline or refrigerated PBS (2-8 °C) and ship with refrigerant gels.

- For histopathological and immunohistochemistry analyses, tissue samples (approximately 1 cm^3) must be fixed in buffered formalin and sent to a pathology laboratory at room temperature. Liver is the tissue of choice for histopathological and immunohistochemistry analyses. Spleen and kidney 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:

- The cold chain should be maintained with dry ice (if possible) or with refrigerant gel. Triple packaging should always be used.

- Samples should be shipped, if possible, within the first 48 hours.

- The original samples must be packaged, marked, labeled (if dry ice is used) and registered as **category B**.

- The shipment must be accompanied by the complete clinical and epidemiological record.

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