There are three well-established procedures for the laboratory diagnosis of yellow fever. These are:

(a) isolation of virus from the blood of a patient early in the disease;
(b) demonstration of the development of specific antibody to yellow fever virus in the serum of a patient during illness; and
(c) demonstration of the pathognomonic histological lesions of yellow fever in a piece of liver obtained during the course of illness or at autopsy.

1. Virus isolation

To isolate virus from the serum of a patient with yellow fever, the blood should be taken as soon as possible after the onset of fever, preferably during the first four days. The serum should be inoculated subcutaneously into susceptible monkeys, or intracerebrally into baby mice 1-3 days old.

Any available susceptible non-human primate can be used, provided that the test animals are known to be non-immune to yellow fever. Rhesus monkeys, however, are the most sensitive primates to use as they develop a febrile reaction while the virus is circulating in their blood.

However, because of the high cost involved in securing monkeys, mice are usually preferred for virus isolation. When mice are used the procedure includes the inoculation of undiluted serum intracerebrally into a litter of five baby mice 1-3 days old. It is also wise to inoculate 1:10 and 1:100 of the serum each into a similar group of mice. The reason for testing serum diluted 10-fold and 100-fold, as well as undiluted, is that the latter sometimes produce non-fatal encephalitis in the first passage, whereas the same serum diluted as above produces fatal encephalitis under the same conditions. The three groups of mice are examined for signs of encephalitis daily for 21 days. Passages into fresh mice are made with the brain suspension of any mice that sicken during the period of observation, provided the illness is not due to iatrogenically induced toxic or bacterial contamination.

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Identification of the virus

Once the virus has been established by two or three passages, it is identified by means of a neutralization or mouse protection test. Such a test is done by making a suspension of the brains of mice ill or dying of encephalitis caused by the unknown agent, diluting the suspension in a suitable diluent, and then mixing the aliquots of the material with known normal serum and with a known hyperimmune serum. After incubation, the mixtures are injected into two groups of mice. If mice that receive the mixture of virus and normal serum die, while those which receive the known yellow fever immune serum survive, the virus in question is identified as yellow fever virus.

Failure to isolate virus from the serum of a suspected case, even though the specimen is apparently taken at the proper stage of the disease and is properly handled afterwards, does not prove that the case was not of yellow fever.

2. Demonstration of the development of specific antibody to yellow fever virus

Yellow fever virus neutralizing and haemagglutination-inhibition antibodies develop during the acute phase and can be detected about five days after onset, reaching maximal levels after 3-4 weeks. Complement fixing antibody also develops in the second week after onset, but does not last nearly as long, probably six months. Accordingly, if both virus neutralizing and complement fixing antibody are found in a serum, infection is probably fairly recent.

In the light of the above information the diagnostic procedure in this respect involves the collection of acute and convalescent sera in order to demonstrate the appearance and subsequent rise in titre of neutralizing, haemagglutination-inhibition and complement fixing antibodies. The first specimens should be collected as early as possible in the course of illness and the second two weeks or more after the onset.

If the patient is not seen until the fifth or sixth day of illness, it is not too late to take the first specimen. Under such conditions, the second specimen may be taken 3-4 weeks instead of two, after onset. Where possible, the acute and convalescent sera should be tested at the same time in order to ensure that the tests are done under the same conditions. The neutralization test is carried out in mice as already described above.

If the first specimen of serum contains no antibodies mentioned above, while the second specimen does contain them, positive proof is at hand that the patient has had yellow fever infection. Equally conclusive is the demonstration of a four-fold rise in antibody titre in the convalescent serum.

If a patient is first seen when convalescent, it is still worth while to test a specimen of serum. If it contains no antibody there is proof that the illness was not yellow fever. However, if the serum is positive for antibody, all the test indicates is that at some time in the past the patient suffered from yellow fever.

Because yellow fever virus belongs antigenically to arbovirus Group B, most closely related to dengue viruses, detection of antibody rise by haemagglutination-inhibition or complement fixing tests is not sufficient to establish a diagnosis. Either a virus must be isolated and identified as yellow fever, or in vitro serological titres and neutralization indices must be greater for yellow fever than other Group B arboviruses.

3. Demonstration of the histological lesions

In fatal cases yellow fever diagnosis can be established by examining a piece of liver, obtained by viscerotome or autopsy, for histological lesions which are sufficiently characteristic in themselves to confirm the diagnosis.
The most prominent histopathological change is necrosis of the parenchymal cells, principally in the midzonal region of lobules. All stages of cellular damage may be seen, from cloudy swelling and fatty change, the coagulative necrosis with margination of chromatin, rupture of nuclear membranes with scattering of nuclear fragments, and eventual hyalinization of entire cells which then become the brightly eosinophilic Councilman bodies. In the early stages of yellow fever, small acidophilic granules, termed Tornes bodies, may be observed. In non-fatal cases, regeneration of liver cells appears to be rapid and complete. No cirrhosis develops following yellow fever infection.