# Yellow Fever Testing Algorithm for **Routine Surveillance**

Version 22Nov2022

- ❑ Serology available
- ❑ RT-qPCR available
- ❑ IHC available

A suspected YF case AND/OR clinical suspicion of YF[1]

Non-fatal

Fatal

Serum or plasma (≥ 2ml), or whole blood[2] (≥ 4ml)

Fresh Tissue[10]

Fixed Tissue[10]

Collected **>14 days** post symptom onset[3]

Collected **≤14 days** post symptom onset[3]

YF RT-qPCR

YF RT-qPCR

YF IHC

RT-qPCR not available

Negative

Positive

Negative

Positive

Report as: **CONFIRMED YF INFECTION**[4]

Report as: **EXCLUDE YF INFECTION**[11]

Report as: **CONFIRMED YF INFECTION**[4]

YF IgM[5]

Positive

Equivocal[6]

Negative

Re-test[7]

Equivocal[6]

Collected ≤7 days post symptom onset

Collected >7 days post onset symptoms and/or collected from jaundice or haemorrhagic fever case

**Documented history of YF vaccination?**

Report as: **INCONCLUSIVE**[9]

Report as: **EXCLUDE YF INFECTION**

**YES**

**NO**, Vaccination history unknown, or case known to be unvaccinated

Report as: **PRESENCE OF YF IgM IN VACCINATED INDIVIDUAL. Interpret with care, considering clinical presentation & epidemiological context**[13]

Refer at least 0.5ml (ideally ≥ 1ml) of serum or plasma to RRL for confirmation[8,12] (with mandatory Case Investigation Form which includes vaccination history; AND the specimen referral form mentioning the testing history)

Report as: **PRESUMPTIVE EVIDENCE OF ACUTE YF INFECTION OR OF VACCINATION.**[8] **CONFIRMATORY & DIFFERENTIAL TESTING RESULTS ARE PENDING**[12]

[1] A suspected YF case is any person with acute onset of fever, with jaundice appearing within 14 days of onset of the first symptoms. Clinical suspicion of YF may be made prior to the appearance of jaundice and is based on other clinically compatible symptoms such as fever, headache, myalgia, nausea, vomiting, and fatigue and on epidemiologic factors. Assessment of YF vaccination history, malaria testing history, travel history, and history of contact with a known YF case (if relevant) should be recorded and taken into consideration when interpreting test results.

[2] Applicable to RT-qPCR testing and IgM RDT only. EDTA blood tubes should ideally be used. Avoid using sodium heparin tubes as interfering with PCR.

[3] RT-qPCR sensitivity is higher in the first 10 days from symptom onset, decreasing as viremia is cleared. However, detection up to 14 days has been reported, particularly in severe cases. In immunosuppressed cases, viremia may even last longer. Therefore, RT-qPCR might be attempted in samples collected ≤14 days from onset. A positive result in those samples will confirm a YF infection, whereas a negative result would not exclude the possibility of a YF infection. Samples with negative RT-qPCR results should be referred for IgM testing regardless of the day post-onset of illness that they were collected as a negative molecular result does not rule out YF and serology should be done. For fatal cases, RT-qPCR should be performed on all available samples, independent of the collection date. If the laboratory has capacity to only test by RT-qPCR or IgM serology, all samples at any number of days post-onset should be tested with the assay.

[4] For cases with no history of vaccination, vaccination history unknown or vaccinated >14 days before onset illness. In areas where YF virus infections have not been reported recently, immediate confirmatory testing at a Regional Reference Laboratory (RRL) is required for such cases (need to specifically request it to RRL when referring specimen).

[5] If the IgM results are uninterpretable (UI) due to high background and/or potential inhibitory factors, consider repeating the test, and request a 2nd specimen if repeat result is still UI. If testing is or continues to be UI, treat as negative.

[6] In the case where a MAC-ELISA method is used, an equivocal result is when a valid result falls within the range between a negative or positive result. Refer to the test instructions for the indicated range of equivocality for this specific test. Equivocal does not refer to an UI test result, e.g., equivocal is not due to presence of factors that cause non-specific background reactions.

[7] In the case of a first equivocal test result, the test should be repeated. If a repeated equivocal test result remains equivocal upon re-testing a second sample needs to be requested and tested. If a second sample is not available, the initial sample should be sent to the RRL. If the second sample repeats as equivocal both first and second sample should be sent to the RRL.

[8] A positive IgM result alone is not confirmatory but considered presumptive evidence of infection. Additional clinical and epidemiological criteria, such as history of vaccination, must be used for the final interpretation of the results and classification of the suspected YF case.
To confirm the infection, particularly in areas where no YF virus circulation has been recently described, differential neutralization testing with flaviviruses endemic to the area of exposure or neutralization testing of appropriately paired sample set to demonstrate seroconversion should be performed in an RRL.

[9] Do not delay reporting of the inconclusive result. With an inconclusive result, infection cannot be ruled out, though it is less likely if molecular testing is negative. Whenever possible a second sample taken ≥10 days post onset of illness should be requested and tested to account for possible seroconversion.

[10] Fresh and fixed tissue samples (≥ 1cm$^3$) should be collected (liver and kidney tissue should always be collected; additionally, spleen, lung, brain and heart tissue can be collected) and tested in fatal cases regardless of sampling date after onset of symptoms. If no other specimen is available, paraffin-embedded tissue could also be used for RT-qPCR testing.

[11] A negative RT-qPCR in tissue from fatal cases can be followed up with serology if serum or plasma was collected before death.

[12] Final interpretation to be reported and advice on conclusion should occur after all testing is complete (e.g., malaria, differential IgM and PRNT for other flaviviruses).

[13] In recent vaccinees (<30 days) who develop classical symptoms of YF infection, targeted sequencing or use of discriminatory RT-qPCR should aim to differentiate between infections with wild-type YF and the vaccine virus strain. Note: YF IgM antibodies can persist for months to years post-vaccination.

# Yellow Fever Testing Algorithm during **Outbreak**

Version 22Nov2022

- ❑ Serology available
- ❑ RT-qPCR available
- ❑ IHC available

A suspected YF case AND/OR clinical suspicion of YF[1]

- Non-fatal
  - Serum or plasma (≥ 2ml), or whole blood[2] (≥ 4ml) → YF RT-qPCR[3]
    - RT-qPCR testing not available → YF IgM[5]
    - Negative → YF IgM[5]
    - Positive → Report as: **CONFIRMED YF INFECTION**[4]
  - YF IgM[5]
    - Positive → Documented history of YF vaccination?
      - YES → Report as: **PRESENCE OF YF IgM IN VACCINATED INDIVIDUAL. Interpret with care, considering clinical presentation & epidemiological context**[14]
      - **NO**, Vaccination history unknown, or case known to be unvaccinated → Refer at least 0.5ml (ideally ≥ 1ml) of serum or plasma to RRL for confirmation [8,9,13] (with mandatory Case Investigation Form which includes vaccination history; AND the specimen referral form mentioning the testing history) → Report as: **EVIDENCE OF ACUTE YF INFECTION OR OF VACCINATION.[8] CONFIRMATORY & DIFFERENTIAL TESTING RESULTS ARE PENDING**[9,13]
    - Equivocal[6] → Re-test[7] → Equivocal[6] → Documented history of YF vaccination?
    - Negative
      - Collected ≤7 days post onset symptoms → Report as: **INCONCLUSIVE**[10]
      - Collected >7 days post onset symptoms and/or collected from jaundice or haemorrhagic fever case → Report as: **EXCLUDE YF INFECTION**
- Fatal
  - Fresh Tissue[11] → YF RT-qPCR[3]
    - Positive → Report as: **CONFIRMED YF INFECTION**[4]
    - Negative → Report as: **EXCLUDE YF INFECTION**[12]
  - Fixed Tissue[11] → YF IHC
    - Negative → Report as: **EXCLUDE YF INFECTION**[12]
    - Positive → Report as: **CONFIRMED YF INFECTION**[4]

[1] A suspected YF case is any person with acute onset of fever, with jaundice appearing within 14 days of onset of the first symptoms. Clinical suspicion of YF may be made prior to the appearance of jaundice and is based on other clinically compatible symptoms such as fever, headache, myalgia, nausea, vomiting, and fatigue and on epidemiologic factors. Assessment of YF vaccination history, malaria testing history, travel history, and history of contact with a known YF case (if relevant) should be recorded and taken into consideration when interpreting test results.

[2] Applicable to RT-qPCR testing and IgM RDT only. EDTA blood tubes should ideally be used. Avoid using sodium heparin tubes as interfering with PCR.

[3] Whenever available, RT-qPCR should be the first-line test during an outbreak, irrespective of the number of days since symptoms onset. Therefore, RT-qPCR might be attempted in all samples collected as a primary test. A positive result in those samples will confirm a YF infection, whereas a negative result would not exclude the possibility of a YF infection. Samples with negative RT-qPCR results should be referred for IgM testing regardless of the day post-onset of illness that they were collected as a negative molecular result does not rule out YF and serology should be done. In case of unavailability of the typical blood-based specimens, RT-qPCR on alternative specimen such as saliva, urine, and sputum could be useful to support case confirmation. For fatal cases, RT-qPCR should be performed on all available samples, independent of the collection date. If the laboratory has capacity to only test by RT-qPCR or IgM serology, all samples at any number of days post-onset of illness should be tested with the available assay.

[4] For cases with no history of vaccination, vaccination, history unknown, or vaccinated >14 days before onset illness.

[5] If IgM results are uninterpretable (UI) due to high background and/or potential inhibitory factors, consider repeating the test, and request a 2nd specimen if repeat result is still UI. If testing is or continues to be UI, treat as negative.

[6] In the case where a MAC-ELISA method is used, an equivocal results is when a valid result falls within the range between a negative or positive result. Refer to the test instructions for the indicated range of equivocality for this specific test. Equivocal does not refer to an UI test result, e.g., equivocal is not due to presence of factors that cause non-specific background reactions.

[7] In the case of a first equivocal test result, the test should be repeated. If a repeated equivocal test result remains equivocal upon re-testing a second sample needs to be requested and tested. If a second sample is not available, the initial sample should be sent to the Regional Reference Laboratory (RRL). If the second sample repeats as equivocal both first and second sample should be sent to the RRL.

[8] A positive IgM result alone is not confirmatory but considered presumptive evidence of infection. Additional clinical and epidemiological criteria, such as history of vaccination, must be used for the final interpretation of the results and classification of the suspected YF case.
It is not essential to perform serology testing to differentiate yellow fever and other flaviviruses on specimens where local transmission of YF has already been confirmed.
To confirm the infection, particularly in areas where no YF virus circulation has been recently described, differential neutralization testing with flaviviruses endemic to the area of exposure or neutralization testing of appropriately paired sample set to demonstrate seroconversion should be performed in an RRL.

[9] Once an outbreak of YF has been confirmed in a specific area, the decision might be made to not refer subsequent samples from unvaccinated cases of the same area to a RRL for confirmatory testing but to report as Presumptive infection based on a YF IgM positive result. However, cases from a new geographic area, particularly if adjacent or linked to outbreak areas, or with atypical or unusual clinical presentation, should be referred to RRL for further confirmatory testing.

[10] A second sample taken ≥10 days post onset of illness should only be requested and tested if the case is from district where there is still no sign of an outbreak.

[11] Fresh and fixed tissue samples (≥1cm$^3$) should be collected (liver and kidney tissue should always be collected; additionally, spleen, lung, brain and heart tissue can be collected) and tested in fatal cases regardless of sampling date after onset of symptoms. If no other specimen is available, paraffin-embedded tissue could also be used for RT-qPCR testing.

[12] A negative RT-qPCR in tissue from fatal cases can be followed up with serology if serum or plasma was collected before death.

[13] Final interpretation to be reported and advice on conclusion should occur after all testing is complete (e.g., malaria, differential IgM and PRNT for other flaviviruses).

[14] In recent vaccinees (<30 days) who develop classical symptoms of YF infection, targeted sequencing or use of discriminatory RT-qPCR should aim to differentiate between infections with wild-type YF and the vaccine virus strain. Note: YF IgM antibodies can persist for months to years post-vaccination.