Yellow Fever **CONFIRMATORY** Testing Algorithm for **Regional Reference Laboratories**

- ❑ Serology
- ❑ RT-qPCR
- ❑ PRNT
- ❑ Virus isolation

Positive or Equivocal IgM result from national labs

National labs refer specimen to RRL for confirmation
(It is mandatory to include Case Investigation Form (with vaccination history); and Specimen referral form (with testing history)

RT-qPCR not conducted or result not available[1]

Perform RT-qPCR for YF[2]

Positive (+)[6]

Report as: **CONFIRMED YF INFECTION**[3]

Negative (-)

Negative RT-qPCR result documented[1]

Perform IgM ELISA for YF, Dengue, Zika, West Nile, and relevant additional arboviruses[4]

Positive RT-qPCR result documented[1,6]

Re-testing by RT-qPCR is not required[8]

Report as: **CONFIRMED YF INFECTION**[3]

Documented history of YF vaccination

Never vaccinated, or YF vaccination history unknown

YF IgM (+) (or Equivocal (Eq)) AND IgM (-) for all other flavivirus

Report as: **PRESENCE OF YF IGM IN VACCINATED INDIVIDUAL**[5,9] Interpret with care, considering clinical presentation & epi. context

YF IgM (+) (or Eq) AND IgM (+) (or Eq) for at least one other flavivirus

Report as: **PRESENCE of IgM for YF and at least for one OTHER FLAVIVIRUS in a YF-VACCINATED INDIVIDUAL**[5,9] Interpret with care, considering clinical presentation & epi. context

YF IgM (-) AND IgM (+) (or Eq) for at least one other flavivirus[7]

Report as: **NO EVIDENCE OF RECENT YF INFECTION. Presumptive evidence of recent flavivirus infection**[5] Clinical correlation required

IgM (-) for all flaviviruses (incl. YF)

Report as: **NO EVIDENCE OF RECENT INFECTION WITH ANY OF THE VIRUSES LISTED, INCLUDING YF**[5] If specimen was collected ≤7 days post symptom onset, the lack of serologic evidence of infection may reflect testing of acute-phase specimen(s) obtained before development of an antibody response. Consider collecting a convalescent specimen

YF IgM (+) (or Eq) AND IgM (+) for at least one other flavivirus

PRNT[11] routinely performed in the Regional lab network

Perform concurrent PRNT[11] for any IgM (+) flavivirus(es)

Report as per Differential PRNT[11] interpretation Table on the NEXT PAGE

YF IgM (+) (or Eq) AND IgM (-) for all other flaviviruses

PRNT[11] NOT routinely performed in the Regional lab network

Report as: **PRESUMPTIVE EVIDENCE OF RECENT FLAVIVIRUS INFECTION OR OF VACCINATION**[10]

Report as: **PRESUMPTIVE EVIDENCE OF RECENT YF INFECTION OR OF VACCINATION**[5]

[1] Laboratories must be part of the GYFLaN and accredited by WHO to perform RT-qPCR. If patient specimen was collected ≤14 days from symptom onset and tested for YF RT-qPCR by the national lab, there is no need for the RRL to repeat the RT-qPCR, unless specifically requested by national lab. However, if there are no documented RT-qPCR results from national lab, the RRL should perform the RT-qPCR for YF. In recent vaccinees (<30 days) who develop classical symptoms of YF infection, and for which a positive RT-qPCR result from the national lab is documented, the RRL should perform targeted sequencing or use of discriminatory RT-qPCR in order to differentiate between infections with wild-type YF virus and the vaccine virus strain.

[2] Whenever available, RT-qPCR should be the first-line test, irrespective of the number of days since symptoms onset. A positive result in those samples will confirm a YF infection, whereas a negative result would not exclude the possibility of a YF virus 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. For fatal cases, RT-qPCR should be performed on all available samples, independent of the collection date.

[3] Clinical correlation required. For cases with no history of vaccination, vaccination history unknown, or vaccinated >14 days before symptom onset, this YF RT-qPCR positive results supports the evidence of active YF virus circulation.

[4] IgM testing for YF, Dengue, Zika, and West Nile are part of the minimum package for YF surveillance purposes. Testing for other arboviruses with similar clinical presentation or in the same genus can be added, if these arboviruses infections are common in this region and therefore epidemiological relevant and specific tests are available (ex: Rift Valley fever, Crimean-Congo Haemorrhagic Fever, etc.)

[5] 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, etc.)

[6] Virus isolation can be attempted following RT-qPCR positive result if the Ct value is <30 to support forward-thinking efforts requiring strain characterization or virus isolate for banking purpose. The outcome of the viral isolation should not delay or affect YF case surveillance reporting.

[7] Further PRNT testing for other flaviviruses with IgM positive or equivocal result can be attempted but is not mandatory as part of YF surveillance. Reporting on the absence of evidence of recent YF virus infection should not be delayed.

[8] Routine RT-qPCR testing is only conducted by accredited national laboratories. Additionally, considering the risk of specimen degradation during transportation, re-testing by RT-qPCR by RRL is not required

[9] 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. Consider documented cross-reactivity of IgM detection among flaviviruses.

[10] Consider documented cross-reactivity of IgM detection among flaviviruses. However, in areas where no YF circulation has been described recently, this result does not rule out yellow fever. Consider performing PRNT in a Regional Reference Laboratory. This should also prompt further clinical and epidemiological investigation.

[11] PRNT= Plaque Reduction Neutralization Test

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Table for the interpretation of PRNT[6] results for IgM positive specimen[4]

Yellow Fever[4] (YF) PRNT result	Dengue[4] (D) PRNT result	Zika[4] (Z) PRNT result	West Nile[4] (WN) PRNT result (If tested)	Differential Interpretation[1]
+	–	–	–	**Evidence of recent YF virus infection[2,3]**
+	+	–	–	**Differential diagnosis considering YF IgM positive result and differences in PRNT titres among IgM-positive viruses tested[5]** - If the YF titre is positive, and at least 4-fold higher than any of the D/Z/WN positive PRNT titres, the interpretation is **Evidence of recent YELLOW FEVER virus infection[2].** - If there is a <4-fold difference between any of the 2 highest positive PRNT titres, the interpretation is **Evidence of recent FLAVIVIRUS infection**. - If the YF PRNT titre is positive but one of D, Z or WN has a positive PRNT titre at least 4-fold higher than all the other PRNT titres, then the interpretation is **Evidence of recent infection of that SPECIFIC FLAVIVIRUS (D, Z or WN)**. - If the YF titre is positive, but at least 4-fold lower than the closest other positive PRNT titre, AND where two or more of the non-YF viruses have a difference in titres less than 4-fold from one another, then the interpretation is **Evidence of recent FLAVIVIRUS infection**.
+	+	–	+	
+	+	+	–	
+	+	+	+	
+	–	+	+	
+	–	–	+	
+	–	+	–	
–	+	–	–	**Evidence of recent Dengue virus infection**
–	+	–	+	**Evidence of recent Flavivirus infection** (unless D or WN has its titre ≥4 greater than the other one)
–	+	+	–	**Evidence of recent Flavivirus infection** (unless D or Z has its titre ≥4 greater than the other one)
–	+	+	+	**Evidence of recent Flavivirus infection** (unless D or Z or WN has its titre ≥4 greater than the other one)
–	–	+	+	**Evidence of recent Flavivirus infection** (unless Z or WN has its titre ≥4 greater than the other one)
–	–	–	+	**Evidence of recent West Nile virus infection**
–	–	+	–	**Evidence of recent Zika virus infection**
–	–	–	–	**Positive YF IgM result not confirmed by neutralization testing, suggesting a non-specific IgM result.**

[1] Final interpretation to be reported and advice on conclusion should occur after all testing is complete.

[2] Case classification to consider the epidemiologic context of co-circulation of other flaviviruses and previous vaccination of the Individual. Also, malaria and rheumatic diseases should also be considered as there is documented cross-reactivity affecting the specificity of the PRNT result.

[3] Interpretation also valid for YF-only PRNT (i.e., non-differential PRNT) if all IgM test results for other flaviviruses were all negative. Note that performing concurrent PRNT for any IgM-positive flavivirus(es) remains mandatory for correct differential interpretation.

[4] PRNT testing for YF must always be accompanying with **concurrent** PRNT testing for any other IgM-Positive flaviviruses tested as part of the differential IgM scheme. The interpretation of a YF PRNT result can ONLY be done as part of a differential PRNT interpretation due to known cross-reactivity among flaviviruses.

[5] Interpretation provided are considering a YF or differential PRNT SOP where the titre difference criteria between viruses tested is set to a ≥4-fold difference.

[6] PRNT= Plaque Reduction Neutralization Test