Laboratory diagnosis of Dengue, Chikungunia, Zika and Yellow fever viruses

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Laboratory Diagnosis of Dengue Virus Infections

- Virus Isolation
- RT-PCR
- NS1 Antigen Capture
- Serology

Virus Isolation

- Traditional diagnostic method
- Variety of cell lines of either mosquito (AP61, Tra-284, AP64, C6/36, and CLA-1 cells) or mammalian (LLCMK2, Vero, and BHK-21 cells) origin or in live mosquitoes .
- Blood samples taken from infected patients experiencing febrile illness up to 5 days after the onset of disease yield the most successful results.
- Although detection of DENV by virus isolation is definitive, it is not particularly practical, as isolation can take days to weeks to perform

RT-PCR

- RT-PCR assay that was highly sensitive.
- A major advantage of PCR-based techniques is that viral RNA can be detected from the onset of illness and is sensitive, specific, fast, less complicated, and cheaper than virus isolation methods.
- For patients with suspected dengue virus disease, NAATs are the preferred method of laboratory diagnosis.
- NAATs should be performed on **serum** specimens collected 7 days or less after symptom onset.

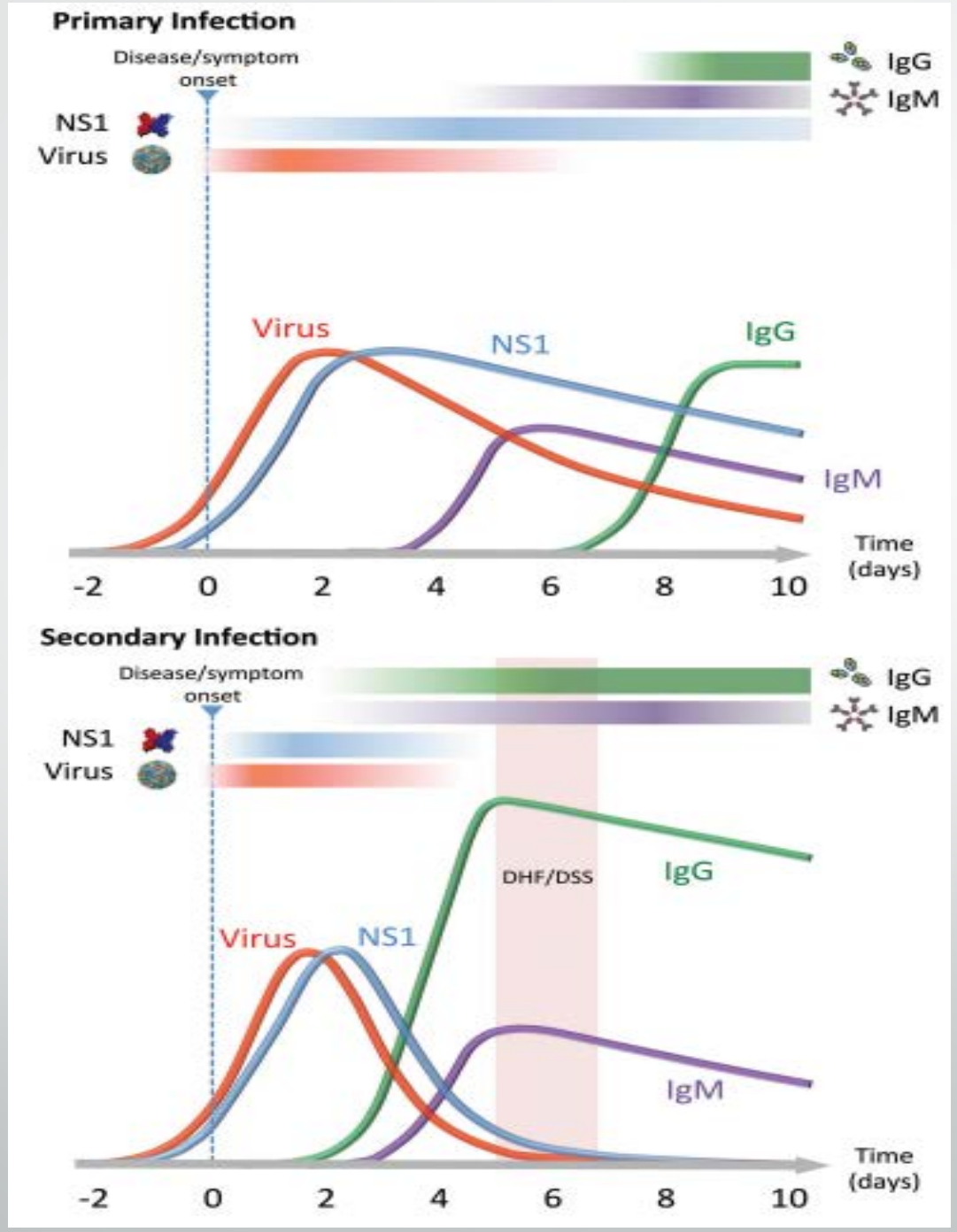
NS1 Antigen Capture

- **The viral protein NS1 is an ideal diagnostic target because it is secreted from infected cells, is found at high levels circulating in the blood of infected individuals, and can be detected from the onset of symptoms through to 9 days or longer after disease onset, at least in primary infections.**

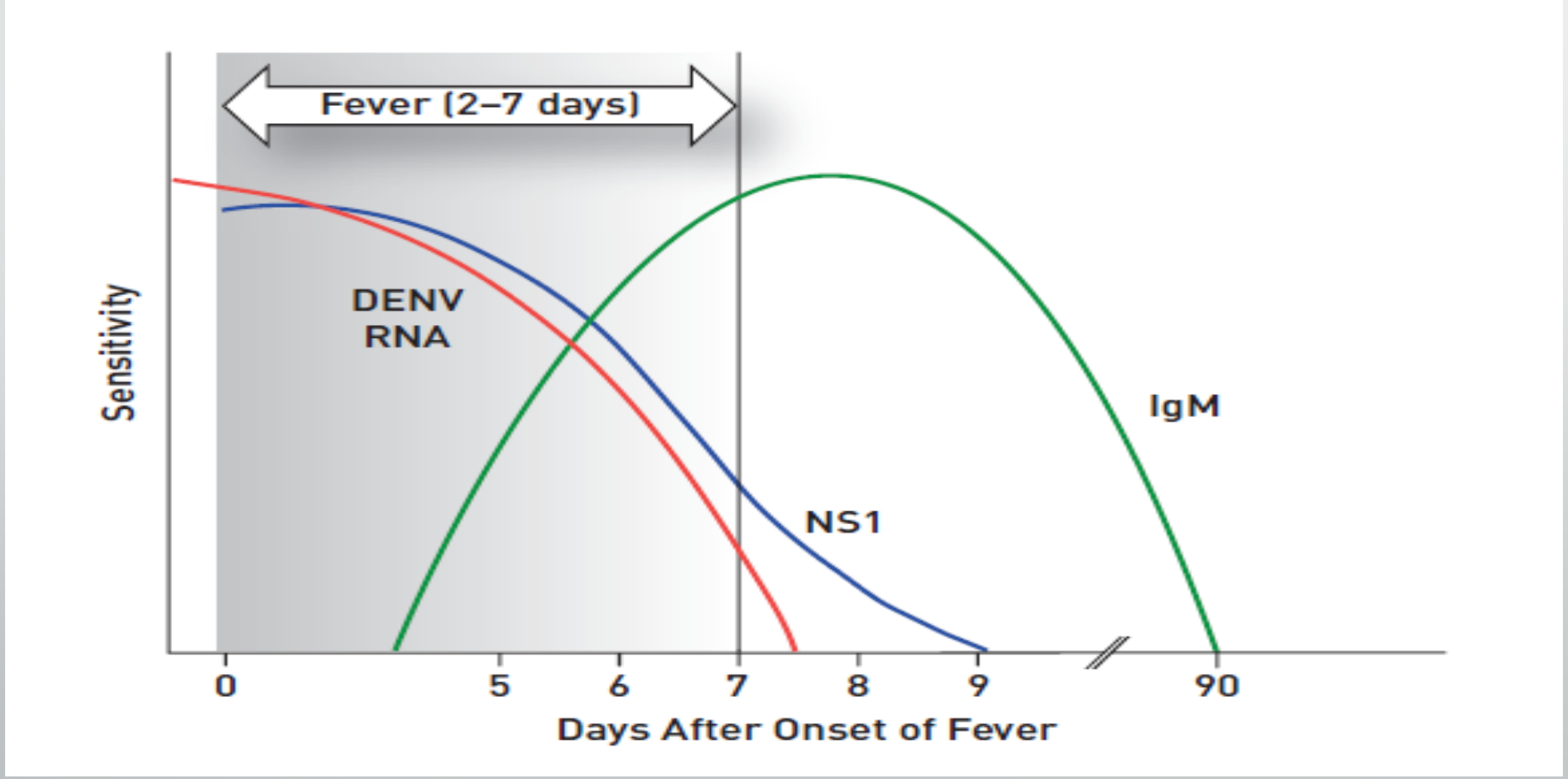
- **Laboratory confirmation can be made from a single acute-phase serum specimen obtained early (≤7 days after fever onset) in the illness by detecting viral genomic sequences with rRT-PCR or dengue nonstructural protein 1 (NS1) antigen by immunoassay.**
- **Presence of virus by rRT-PCR or NS1 antigen in a single diagnostic specimen is considered laboratory confirmation of dengue in patients with a compatible clinical and travel history.**

Serology

- Hemagglutination inhibition (HI) assays, CF, dot-blot assays, Western blotting, IFA and PRNT, as well as IgM and IgG antibody-capture ELISAs.
- HI assays along with IgM and IgG antibody-capture ELISAs have proven to be the most useful serological diagnostic methods for routine DENV detection.
- IgM antibody testing can identify additional infections and is an important diagnostic tool.
- However, interpreting the results is complicated by cross-reactivity with other flaviviruses, like Zika, and determining the specific timing of infection can be difficult.


Primary Infection
Disease/symptom onset
IgG
IgM
NS1
Virus
Virus
NS1
IgG
IgM
Time (days)
-2
0
2
4
6
8
10
Secondary Infection
Disease/symptom onset
IgG
IgM
NS1
Virus
DHF/DSS
IgG
Virus
NS1
IgM
Time (days)
-2
0
2
4
6
8
10

Relative sensitivity of detection of Dengue virus nucleic acid, antigen, and IgM



Serology

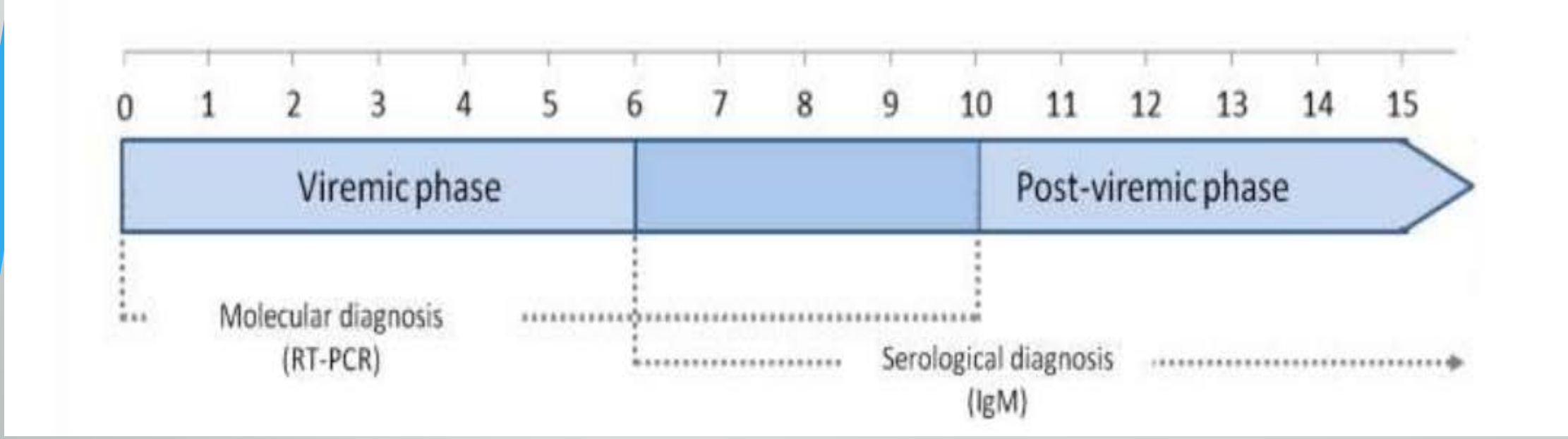
- **PRNTs can resolve false-positive IgM antibody results caused by non-specific reactivity, and, in some cases, can help identify the infecting virus.**
- **However, in areas with high prevalence of dengue and Zika virus neutralizing antibodies, PRNT may not confirm a significant proportion of IgM positive results.**

Laboratory Diagnosis of Yellow Fever Virus Infections

Virological diagnosis

- Molecular diagnostics
- Viral isolation
- Immunohistochemistry

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



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 conventional (end-point) or RT-PCR.**
- **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

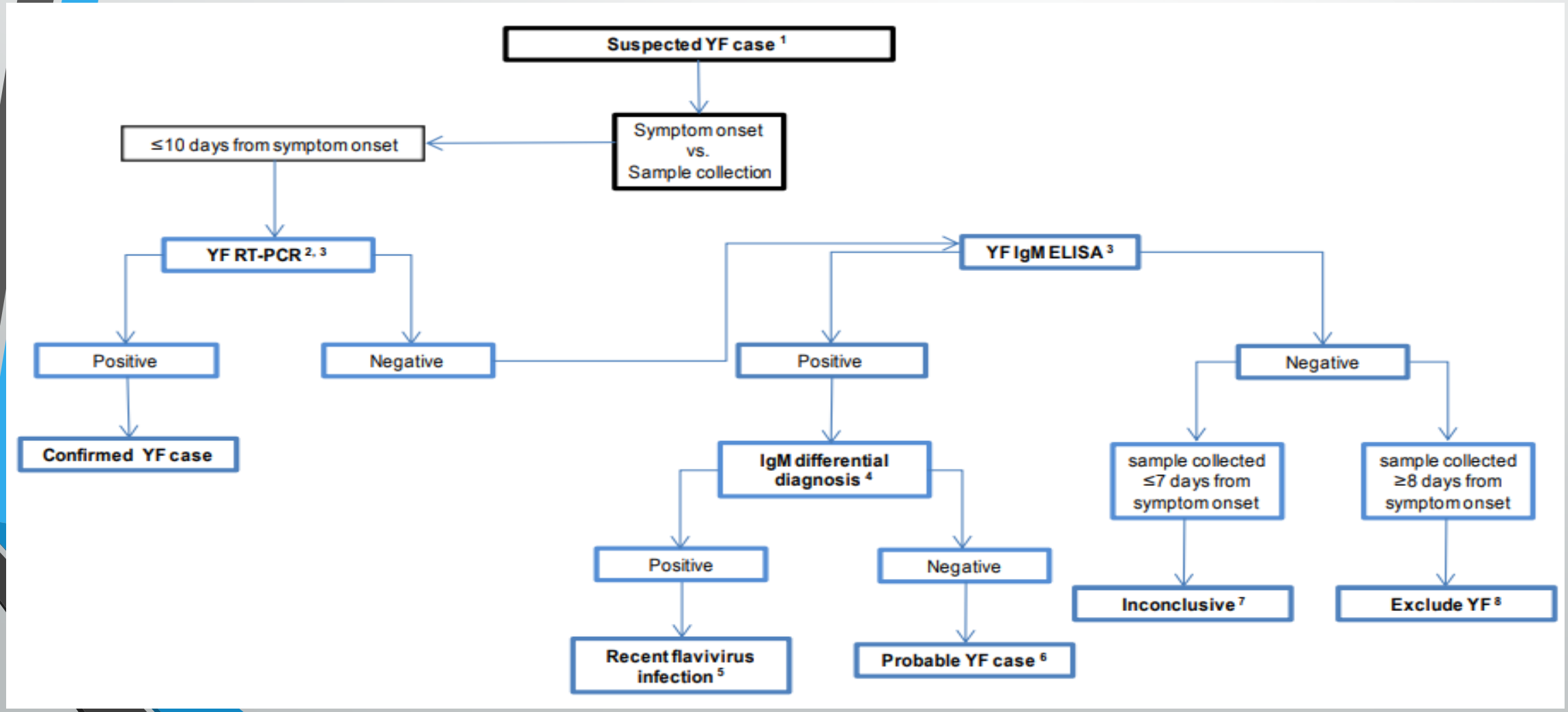
- Histopathological analysis with immunohistochemistry performed on liver sections (and other tissues) is considered the "gold standard" assay for the diagnosis of yellow fever in fatal cases.
- Additionally, molecular detection can also be performed in fresh or formalin fixed (paraffin-embedded) tissue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

Serological diagnosis

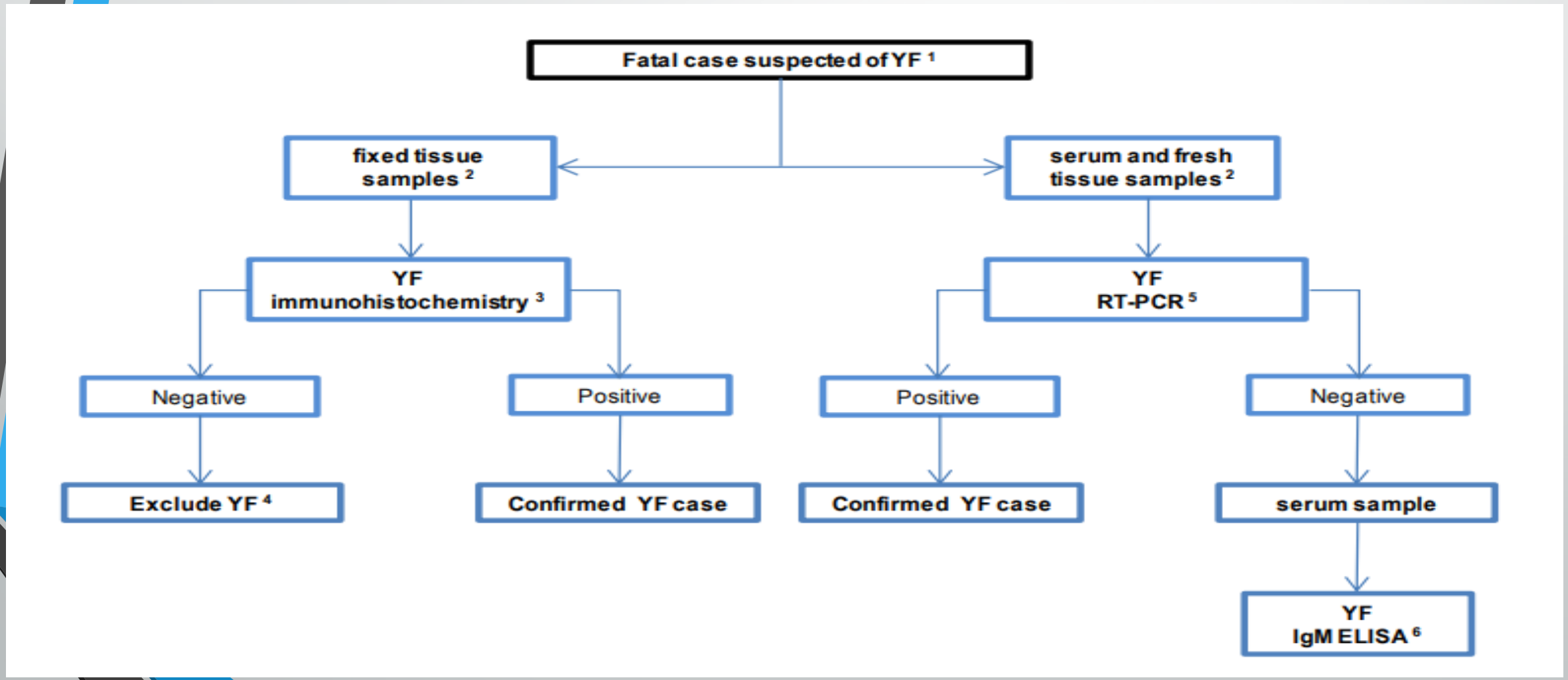
- **IgM detection**
- **Anti-YFV IgM antibodies can be detected by ELISA (mainly IgM antibody-capture, MAC-ELISA) or any other immunoassay (e.g., indirect immunofluorescence).**
- **In areas where other flaviviruses co-circulate (especially dengue and Zika viruses), the probability of cross-reactivity is high.**

- 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.

Algorithm for laboratory confirmation of yellow fever (YF) cases



Algorithm for laboratory confirmation of fatal yellow fever cases



Laboratory Diagnosis of Chikungunya Virus Infections

Chikungunya virus detection

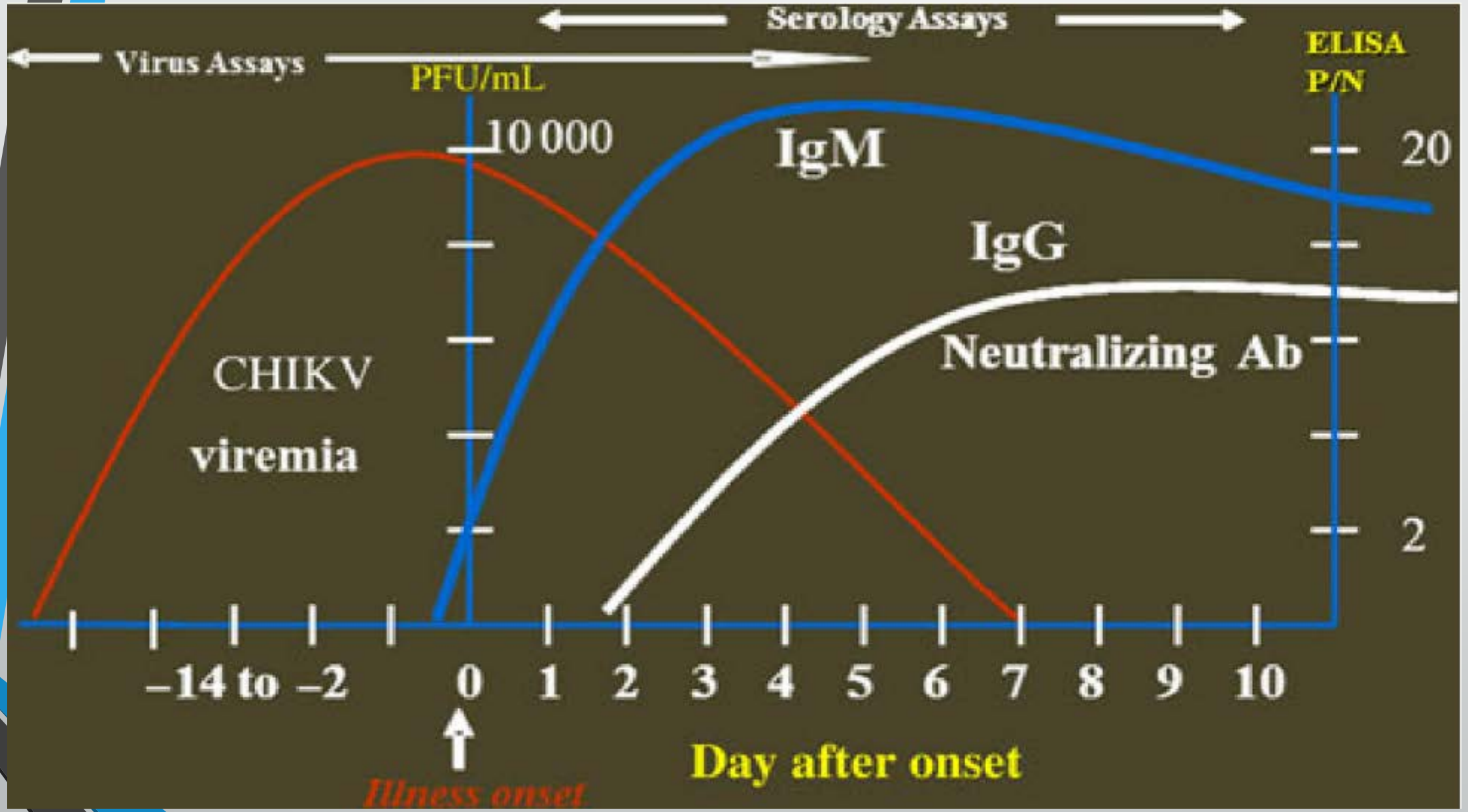
- **Laboratory diagnosis of CHIKV infection is accomplished by testing serum or plasma**
  - **Serologic methods**
  - **Virus isolation and**
  - **Viral RNA detection by RT-PCR.**

Chikungunya virus detection

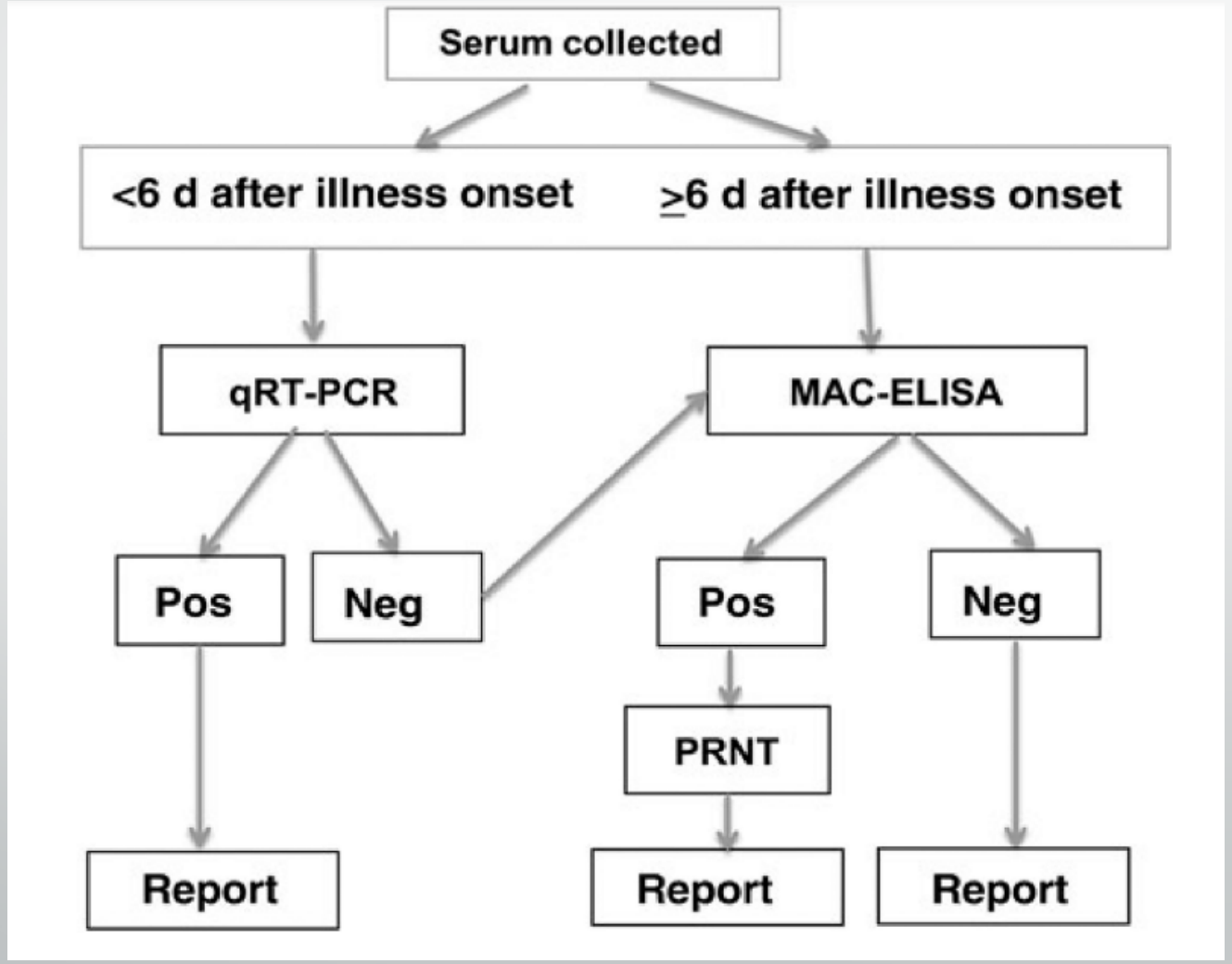
- **Viral culture may detect virus in the first 3 days of illness; however, chikungunya virus should be handled under biosafety level (BSL) 3 conditions. During the first 8 days of illness, chikungunya viral RNA can often be identified in serum.**
- **Chikungunya virus antibodies normally develop toward the end of the first week of illness. Therefore, to definitively rule out the diagnosis, convalescent-phase samples should be obtained from patients whose acute-phase samples test negative.**

Chikungunya virus detection

- **Real-time PCR assay has many advantages over conventional PCR methods, including:**
  - **Rapidity**
  - **quantitative measurement**
  - **low risk of contamination**
  - **high sensitivity**
  - **high specificity and**
  - **ease of standardization**


Serology Assays
Virus Assays
PFU/mL
ELISA
P/N
10 000
20
IgM
IgG
Neutralizing Ab
CHIKV
viremia
2
–14 to –2
0
1
2
3
4
5
6
7
8
9
10
Illness onset
Day after onset

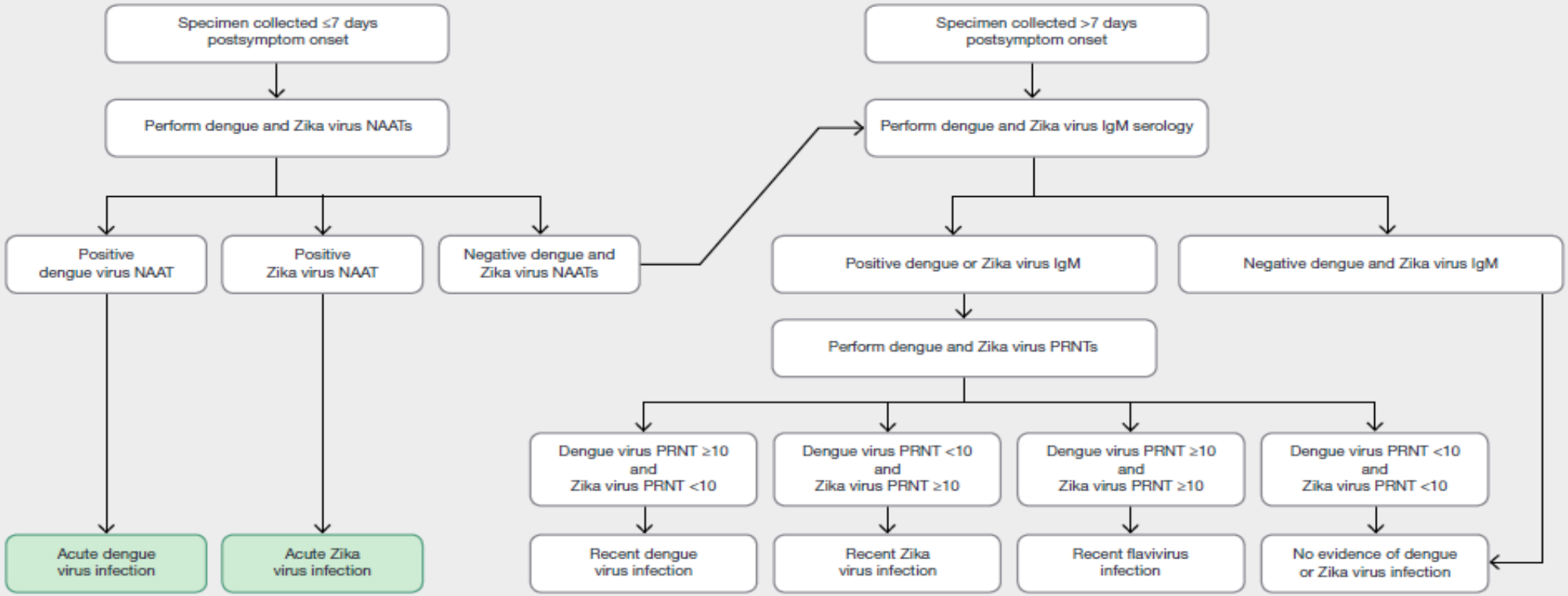
The CDC diagnostic testing algorithm for detection of CHIKV infection



Dengue and Zika Virus Testing Guidance: Symptomatic Non-Pregnant People with a Clinically Compatible Illness and Risk for Infection with Both Viruses*

Accessible version: https://www.cdc.gov/mmwr/volumes/68/rr/rr6801a1.htm?s_cid=rr6801a1_w

Testing Guidance and Interpretation of Results for Healthcare Providers



Dengue and Zika Virus Testing Guidance: Symptomatic Pregnant Women with a Clinically Compatible Illness and Risk for Infection with Both Viruses*

Accessible version: https://www.cdc.gov/mmwr/volumes/68/rr/rr6801a1.htm?s_cid=rr6801a1_w

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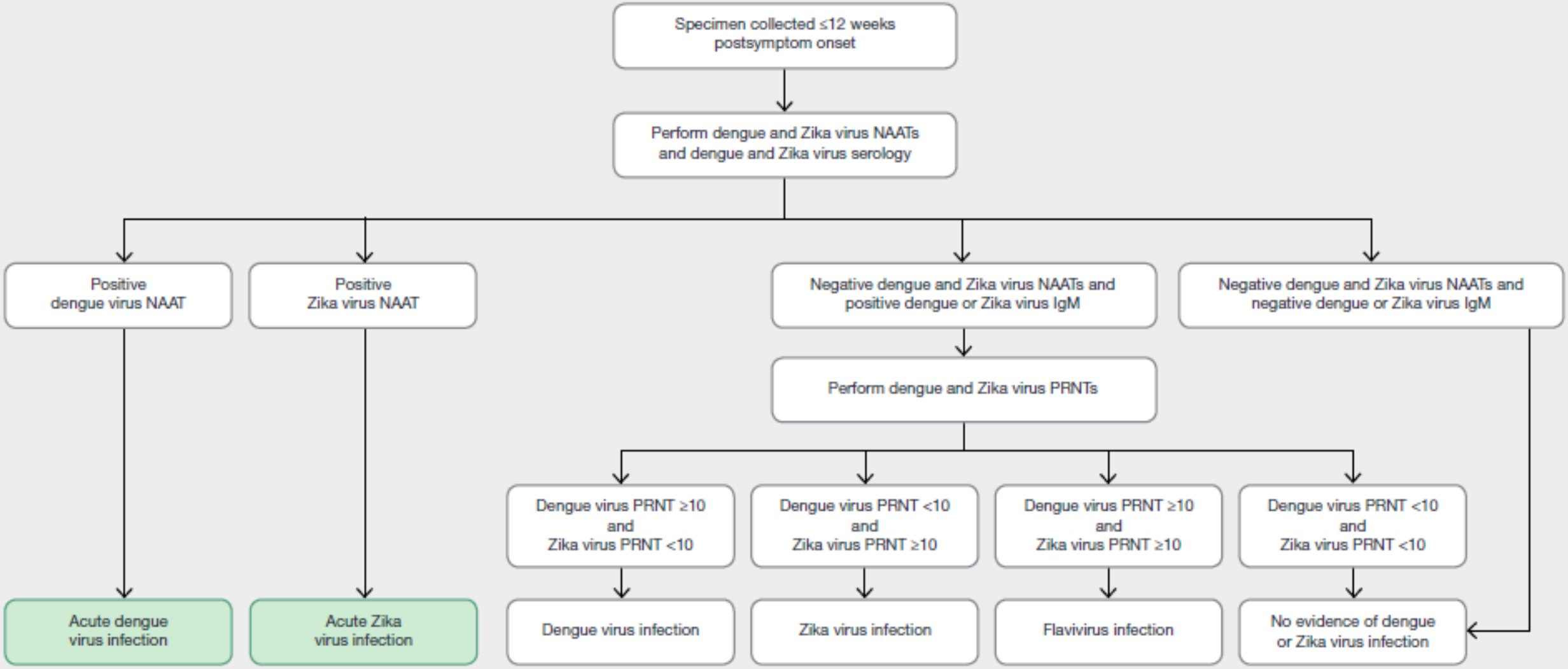

Thanks for your attention

Zika Virus Testing Guidance: Asymptomatic Pregnant Women with Possible Zika Virus Exposure

Accessible Version: https://www.cdc.gov/mmwr/volumes/66/wr/mm6629e1.htm?s_cid=mm6629e1_w

Testing Guidance and Interpretation of Results for Healthcare Providers

ASK PREGNANT WOMEN ABOUT

Travel to or residence in any areas with risk for Zika virus transmission *before* and *during* the current pregnancy[1,2]
Possible sexual exposure *before* and *during* the current pregnancy • A diagnosis of laboratory-confirmed Zika virus infection before current pregnancy[3] • Symptoms of Zika virus disease during current pregnancy (e.g., fever, rash, conjunctivitis, arthralgia) • If symptoms are reported, refer to symptomatic algorithm.

Before testing, discuss testing limitations and potential risks of misinterpretations for test results.

WHOM to test?

Asymptomatic pregnant women ***with ongoing*** possible Zika virus exposure[4]

Asymptomatic pregnant women with recent possible Zika virus exposure, **without ongoing exposure: Testing not routinely recommended, but should be considered.**

If considering testing, base decisions on patient preferences and values, clinical judgment, a balanced assessment of risks and expected outcomes, and jurisdiction's recommendations

If testing is conducted, test for Zika only (not dengue) following the algorithm for symptomatic pregnant women using timeframe from last possible exposure.

WHEN to test?

Three times during pregnancy[5]
First test at initiation of prenatal care.

WHICH tests?

Zika virus NAT (serum and urine)

RESULTS

Positive Zika virus NAT[6]

Negative Zika virus NAT

INTERPRETATION

ACUTE ZIKA VIRUS INFECTION

NO ZIKA VIRUS RNA DETECTED. ZIKA VIRUS INFECTION DURING PREGNANCY CANNOT BE RULED OUT.[7]