



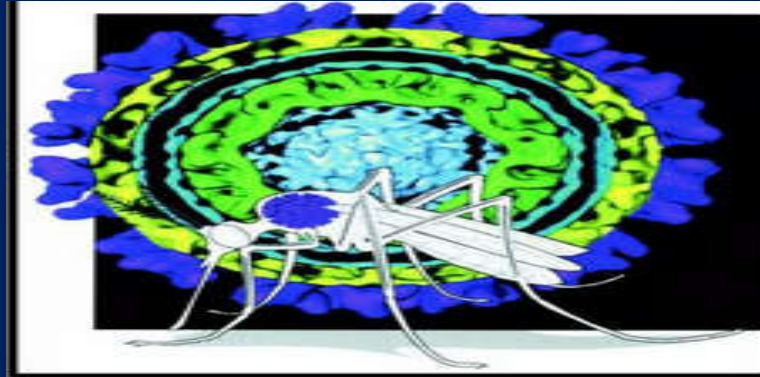
دانشگاه علوم پزشکی گیلان
دانشکده پیراپزشکی
آزمایشگاه تحقیقاتی میکروبیولوژی و ایمنولوژی بیماریهای عفونی



نگرشی بر تشخیص آزمایشگاهی
بیماریهای ویروسی منتقله از طریق پشه آئدس
Dengue, Chikungunia, Zika
and Yellow fever viruses

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Dengue, Chikungunya, Zika and Yellow fever viruses



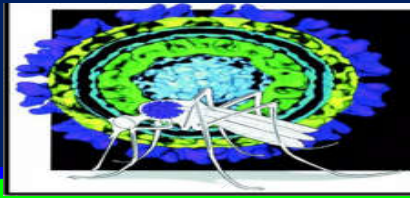
Dengue virus (DENV)

Zika virus (ZIKV)

Flavivirus (ss RNA), Flaviviridae

Yellow Fever virus (YFV)

Chikungunya virus(CHIKV) *Alphavirus (SS RNA) ,Togaviridae*



Dengue, Chikungunya, Zika and Yellow fever viruses

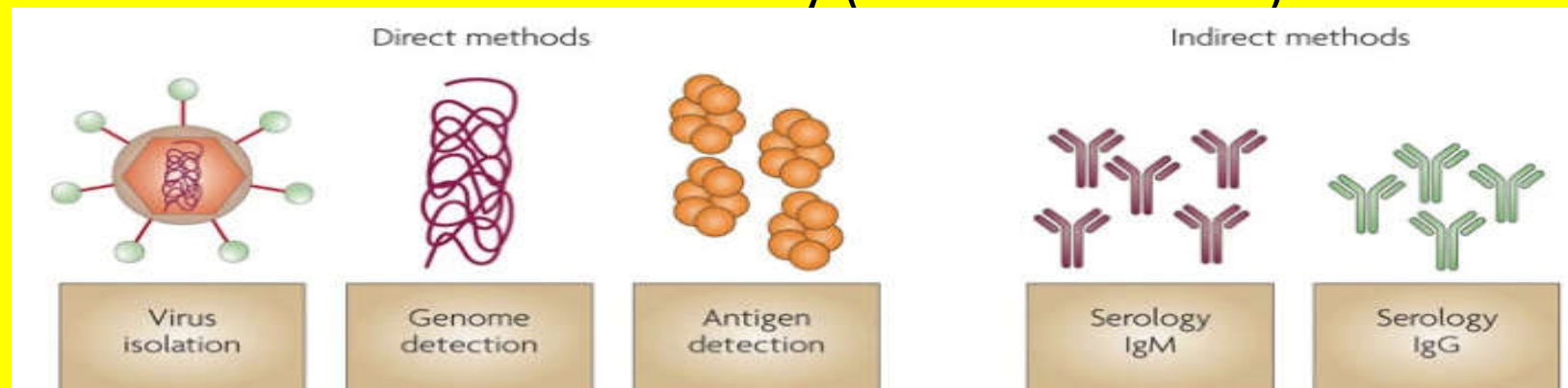
Laboratory diagnosis Of Viruses

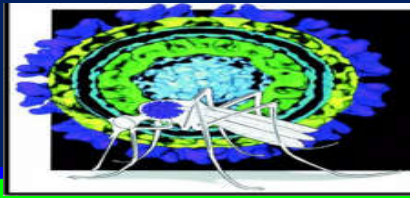
Two Basic Methods:

1. Detection of Viruses (Direct Method)

- .Isolation of Virus by Cell Culture
- .Detection of Viral Nucleic Acid
- .Detection of Viral Antigens

2. Detection of Anti Viral Antibody (Indirect Method)





Dengue, Chikungunya, Zika and Yellow fever viruses

Laboratory diagnosis Of Viruses

Detection of Viruses (Direct Method)

Isolation of Virus by Cell Culture

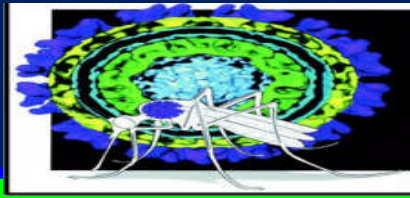
Virus isolation can be performed in mammalian(Vero-Hela-BHK..) or mosquito (AP61, Tra-284, AP64, C6/36, and CLA-1) cell Lines :

It is intensive and is slower to generate results

Therefore it is **rarely used for diagnosing infections**

However, it remains the gold standard

The capacity should be maintained in arbovirus reference laboratories for studies of pathogenesis, virulence, epidemic potential, or other studies of viral characterization



Dengue, Chikungunya, Zika and Yellow fever viruses

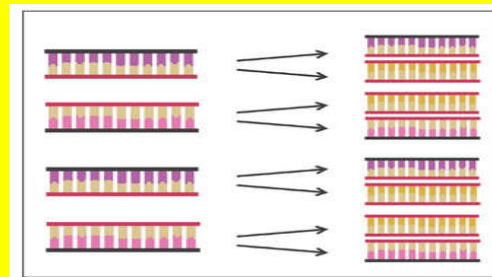
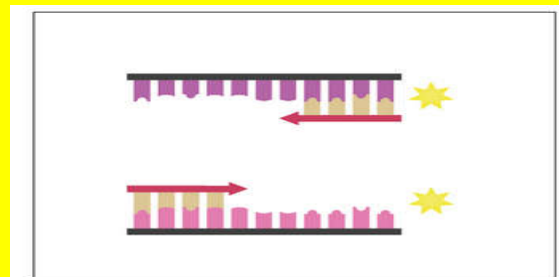
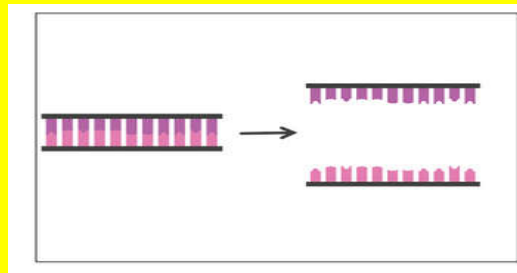
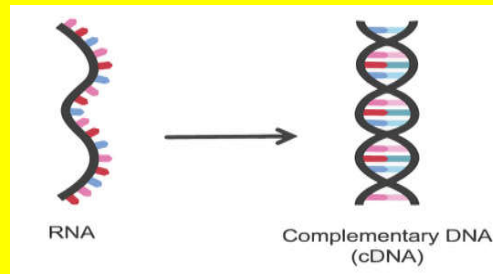
Laboratory diagnosis Of Viruses

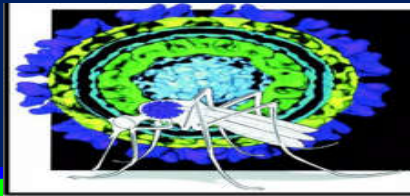
Detection of Viruses (Direct Method)

Detection of Viral Nucleic Acid (Nucleic Acid Amplification Test : NAATs)

Reverse Transcription PCR [RT-PCR]

Real Time PCR



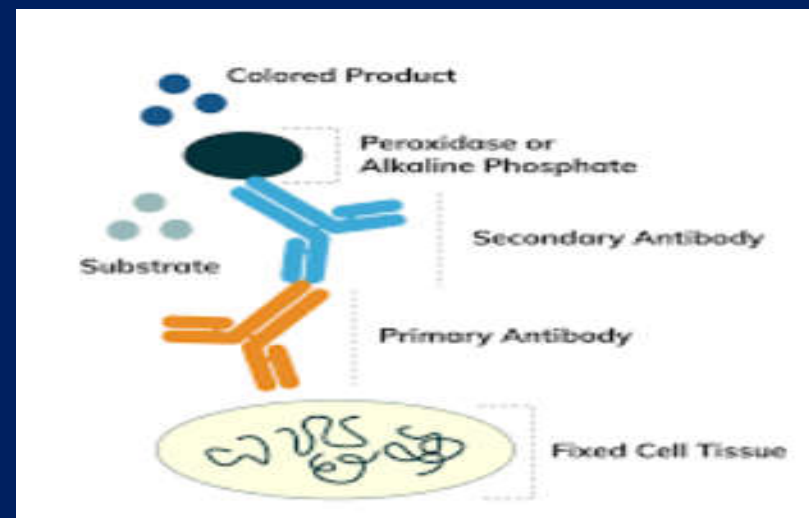
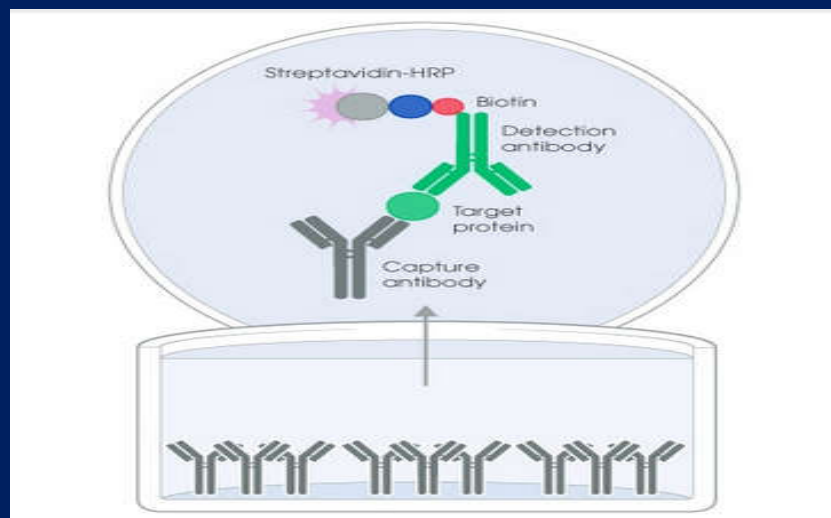


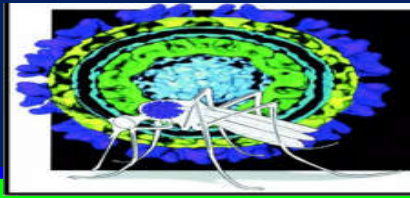
Dengue, Chikungunya, Zika and Yellow fever viruses

Detection of Viral Antigens

Enzyme-linked ImmunoSorbent Assays (ELISA)

Immunohistochemical (IHC) Analysis for Fixed Tissue Specimens





Dengue, Chikungunya, Zika and Yellow fever viruses

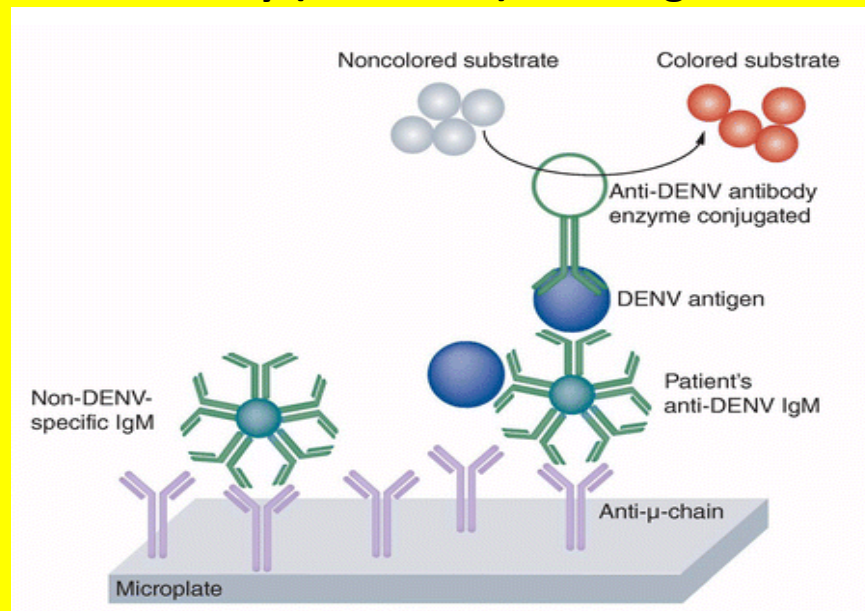
Laboratory diagnosis Of Viruses

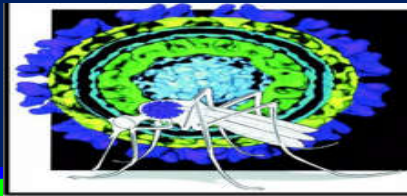
2. Detection of Anti Viral Antibody (Indirect Method)

Enzyme-linked ImmunoSorbent Assays (ELISA)

IgM antibody capture enzyme-linked immunosorbent assay (Mac-Elisa) and IgG Elisa

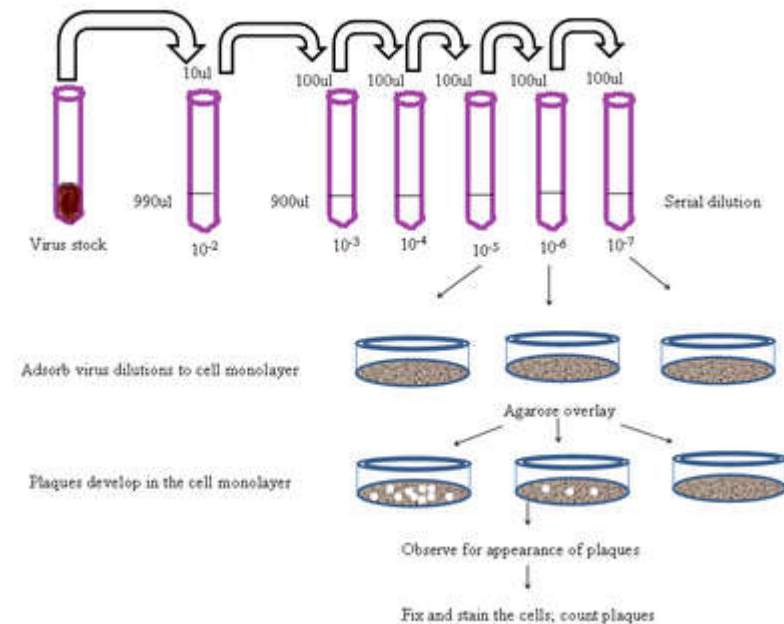
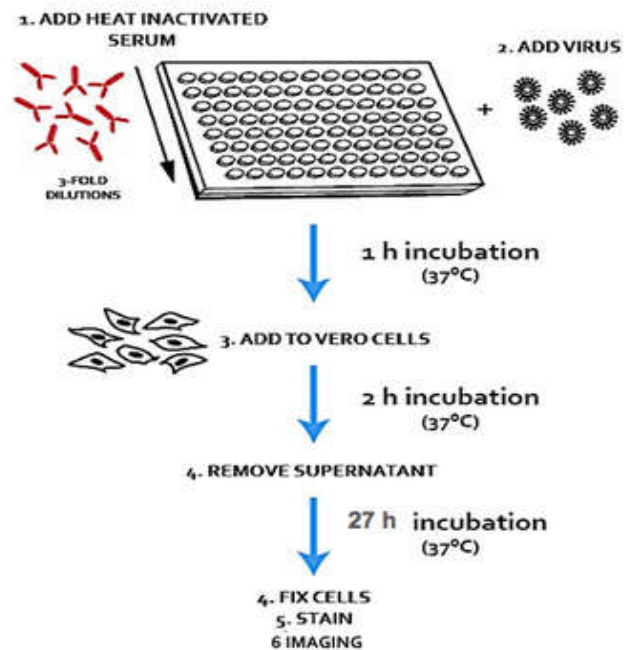
- Antibody detection
- Antibody response to infection differs according to the immune status of the host.
- First antibody to appear – IgM
- Secondary antibody – IgG



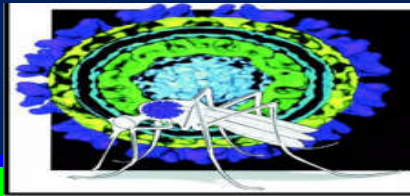


Dengue, Chikungunya, Zika and Yellow fever viruses

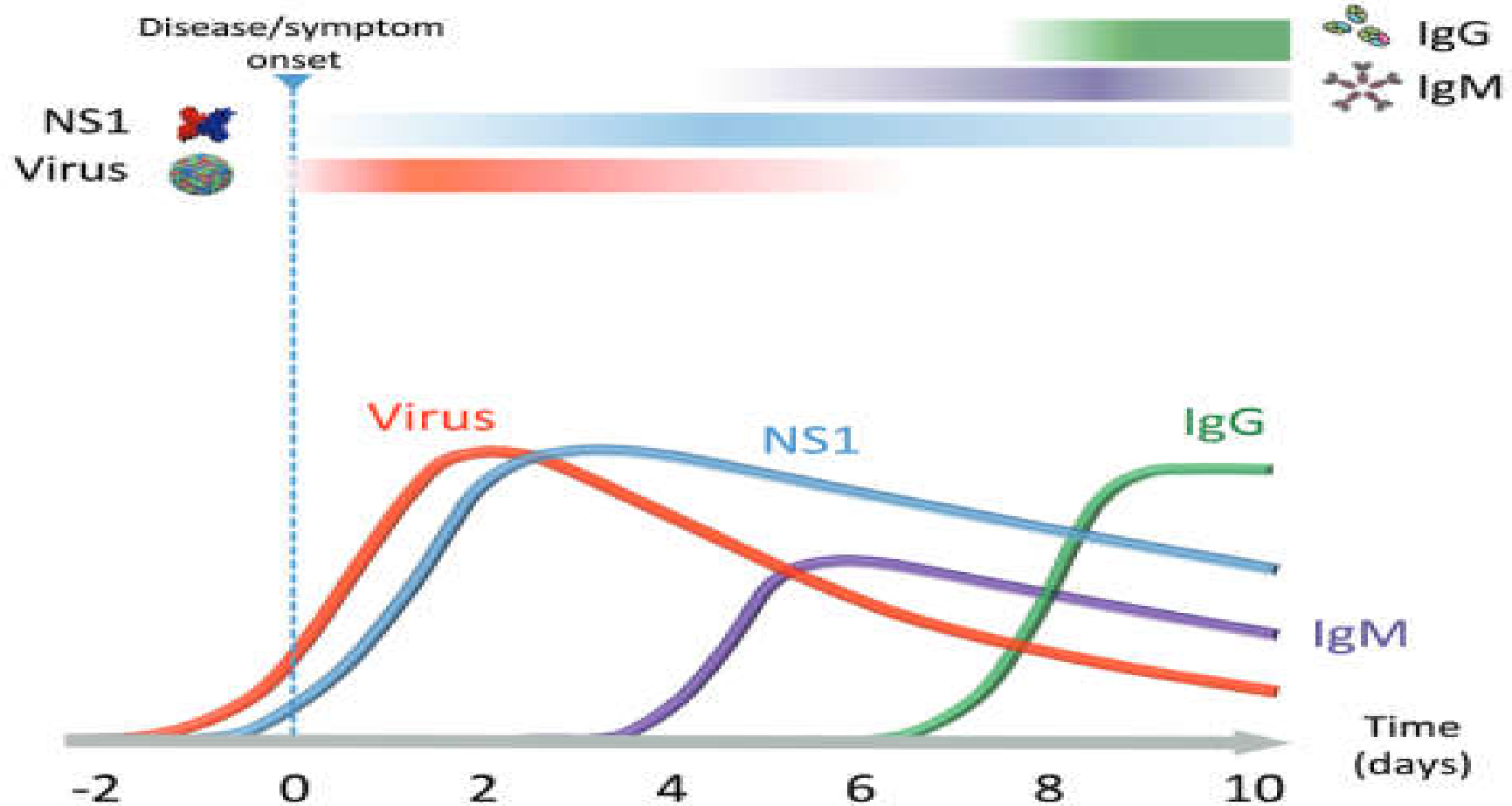
Plaque Reduction Neutralization Tests (PRNTs)

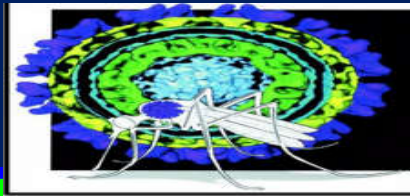


PRNT overview



Dengue, Chikungunya, Zika and Yellow fever viruses





Dengue, Chikungunya, Zika and Yellow fever viruses

Preservation of samples

- Keep refrigerated (2-8 °C) – if it is to be processed or sent to reference laboratory within 48 hrs
- Keep frozen (-10 to -20 °C) – if it is to be processed after 48 hrs to 7 days
- Keep frozen (-70 °C) – if it is to be processed after a week



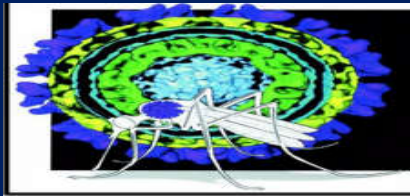
Laboratory diagnosis Of Dengue Fever

Diagnostic Tests for Dengue and Specimens

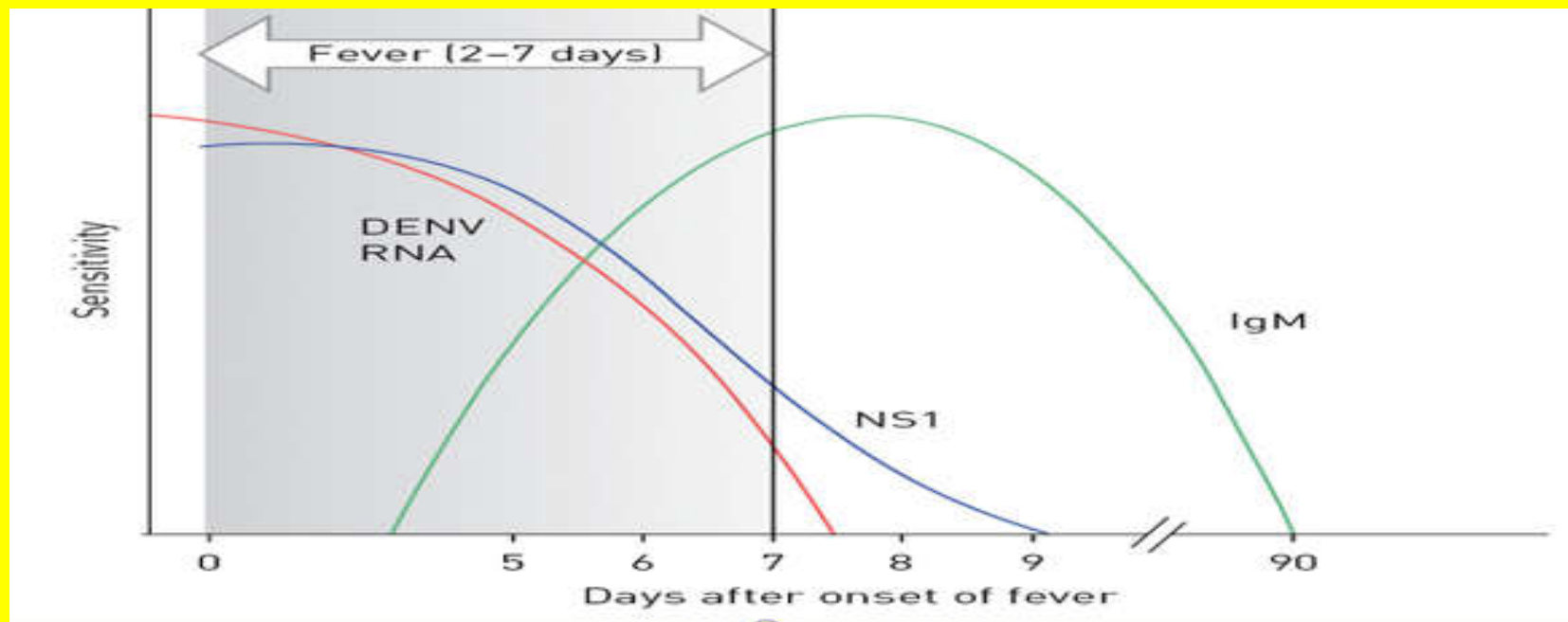
| Diagnostic Test | ≤ 7 Days After Symptom Onset | > 7 Days Post Symptom Onset | Specimen Types |
|--|------------------------------|-----------------------------|--|
| Molecular Tests | ✓ | — | Serum, plasma, whole blood, cerebrospinal fluid* |
| Dengue Virus Antigen Detection (NS1) | ✓ | — | Serum |
| Serologic Tests | ✓ | ✓ | Serum, cerebrospinal fluid* |

The initial 1-7 days after symptom onset are referred to as the acute phase of dengue.

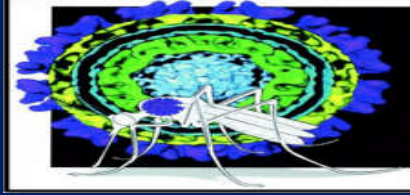
The period beyond 7 days following symptom onset is referred to as the convalescent phase of dengue.



Laboratory diagnosis Of Dengue



Abbreviations: DENV, dengue virus; NS1, nonstructural protein 1
DENV RNA and NS1 are detectable during the first week of illness. DENV IgM is detectable starting ≈5 days after illness onset. Although most cases only have detectable DENV IgM for 14–20 days after illness onset, in some cases IgM might be detectable for up to 90 days.



Laboratory diagnosis Of Dengue

Interpretation of results

Positive IgM: Patients with a positive IgM test result are classified as recent dengue virus infections.

Negative IgM: Patients with negative IgM results before day 8 of illness and absent or negative RNA or NS1 results are considered unconfirmed cases.

A second sample should be obtained after day 7 of symptoms for additional serologic testing.

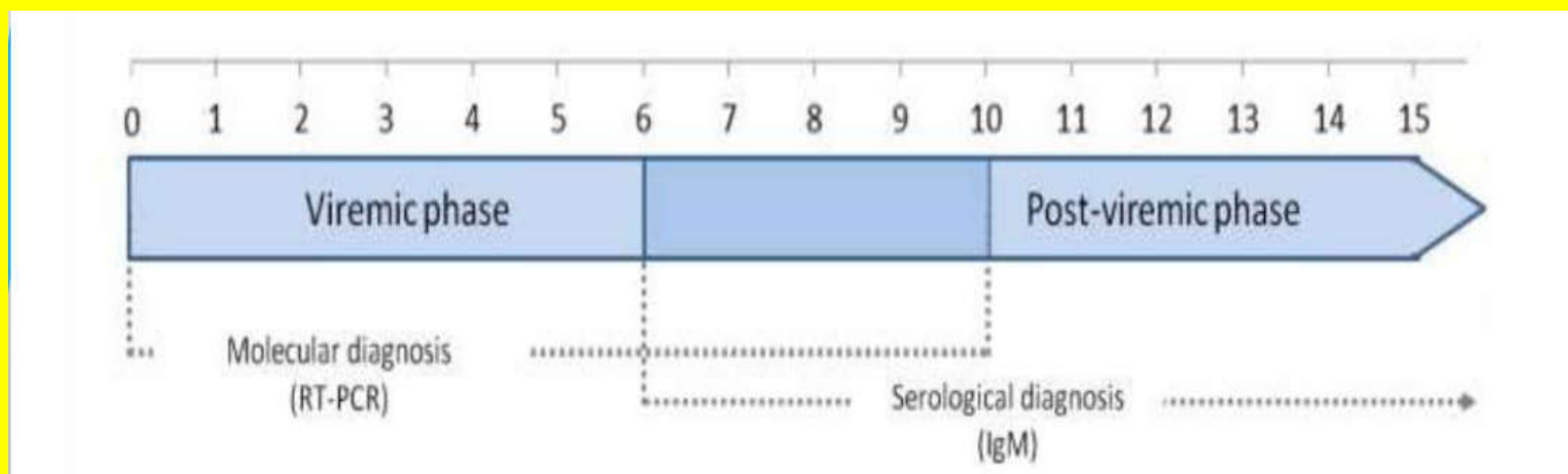
Patients with negative IgM results after 7 days of symptoms, and absent or negative NAAT or NS1 (dengue virus antigen detection) are classified as **negative for recent infection**.

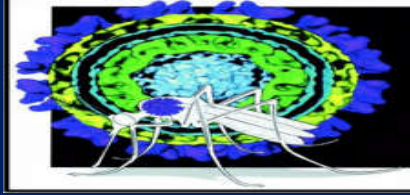
Patients with a **change from negative to positive IgM** results are classified as **current dengue infections**.



Laboratory diagnosis Of Yellow fever

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



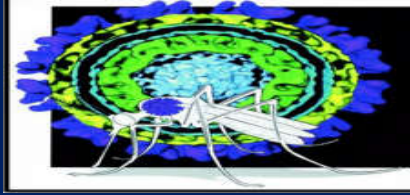


Laboratory diagnosis Of Yellow fever

Detection of Viral Nucleic Acid

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 RT-PCR

A positive result by molecular testing confirms the diagnosis of YFV infection.



Laboratory diagnosis Of Yellow fever

Detection of Viral Antigens

Immunohistochemical (IHC) Analysis for Fixed Tissue Specimens

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.



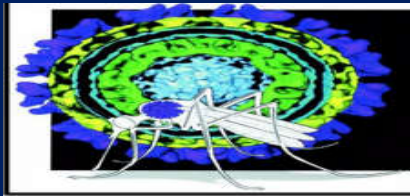
Laboratory diagnosis Of Yellow fever

Detection of Anti Viral Antibody (serology)

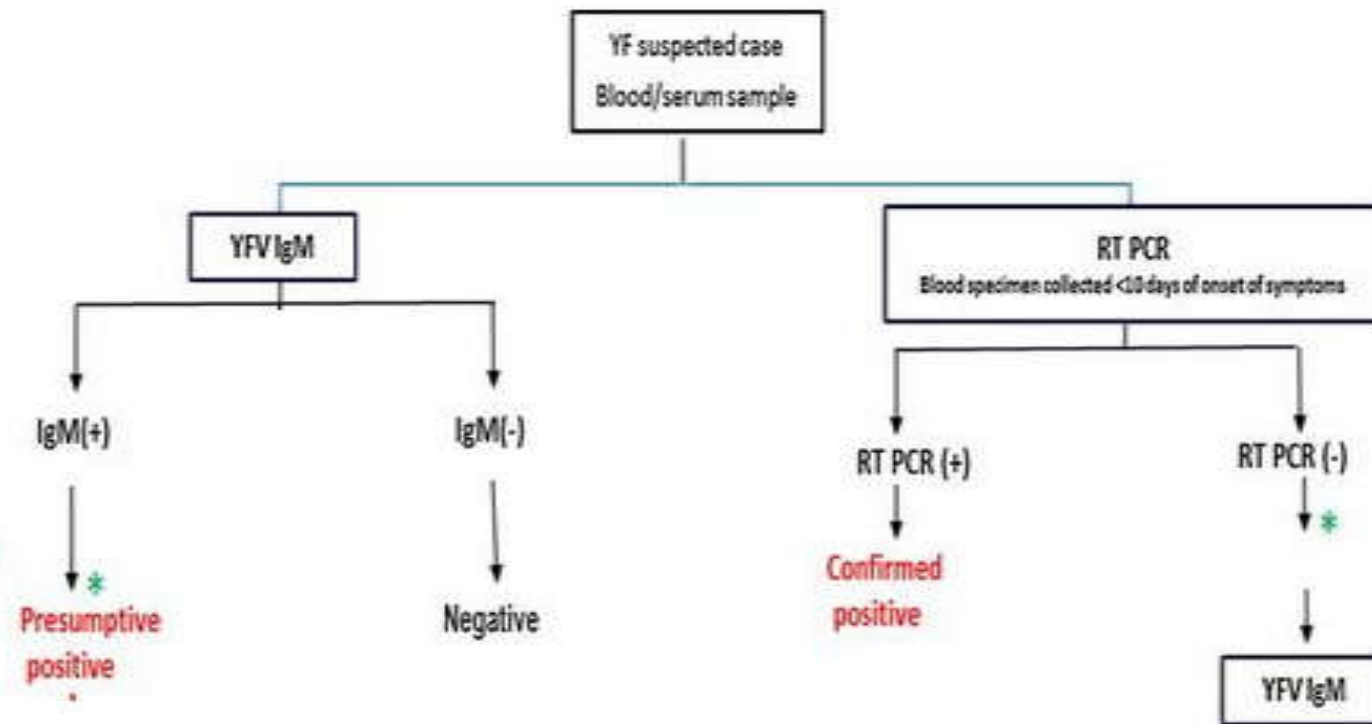
IgM detection

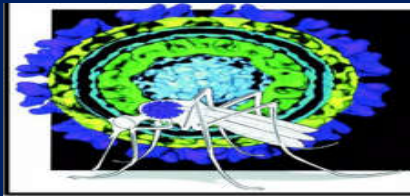
Anti-YFV IgM antibodies can be detected by ELISA

As with any IgM test, a positive result in a single sample is only presumptive of a recent infection.

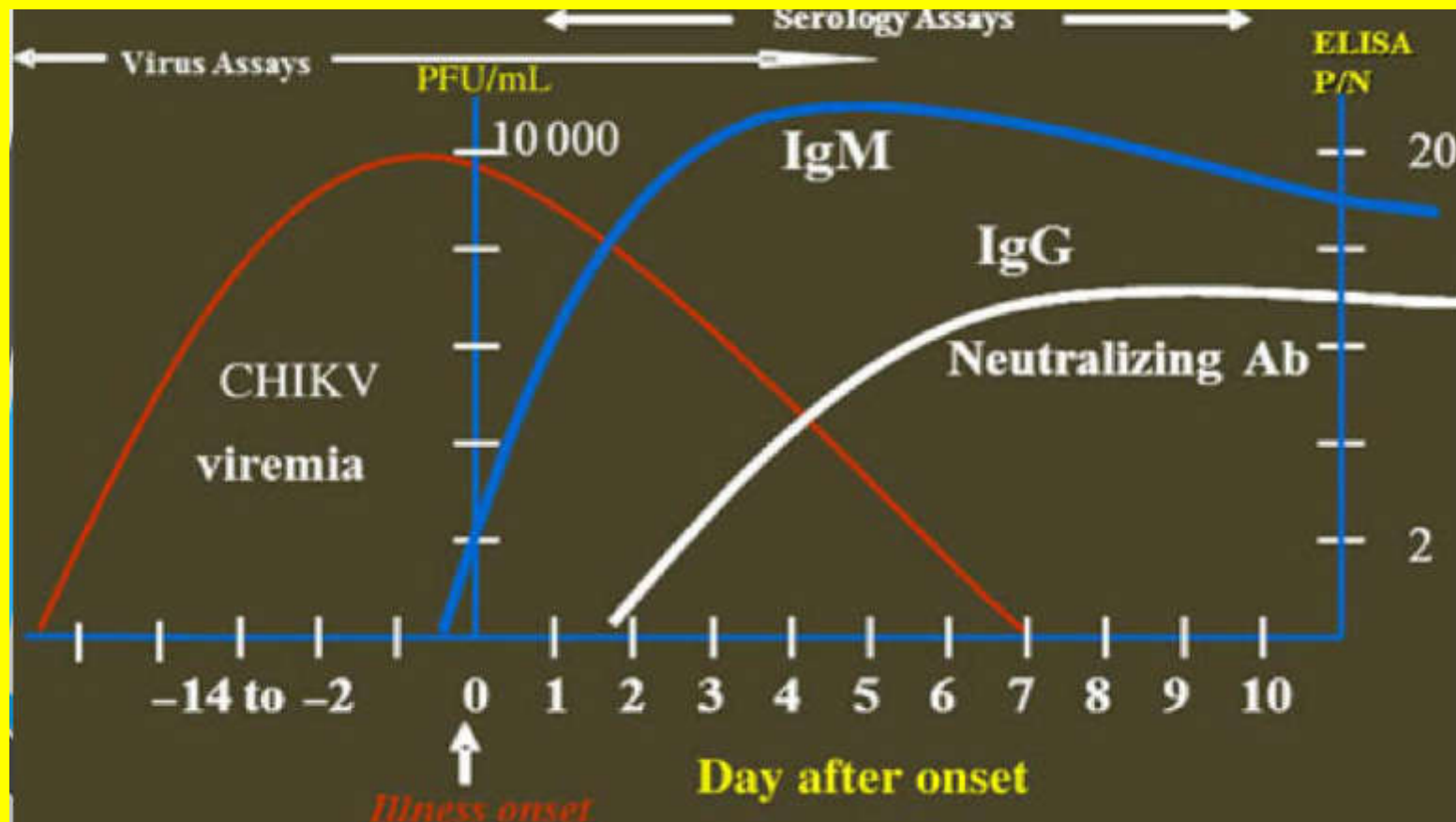


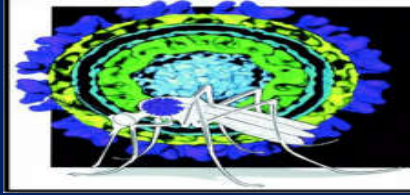
Laboratory diagnosis Of Yellow fever





Laboratory diagnosis Of Chikungunya

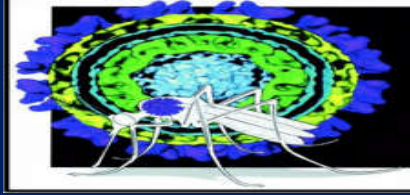




Laboratory diagnosis Of Chikungunya

Detection of Viral Nucleic Acid

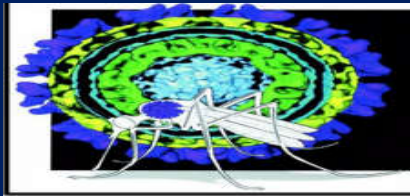
During the first 8 days of illness, chikungunya viral RNA can often be identified in serum by Real-time PCR assay



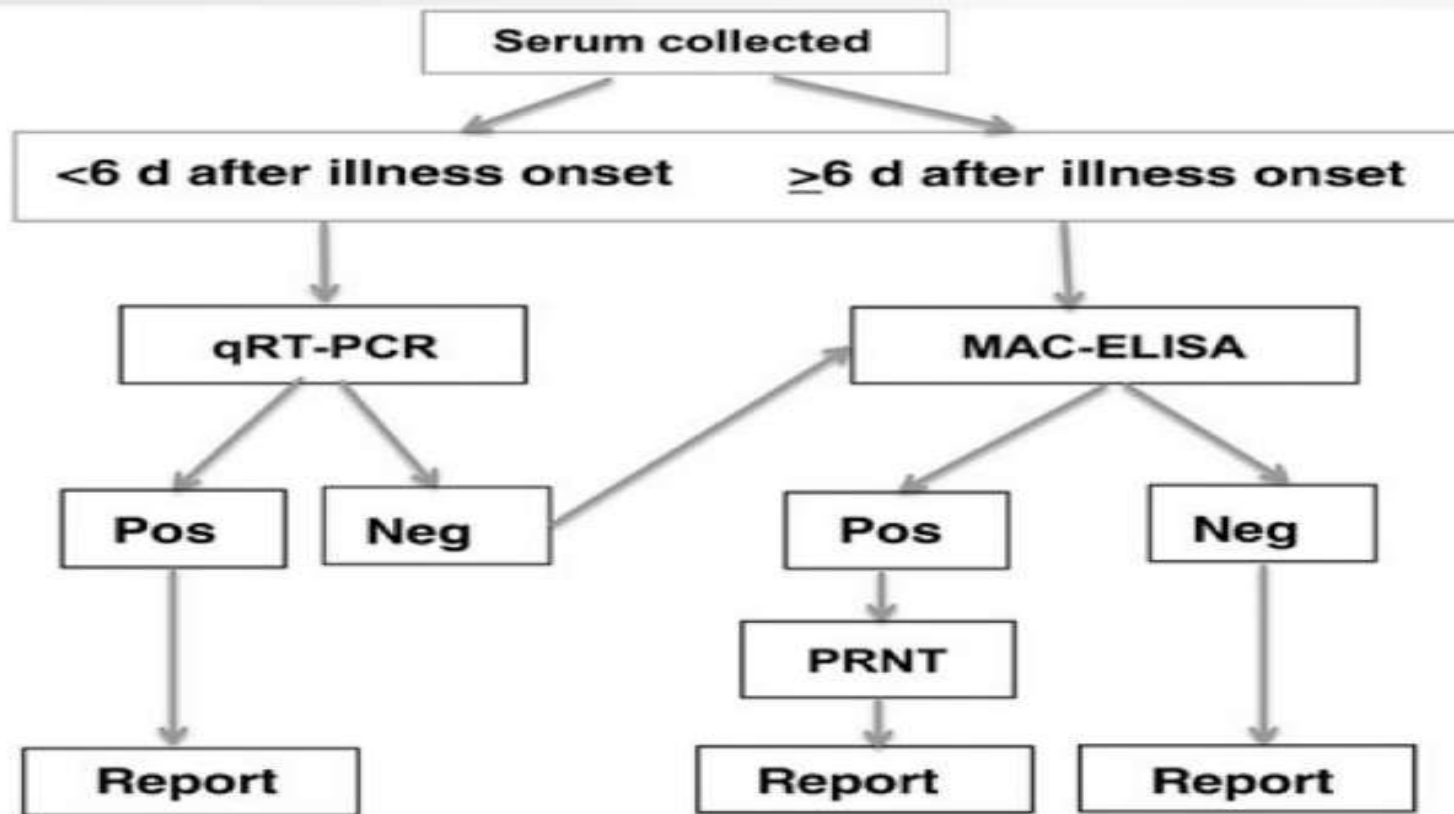
Laboratory diagnosis Of Chikungunya

Detection of Anti Viral Antibody (serology)

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.



Laboratory diagnosis Of Chikungunya





Laboratory diagnosis Of Zika

RT-PCR is the main test for detection of viral nucleic acid of Zika during the initial viremic phase.

NAAT (nucleic acid amplification testing) and antibody testing can be performed on serum and CSF.

Zika IgM antibodies can persist for months to years following infection. Therefore, detecting Zika IgM antibodies might not indicate a recent infection.



Laboratory diagnosis Of Zika

Symptomatic pregnant patients:

For symptomatic pregnant women who had recent travel to areas with active dengue transmission and a risk of Zika,

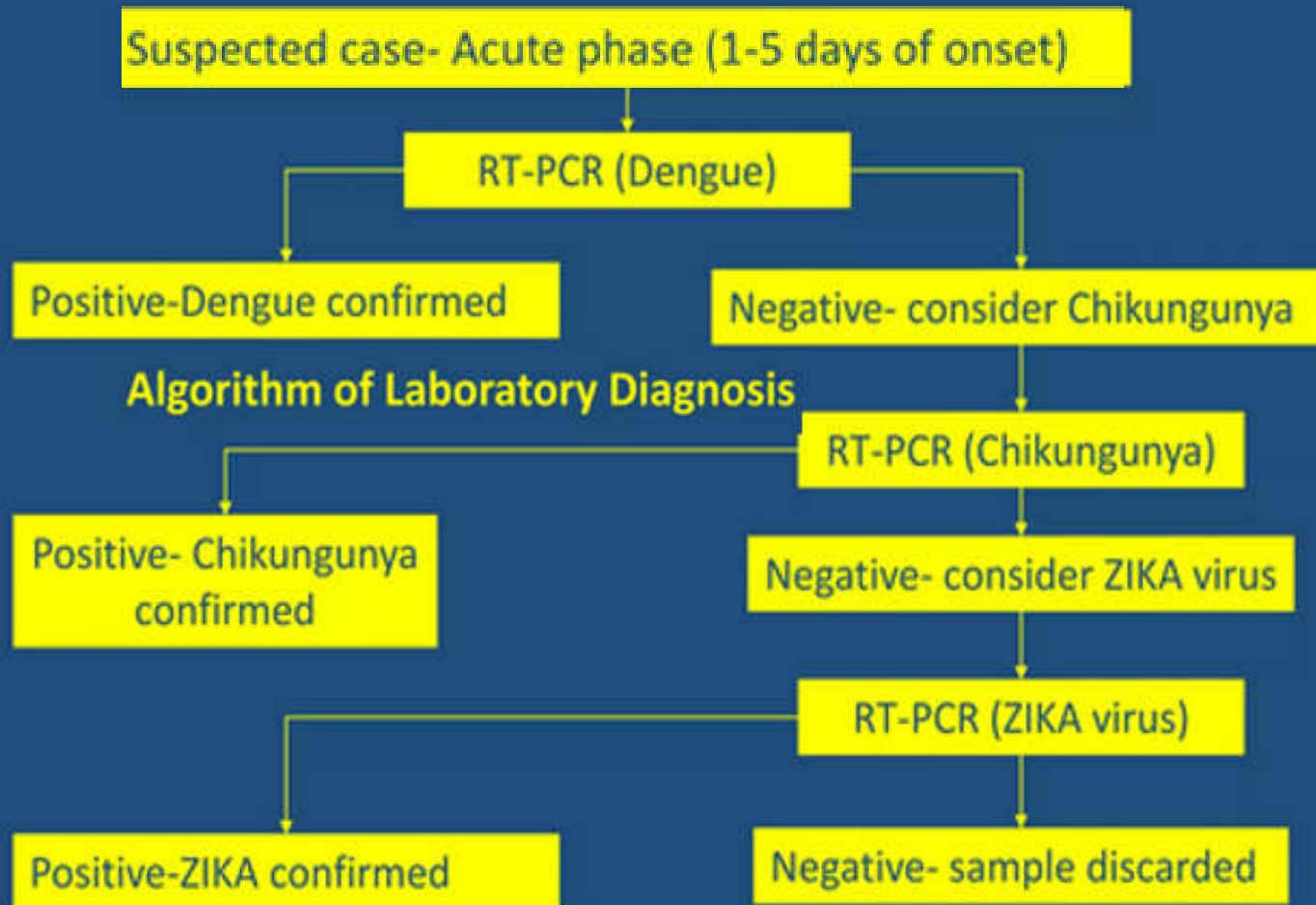
Specimens should be collected as soon as possible after the onset of symptoms up to 12 weeks after symptom onset.

The following diagnostic testing should be performed at the same time:

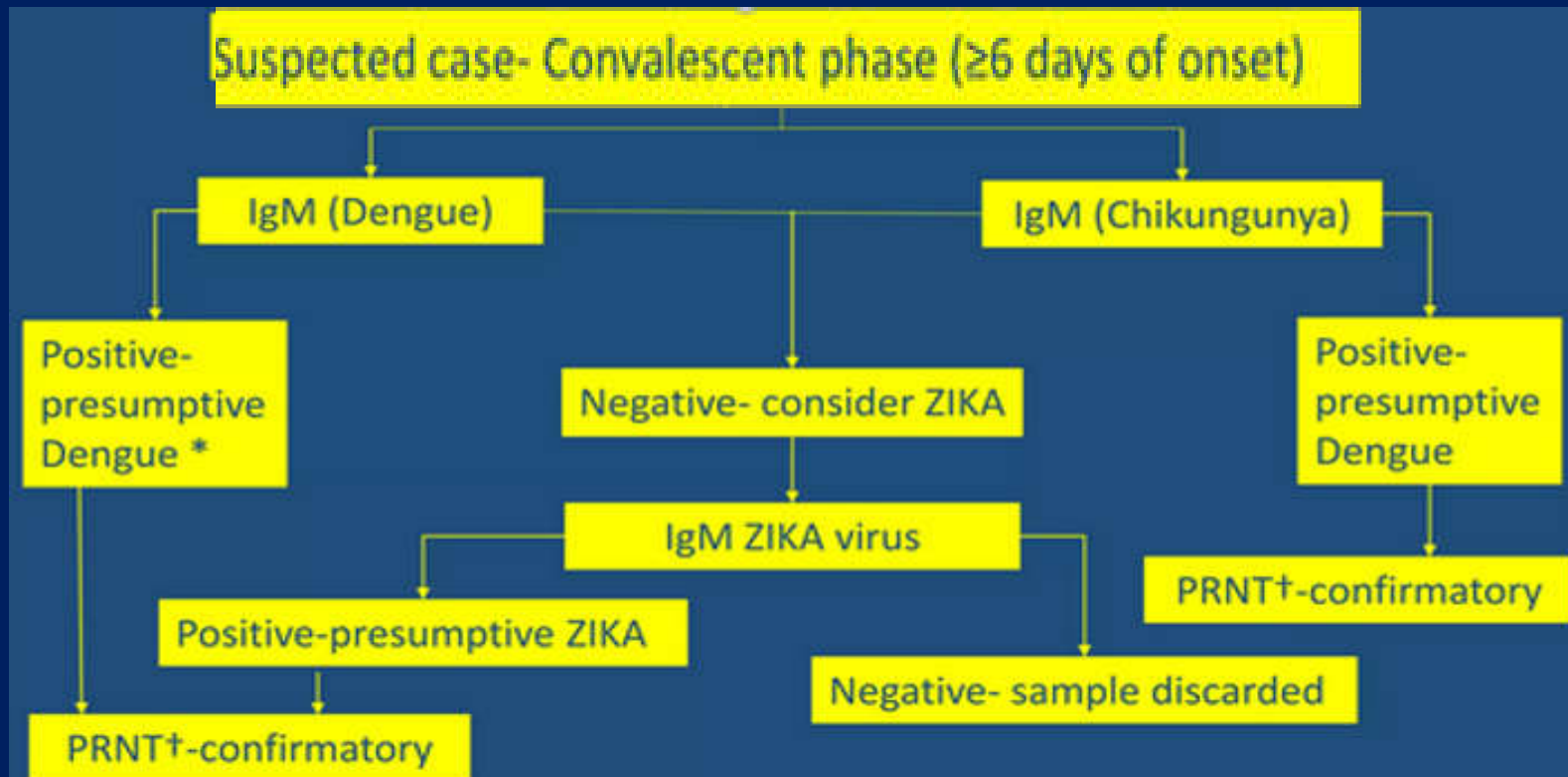
Dengue and Zika virus NAAT testing on a serum specimen, and Zika virus NAAT on a **urine specimen**, and IgM testing for dengue only.

Zika IgM antibodies can persist for months to years following infection. Therefore, detecting Zika IgM antibodies might not indicate a recent infection.

Algorithm of Laboratory Diagnosis



Algorithm of Laboratory Diagnosis



* Dengue virus IgM assay could be positive in ZIKA virus infected patients, thus an extensive cross reactivity would be expected in Dengue virus circulation areas. Therefore molecular detection in acute sample should be prioritized. † PRNT: Plaque Reduction Neutralization Test



با تشکر