

Arthropod-borne Viruses

Dr. Ommar Swe Tin
Consultant Microbiologist
Virology Section
National Health Laboratory

Arboviral Diseases prevalent in Myanmar

1. Dengue
2. Japanese B Encephalitis (JBE)
3. Chikungunya
4. Zika

Dengue



Dengue Virus belongs to the Arbovirus Group B

Dengue virus 1,2,3,4

Causes Dengue (breakbone fever)

Transmitted by the bite of infected female

Aedes aegypti (and *Aedes albopictus* in some places)

Clinical features

- (1) Dengue Fever
- (2) Dengue Haemorrhagic Fever (DHF)
- (3) Dengue Shock Syndrome (DSS)

Aedes aegypti



Lab diagnosis

- (1) Isolation of virus
- (2) Detection of dengue antibodies (Serology)
- (3) Detection of viral RNA by Polymerase Chain Reaction (PCR)

(1) Isolation of Dengue Virus

Specimen for culture – serum, plasma, leukocytes

To be transported in Viral Transport Media /
in cold chain

- (1) Mosquito inoculation (best)
- (2) Cell Culture
- (3) Suckling mouse inoculation

(2) Detection of dengue antibodies

Specimen – serum

1. Haemagglutination Inhibition test

Dengue virus + Goose RBC = agglutination

Dengue virus + Dengue Ab + Goose RBC
in serum

= No agglutination

2. MAC-ELISA – IgM Antibody Capture ELISA

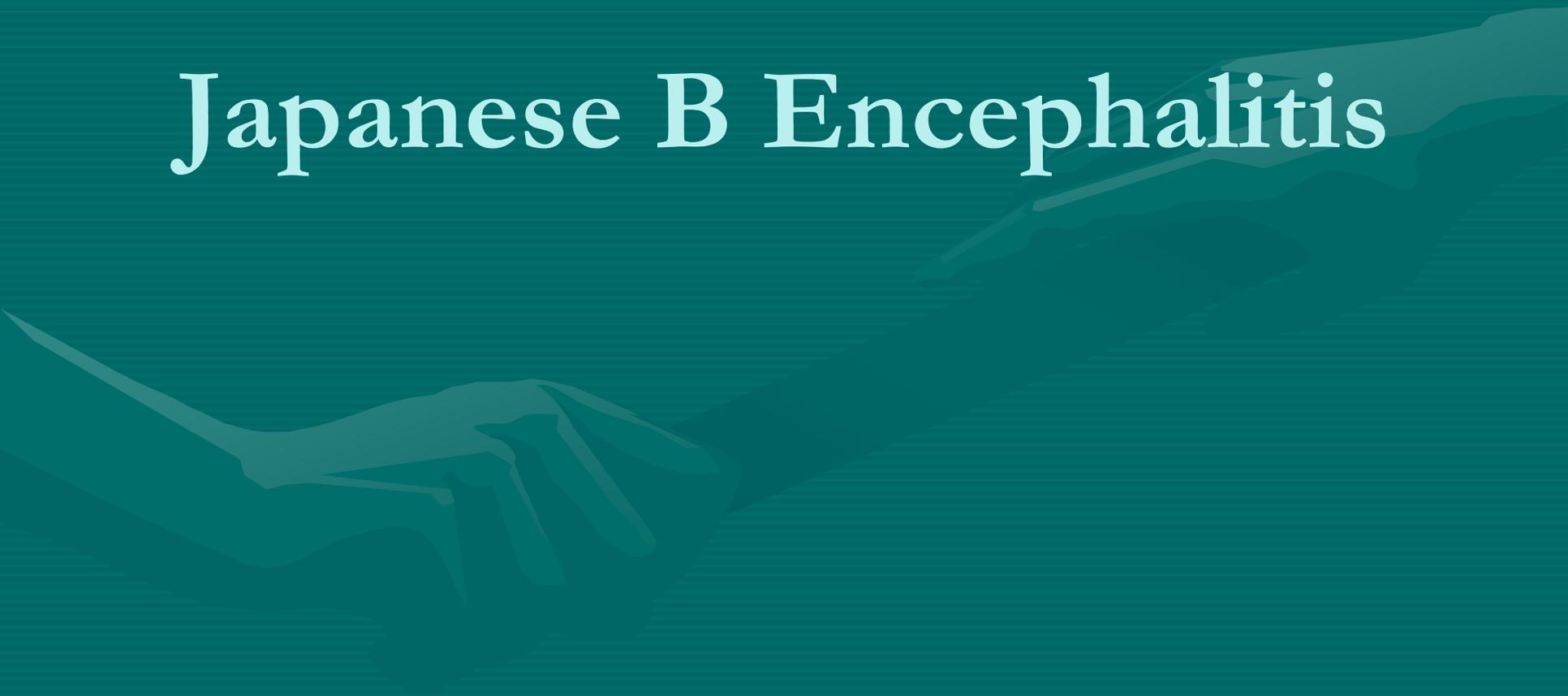
3. Dengue ICT –

- NS1Ag(+ive) = significantly more sensitive for primary than secondary dengue
- IgM (+ive) = Primary dengue infection
- Both IgM and IgG (+ive) = Secondary dengue infection
- IgG (+ive) = Secondary dengue infection

(3) Detection of viral RNA by PCR

- Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) – amplification of viral genetic material
- Detect Dengue serotypes

Japanese B Encephalitis



Japanese B encephalitis

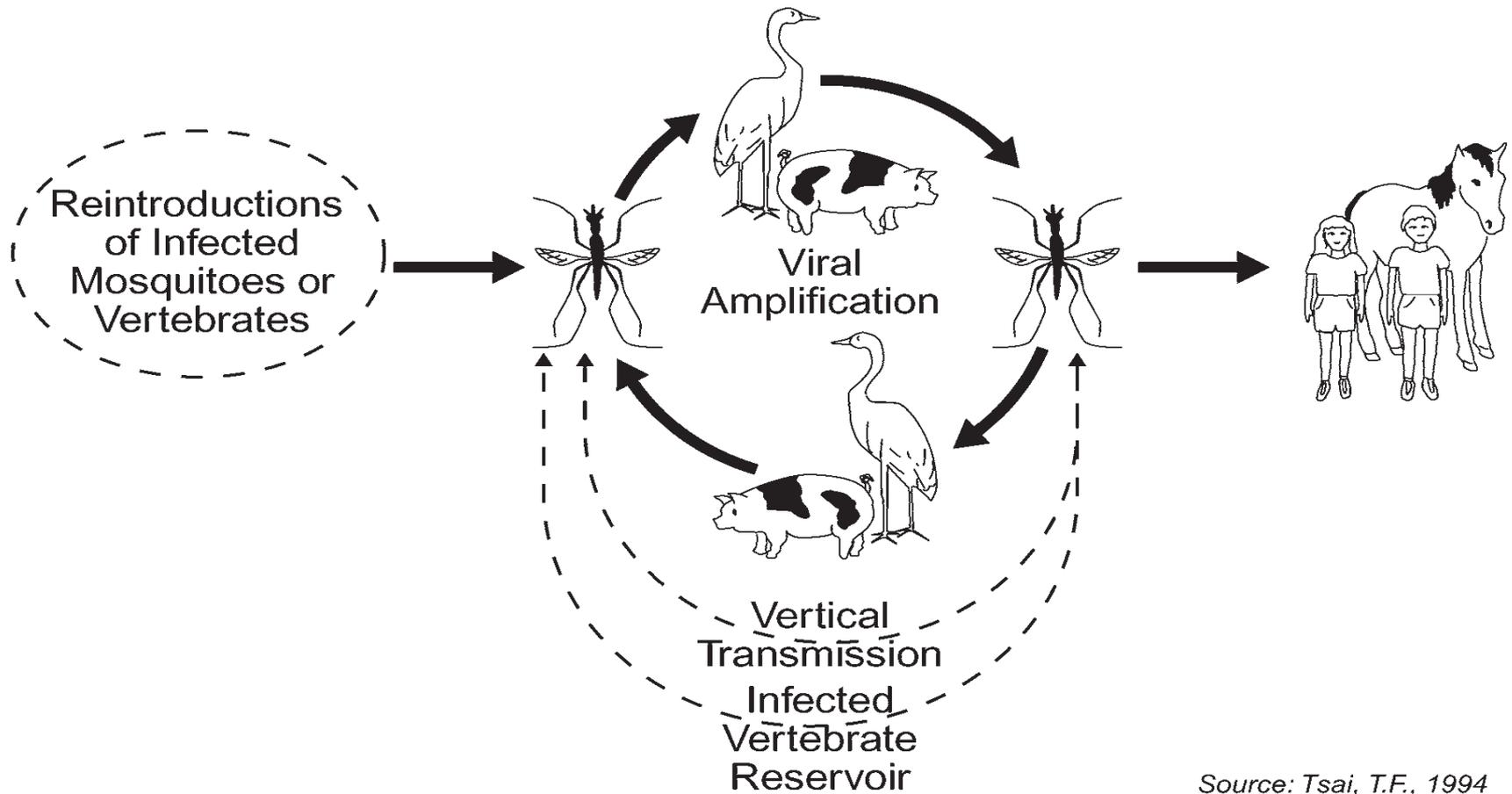
- Causal agent: Japanese encephalitis virus
- Arbovirus -Family Flaviviridae
 - Genus Flavivirus
(JE, Dengue, West Nile, Yellow Fever)

- MoT- through the bite of mosquito- *Culex tritaeniorhynchus*
- Maintained in a cycle of virus transmission between vertebrate amplifying hosts (e.g. pigs, heron, egrets) and several *Culex* mosquito species
- Transmission to humans occurs in rural settings- agricultural practice where vectors can breed or infection to vertebrate hosts
- Urban settings- potential for outbreak is low

Culex tritaeniorhynchus



JE virus transmission cycle



Halstead. In: Vaccines (Eds. Plotkin et al.) 2004:919.

JE infection

- Asymptomatic
- Febrile illness
- Meningitis
- Myelitis
- Encephalitis- most common , indistinguishable from other AES



WHO recommended case definition for suspect Acute Encephalitis Syndrome

Clinical case definition

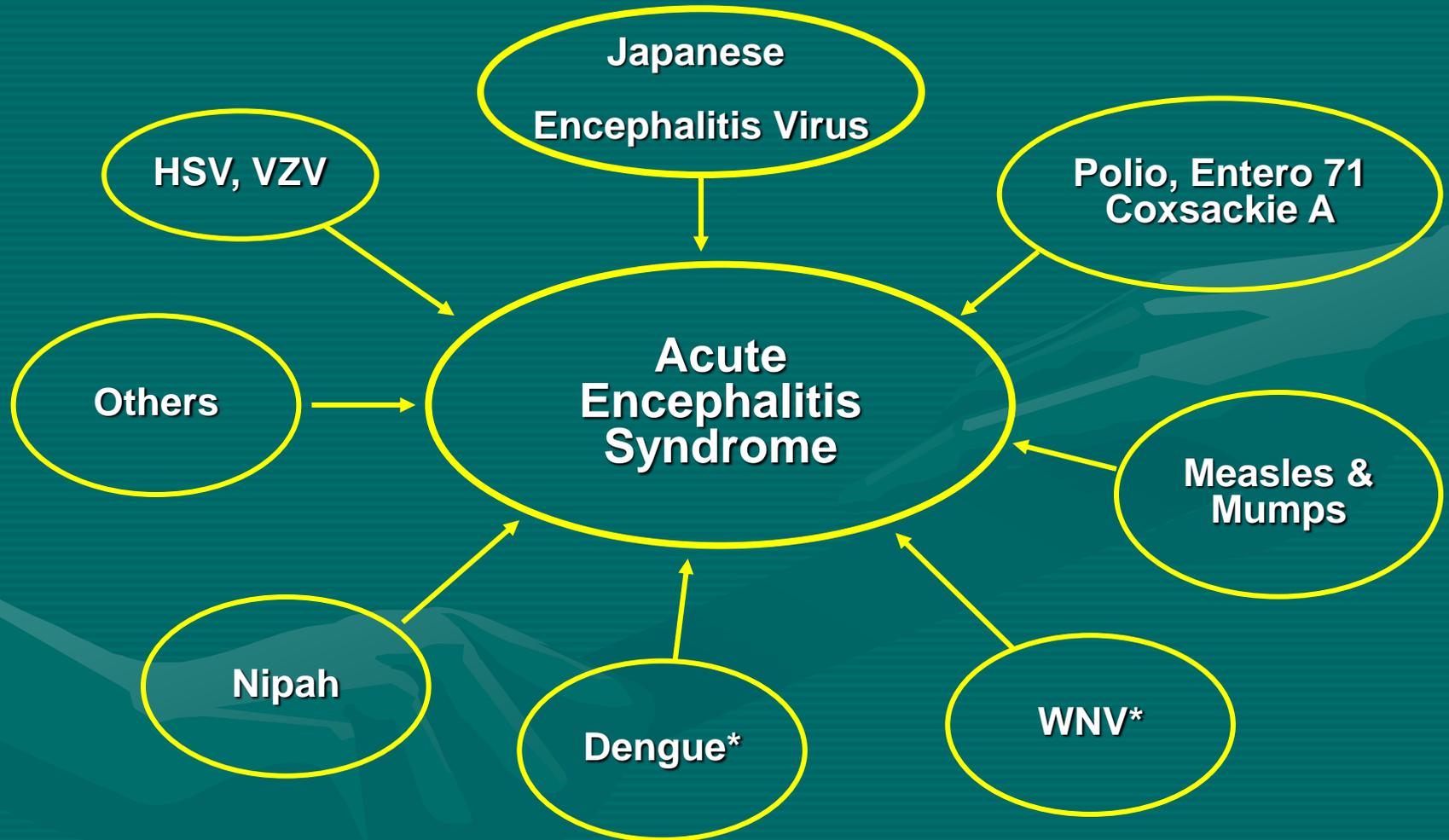
- Clinically, a case of acute encephalitis syndrome is defined as a person of any age, at any time of year with the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) AND/OR new onset of seizures (excluding simple febrile seizures*). Other early clinical findings may include an increase in irritability, somnolence or abnormal behaviour greater than that seen with usual febrile illness.

* A simple febrile seizure is defined as a seizure that occurs in a child aged 6 months to less than 6 years old, whose only finding is fever and single generalized convulsion lasting less than 15 minutes, and who recovers consciousness within 60 minutes of the seizure.

AES

- Any time of the year
- Any age
- Any sex
- Acute onset
- Fever and change in mental status (confusion, disorientation, coma, inability to talk) AND/OR new onset of seizures
- Early clinical findings-increase irritability, somnolence or abnormal behavior more than usual febrile illness.

Multiple causal agents of AES



* Flaviviruses with antigenic cross reactivity with JE

Specimens

- Serum for JE IgM detection and/or
- CSF for JE IgM detection



Specimen Collection

Serum

- A serum sample should be obtained at admission. Because it may not be positive in a JE-infected person, a second serum sample should be collected at discharge or on the 10th day of illness onset or at the time of death.

- Collect 5ml of blood in a sterile plain tube
- Label the tube with the patient's name, age, sex, outbreak ID number, specimen number, date of collection and specimen type.
- **Bottles are without label. We cannot do the samples without label.**
- Transport the whole blood specimen to NHL if it can reach within 24 hours.
- If it cannot reach NHL within 24 hours, do separation of serum

- Separate serum after clotting, and transfer into a new sterile bottle or microvial and send to NHL.
- To prevent insufficiency, collect **5 ml of blood or 2 ml of serum** in a sterile bottle
- For outbreak, **5 cases** enough.
- Before transport, in the hospital laboratory, they should be kept at 4-8°C.
- The specimens should be sent to NHL in cold box with laboratory request form.
- The serum/ blood samples should not be haemolysed samples (Prevent hemolysis of samples – narrow needle, rapid suction, rapid pushing blood out of syringe, wet container should not be used)

CSF

- The collection of CSF is an invasive technique that should only be performed by experienced personnel using appropriate equipment under aseptic conditions.
- The CSF can be aseptically divided into separate aliquots for examination for cells, biochemistry, microbiology and virology.

- For virological investigations, collect a minimum of 0.5ml of CSF in a dry, sterile, screw cap container.
- Before transport, in the hospital laboratory, they should be kept at 4-8°C.
- The specimens should be sent to NHL **in cold box** with laboratory request form.

Lab Diagnosis

- Serum – JE IgM Ab detection by rapid test, ELISA
- CSF - JE IgM Ab detection by ELISA

Chikungunya



Clinical Features

Causes acute dengue-like fever of sudden onset, intense joint and muscle pain, and rash

MoT- through the bite of mosquito- *Culex*

Lab Diagnosis

- Specimen – Serum
 1. Rapid test – Chikungunya IgM Ab
 2. ELISA - Chikungunya IgM Ab
 3. PCR



Zika

PROTECT YOUR FAMILY AND COMMUNITY: HOW ZIKA SPREADS

Most people get Zika from a mosquito bite



More members in the community become infected



A mosquito bites a person infected with Zika virus



The mosquito becomes infected



A mosquito will often live in a single house during its lifetime



More mosquitoes get infected and spread the virus



The infected mosquito bites a family member or neighbor and infects them

Other, less common ways, people get Zika:



During pregnancy
A pregnant woman can pass Zika virus to her fetus during pregnancy. Zika causes microcephaly, a severe birth defect that is a sign of incomplete brain development



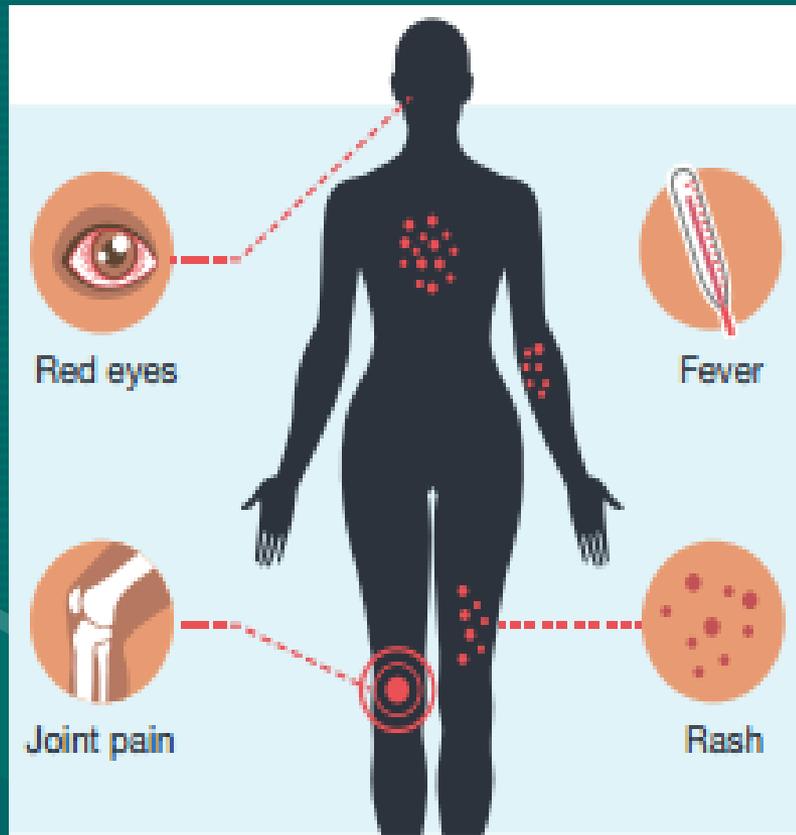
Through sex
Zika virus can be passed through sex from a person who has Zika to his or her sex partners



Through blood transfusion
There is a strong possibility that Zika virus can be spread through blood transfusions



When to test for Zika virus



If your patient is

- Experiencing or has recently experienced symptoms of Zika
- An asymptomatic pregnant woman

When to test for Zika virus (contd)

Ask the following questions:

- Does the patient live in or has the patient recently traveled to an area with Zika?
- Has the patient had unprotected sex with a partner who has lived in or traveled to an area with Zika?

Yes



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graph TD; A(Yes) --> B[ ]
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CDC does not recommend Zika virus testing for **asymptomatic**

- Men
- Children
- Women who are not pregnant

Testing Indications

- The incubation period for Zika virus infection is approximately 3 days to 2 weeks
- Patients with current symptoms compatible with Zika virus infection should be tested by PCR if:
 1. They are within 14 days of symptom onset, AND
 2. Onset was while in an endemic or currently affected area, or within 14 days of departure from an endemic or currently affected area, OR
 3. Onset was within 14 days of sexual contact with a confirmed case of Zika virus disease

WHO Testing algorithms for suspected cases identified within seven days of onset of symptoms

Collect blood and Urine



RT-PCRs for DENV, CHIK, ZIKV sequential or parallel testing



Result for multiplex reactions	Interpretation
ZIKV pos, DENV neg, CHIK neg	ZIKV confirmed case
ZIKV neg, DENV pos, CHIK neg	DENV confirmed case
ZIKV neg, DENV neg, CHIK pos	CHIK confirmed case
ZIKV pos and/or DENV pos or CHIK pos	Co-detection and ZIKV confirmed case

* A negative result for any PCR test does not conclusively rule out the infection

WHO Testing algorithms for suspected cases identified more than one week after onset of symptoms

Collect blood (serology and RT-PCR) and urine (only RT-PCR)
If collecting paired serum samples, allow 2-3 weeks between samples

RT-PCR for ZIKV virus on
urine and blood

Test for IgM to DENV,
CHIK and ZIKV (ELISA)

Positive PCR -

Zika confirmed case

Negative PCR -

Zika virus infection not definitely ruled
out

*For paired serum samples, a four-fold rise in IgM in the absence of a rise in antibody titre to other flaviviruses is further evidence of recent Zika virus infection

Zika Virus IgM Antibody Testing

Test for IgM to DENV, CHIK and ZIKV,



Result	Laboratory Interpretation*
ZIKV pos, DENV neg, CHIK neg	ZIKV probable case
ZIKV neg, DENV pos, CHIK neg	DENV probable case
ZIKV neg, DENV neg, CHIK pos	CHIK probable case
ZIKV pos, DENV neg, CHIK pos	ZIKV Probable case + CHIK co-infection
ZIKV pos, DENV pos and/or CHIK pos	Probable flavivirus infection+ CHIK co-infection

* Final interpretation of result should be done in conjunction with clinical presentation

Zika Virus IgM Antibody Testing

- Serology is the preferred method in specimens from patients with onset of symptoms >7 days
- A reactive result for Zika virus IgM in the absence of IgM to dengue or other flaviviruses suggests recent exposure to Zika virus
- Zika virus IgM ELISA can provide false-positive results because of cross-reacting IgM antibodies against related flaviviruses or nonspecific reactivity
- To resolve false-positive results, samples with presumptive positive, equivocal, or inconclusive IgM test results must be forwarded for confirmation by PRNT against Zika, dengue, and other flaviviruses to which the person might have been exposed
- **PRNT** is performed by CDC or a CDC-designated confirmatory testing laboratory

Samples collection and transportation

Serum and urine are the primary diagnostic specimens for Zika virus infection



Samples collection and transportation

Acute serum

- Collect 5ml of blood in a sterile plain tube (red capped tube)
- Label the tube with the patient's name, age, sex, date of collection and specimen type
- Transport the whole blood specimen to NHL if it can reach within 24 hours
- If it cannot reach NHL within 24 hours, do separation of serum
- Separate serum after clotting, and transfer into a new sterile bottle or microvial and send to NHL
- Before transport, in the hospital laboratory, they should be kept at 2-8°C
- The specimens should be sent to NHL in cold box with laboratory request form

Convalescent Serum - 2-3 weeks after first serum

Samples collection and transportation (contd)

- Samples should be tested within 48 hours
- If there is a delay of more than 48 hours before testing, serum should be separated and stored separately
- All types of specimens may be kept frozen at -20°C for up to 7 days
- For storage longer than 7 days, specimens should be frozen at -70°C
- Repeated freezing and thawing of specimens should be avoided

Samples collection and transportation (contd)

Urine

- 10-20ml of urine collected in a sterile screw-capped container
- Label the tube with the patient's name, age, sex date of collection and specimen type
- Before transport, in the hospital laboratory, they should be kept at 4-8°C
- Urine should be sent to NHL within 24 hours after collection (in cold box) with laboratory request form
- Please do not submit urine in urine collection cups for Zika virus testing

Thank you

