

## **4. LABORATORY**

### **4A. Types of Laboratory Tests Available and Specimens Required**

Three main types of laboratory tests are used for diagnosing CHIK: virus isolation, reverse transcriptase-polymerase chain reaction (RT-PCR), and serology. Samples collected during the first week after onset of symptoms should be tested by both serological (IgM and IgG ELISA) and virological (RT-PCR and isolation) methods. Specimens are usually blood or serum but in neurological cases with meningoencephalitic features, cerebrospinal fluid (CSF) may also be obtained. Limited information is available for the detection of virus by isolation or RT-PCR from tissues and/or organs. In suspected fatal cases, virus detection can be attempted on available specimens.

Selection of the appropriate laboratory test is based upon the source of the specimen (human or field-collected mosquitoes) and the time of sample collection relative to symptom onset for humans.

#### ***Virus isolation***

Virus isolation can be performed on field collected mosquitoes or acute serum specimens ( $\leq 8$  days). Serum obtained from whole blood collected during the first week of illness and transported cold (between 2-8°C or dry ice) as soon as possible (within 48 hours) to the laboratory can be inoculated into a susceptible cell line or suckling mouse. CHIKV will produce typical cytopathic effects within 3 days post inoculation in a variety of cell lines including Vero,

BHK-21, and HeLa cells. Virus isolation can be performed in T-25 flasks or shell vials (see appendix A). However, recent data suggest that isolation in shell vials is both more sensitive and produces CPE earlier than conventional isolation in flasks<sup>42</sup>. CHIKV isolation must be confirmed either by IFA using CHIKV-specific antiserum or by RT-PCR of the culture supernatant or mouse brain suspension. Virus isolation must only be carried out in BSL-3 laboratories to reduce the risk of viral transmission.

### ***RT-PCR***

Several RT-PCR assays for the detection of CHIKV RNA have been published. Real time, closed system assays should be utilized due to their increased sensitivity and lower risk of contamination. The Diagnostic Laboratory at Centers for Disease Control and Prevention (CDC), Division of Vector-borne Diseases (DVBD), routinely utilizes the published assay in Appendix B<sup>43</sup> which demonstrates a sensitivity of less than 1 pfu or 50 genome copies. Serum from whole blood is used for PCR testing as well as virus isolation.

### ***Serological Tests***

For serological diagnosis, serum obtained from whole blood is utilized in enzyme-linked immunosorbent assay (ELISA) and plaque reduction neutralization testing (PRNT). The serum (or blood) specimen should be transported between 2-8°C and not frozen. Serologic diagnosis can be made by demonstration of IgM antibodies specific for CHIKV or by a four-fold rise in PRNT

titer in acute and convalescent specimens. IgM antibodies specific for CHIKV are demonstrated by using the immunoglobulin M antibody (IgM) capture ELISA (MAC-ELISA)<sup>44</sup> followed by the PRNT (detailed protocols for IgM and IgG ELISAs in Appendix C). As of 2010, there are no WHO-validated commercial IgM ELISAs available. PRNT is required to confirm the MAC-ELISA results since cross-reactivity in the MAC-ELISA between some members of the Semliki Forest virus (SFV) serogroup has been observed. PRNT testing, whether used to confirm the MAC-ELISA or to demonstrate a 4-fold rise in acute/convalescent specimens, should always include other viruses within the SFV serogroup (e.g., Mayaro virus) to validate specificity of reactivity. In situations where the PRNT assay is not available, other serological tests (e.g. hemagglutination-inhibition [HI]) can be used to identify a recent alphavirus infection; however PRNT is required to confirm a recent CHIKV infection.

An acute phase serum should be collected immediately after the onset of illness and the convalescent phase serum 10-14 days later. CHIKV-specific IgM and neutralizing antibodies normally develop towards the end of the first week of illness. Therefore, to definitively rule out the diagnosis, convalescent samples should be obtained on patients whose acute samples test negative.

### ***Collection, storage and transportation of samples***

Proper collection, processing, storage and transportation of the specimens are an essential aspect of the laboratory diagnosis.

### Collection of samples for serology, isolation & molecular diagnosis

**Sample:** Serum

**Time of collection:** Acute: within first eight days of illness; convalescent: 10-14 days after acute specimen collection.

**To collect serum:**

- Aseptically collect 4-5 ml of venous blood in a tube or a vial.
- Allow blood to clot at room temperature, centrifuge at 2,000 rpm to separate serum. Collect the serum in a clean dry vial.
- All clinical samples should be accompanied by the clinical and epidemiological information.

### Other types of specimen for laboratory investigation

**Specimens:** CSF in meningo-encephalitis cases

Synovial fluid in arthritis with effusion

Autopsy material – serum or available tissues

[Note: Mosquitoes collected in the field will also be handled using same techniques described here]

**Transportation of samples**

- Transport specimens to the laboratory at 2-8°C (ice box) as soon as possible.
- Do not freeze whole blood, as hemolysis may interfere with serology test results.

- If more than 24-hour delay is expected before specimens can be submitted to the laboratory, the serum should be separated and stored at refrigerated temperature.
- Serum samples for virus isolation and molecular diagnosis should be stored frozen (either -20°C for short-term storage or -70°C for long-term storage).

#### 4B. Laboratory Surveillance

Prior to identification of CHIKV in a country, laboratory surveillance should be conducted on 3 sets of samples as follows: 1) Dengue negative specimens where the patient exhibits severe joint pain; 2) Samples with clinically compatible illness from new geographic areas without active dengue circulation; 3) Clusters of febrile illness with severe joint pain. The following table (Table 5) outlines the ideal tests to be performed in various epidemiological settings.

<b>Table 5: Laboratory Surveillance for Chikungunya Virus by Epidemiologic Scenario</b>		
<i>Epidemiological scenario</i>	<i>Testing to be performed</i>	<i>Samples to test</i>
No signs of transmission	IgM ELISA, IgG ELISA	All samples from patients exhibiting clinically compatible illness
Suspect CHIKV illness	IgM ELISA, IgG ELISA, real-time RT-PCR, virus isolation, PRNT	All samples from patients exhibiting clinically compatible illness

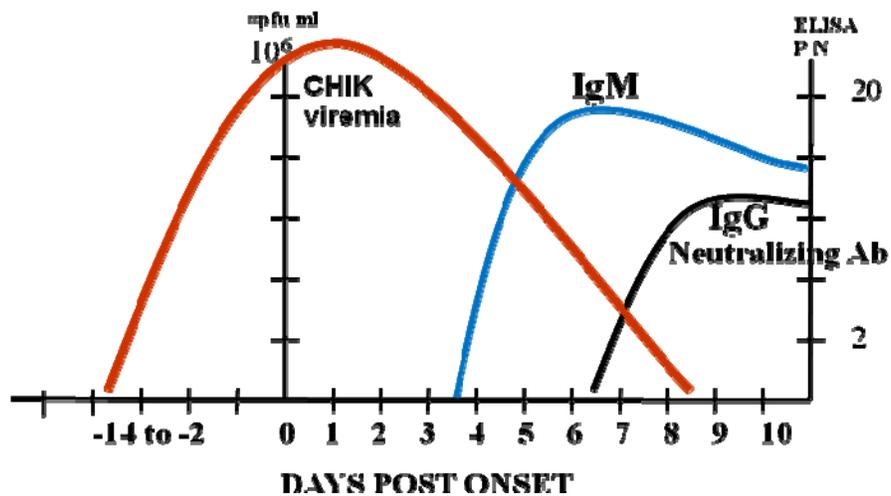
Continued transmission	IgM ELISA, IgG ELISA, real-time RT-PCR; limited virus isolation	Subset samples from classical CHIK cases, as determined by lab constraints and epidemiological status; Samples from all atypical or severe cases should be tested
Periodic outbreaks (once CHIKV has been detected in an area) or active surveillance in areas near CHIKV transmission	IgM ELISA, IgG ELISA, real-time RT-PCR; limited virus isolation	Subset of samples from classical CHIK cases, as determined by lab constraints and epidemiological status; samples from all atypical or severe cases should be tested

During the initial introduction of CHIKV into a new region, comprehensive testing should be completed to confirm that CHIKV is the etiological agent. After CHIKV has been identified limited testing (not testing all specimens or performing fewer assay types) can be considered depending upon the capacity of the lab and the epidemiological situation.

#### **4C. Interpretation and Reporting of Results**

Typical viremia and antibody response in humans is shown in Figure 2 and Table 6 describes the typical results of testing samples at various time points.

**Figure 2: Viremia and Immune Response following Chikungunya Virus Infection**



**Table 6: Typical Results of Samples Tested at Various Time Points Post Infection**

<i>Days post illness onset</i>	<i>Virus testing</i>	<i>Antibody testing</i>
<b>Day 1-3</b>	RT-PCR = Positive Isolation = Positive	IgM = Negative PRNT = Negative
<b>Day 4-8</b>	RT-PCR = Positive Isolation = Negative	IgM = Positive PRNT = Negative
<b>&gt;Day 8</b>	RT-PCR = Negative Isolation = Negative	IgM = Positive PRNT = Positive

The following laboratory test results would confirm a recent CHIKV infection:

- Isolation of CHIKV, including confirmatory identification (either IFA, RT-PCR, or sequencing)
- Detection of CHIKV RNA by real time RT-PCR
- Identification of a positive IgM result in a patient with acute symptoms of CHIK followed by the demonstration of CHIKV-specific antibody determined by PRNT with viruses in the SFV serogroup
- Demonstration of seroconversion or a 4-fold rise in PRNT, HI, or ELISA titers (again using other SFV serogroup viruses) between acute and convalescent specimens

Reporting of autochthonous cases to WHO should be performed, in collaboration with epidemiologist, according to the international health regulations (see section 6F).

#### **4D. Laboratory Network for Diagnosing CHIKV**

Currently the US CDC, DVBD can provide diagnostic testing for CHIKV infection. Reagents and consultations can also be provided by CDC and the Public Health Agency of Canada. Depending on the availability of resources and the epidemiologic situation, PAHO and CDC will be working together in the near future to improve CHIKV detection in the area by training and providing reagents to existing dengue (RELDA) and other arbovirus laboratories in the Americas. Furthermore, proficiency testing is planned to ensure testing quality in the area. A

contingency plan will be developed to ensure the adequate supply of reagents and protocols to all laboratories capable of performing testing in the Americas.

***Summary of Laboratory Section***

- Both molecular and serologic techniques are available for the laboratory diagnostic evaluation of CHIKV infection
- During an outbreak, laboratories will need to develop, with other public health partners, sample triage plans to avoid laboratory overload
- Laboratories have a key role in the surveillance for CHIKV introduction and spread; ongoing training of laboratories for CHIK detection is needed throughout the region
- Collaborations are important in order to share materials among network partner labs in public health role
- Reference laboratories in the region will have a significant role in reagent production and in providing laboratory confirmation of suspected CHIK cases