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# Diagnosis of Chikungunya Infection: A Narrative Review

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**Abstract**

*Chikungunya virus (CHIKV) is an arbovirus infects humans that lead to Chikungunya fever, usually characterized by intense joint pain and arthritis. The first case was identified in 1953 at Tanzania of east Africa. As of December 2024, 119 countries and territories have reported cases of Chikungunya and many cases presumed to be under reporting due to diagnostic limitations. The virus is transmitted by Ades aegypti and Ades albopictus. Bangladesh carries a huge burden of Chikungunya virus since 2007 which is gradually increasing. Many factors influences laboratory identification of Chikungunya virus (CHIKV) includes viral strains, patient immune response, time of sample collection from the onset of infection, test sensitivity and specificity, test compliances. Most reliable test for detection of Chikungunya virus is RT-PCR. Loop-mediated isothermal amplification (LAMP) can also be used in resource limited setting but has less sensitivity. Antibody detection depends on time of collection of sample after onset of infection, patient immune response, type of tests. Anti-Chikungunia IgM antibody becomes positive between 3-8 days of onset of infection. IgM capture ELISA (MAC-ELISA) is more sensitive. A fourfold rising titer of anti-IgG antibody in 3 weeks apart often recommended for diagnosis of recent Chikungunya infection. Serology test are less sensitive to RT-PCR or LAMP. Selecting appropriate tests for laboratory diagnosis of Chikungunya is critical in terms of its timing of test, cost effectivity, resource mobilization and reliability, sensitivity. Hence a combine approach of clinical judgment and laboratory evidence is important for diagnosis of Chikungunya.*

## Introduction

Chikungunya virus (CHIKV) is a mosquito-transmitted pathogen that infects humans and leads to Chikungunya fever, usually characterized by intense joint pain. The first case was identified in 1952-53 at Tanzania of east Africa[1]. Serological evidence indicated that in Asia, CHIKV first emerged in 1954[2]. The first outbreak was documented in 1958 in Thailand[3]. The virus is transmitted by Ades aegypti and Ades albopictus the same vectors which also transmit Dengue and Zika. Both vectors feed outdoor in addition to Ades aegypti that feed indoors also[4]. World health organization reported as of December 2024, 119 countries and territories have reported cases of Chikungunya and many cases presumed to be under reporting due to diagnostic limitations[5]. Chikungunya was epidemic from 1963-1973 followed by a huge outbreak in the Indian Ocean islands from 2004-2007. In Bangladesh Chikungunya was first reported in 2008 followed by outbreaks in 2009, 2011 and 2012[8-9]. However Bangladesh experienced the highest outbreak in 2017 affecting 17 out of 64 districts of the country[10]. The disease is characterized by high fever, rash, disabling arthritis and arthralgia[11-12]. Apart from these severe gastrointestinal and neurological complications like encephalitis, myeilitis, Gullian-Barre syndrome may occur[13]. In the acute phase of infection the diagnostic laboratory tests includes viral isolation, RT-PCR

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for viral RNA, virus specific IgM in acute or convalescence phase and a fourfold rise of IgG antibody titers in three weeks apart often used[11].

**The virus and its genotypes:**

The virus belongs to Togaviridae family having 11-12 kb positive sense RNA encoding four non-structural proteins e.g., nsP1, nsP2, nsP3 and nsP4, several structural proteins namely capsid, E3, E2, 6K and E1 and 5Ê and 3Ê untranslated regions[14]. CHIKV is classified into three main genotypes, namely Asian, West African and East/Central/South African (ECSA) genotypes[15]. It has been demonstrated that adaptive mutations in viral lineages contribute to the transmission potential and fitness of CHIKV in various hosts and vectors[15]. A recent study of viral phylogenetics in Bangladesh identified an E1-K211E substitution, revealing a distinct East-Central-South-African (ECSA) genotype by sequence analysis[16]. Viral structures and genomic diversities have an impact on laboratory diagnosis of Chikungunya.

**Immunopathogenesis:**

After inoculation from mosquito bites virus replicates in fibroblast, mesenchymal cells and osteoblasts. CHIKV induces local cytokine and chemokine response that leads to inflammatory infiltrate of macrophage, monocytes, NK cells, CD4+ and CD8+ T cells. Damage from virus and inflammatory reaction results in myfiber degeneration, mesenchymal damage of the synovium and periosteum and increase osteoclast generation causing bone damage[17-18].

**Role of T lymphocytes:**

CHIKV infection results in the activation of $CD8^+$ T cells (as judged by increased expression of CD69 and HLA-DR) with peak levels in peripheral blood detected soon after symptom onset[19]. In infected individual activated $CD38^+$ $HLA\text{-}DR^+$ $CD8^+$ T cells may persist in the circulation even after 7-10 weeks post infection[20].

**Role of B lymphocytes:**

Anti-CHIKV IgM can be detected within a few days of infection and begins to wane during the second week. This coincides with the development of an IgG response, which increases through the acute phase and remains high during the chronic phase[21]. Neutralizing antibodies target the envelope glycoproteins, which are displayed on the virion surface as trimers of E2/E1 heterodimers[22].

Although acute infection generates effective immune response that eliminates circulating CHIKV but it is evident that infected perivascular macrophages, CD14+ macrophages, activated $CD56^+$ $CD69^+$ NK cells harbor the source of CHIKV in chronic infection[23]. Immune activation from persistent viral RNA and antigen, results in continuous production of inflammatory cytokines and chemokines in synovial and muscle tissue[24].

**Case definition:**

*Possible case:*

A patient meeting clinical criteria (acute onset of fever > 38.5°C and severe arthralgia/arthritis not explained by other medical conditions)[11].

*Probable case:*

A patient meeting both the clinical and epidemiological criteria (residing or having visited epidemic areas, having reported transmission within 15 days before the onset of symptoms)[11]

*Confirmed case:*

A patient meeting the laboratory criteria, irrespective of the clinical presentation.[11]

**Clinical manifestations**

CHIV infection can be classified in 3 phases acute, post-acute or chronic phases. Incubation period is 3-7 days.

Acute phase: Starts with abrupt onset of high grade fever (> $39^0$C ) which lasts for 3-5 days and malaise[26]. 40-75% manifests maculopapular rash and pruritus among 25-50% cases. Acute infection is also manifested with joints symptoms which stipulated in the Table I.

**Table-I:** Joint manifestation of acute Chikungunya[27]

| Feature | Description | | |
|---|---|---|---|
| Onset | 2–5 days after onset of fever | | |
| Pattern | Bilateral and symmetric | | |
| Joints Involved | - Hands | : | 50–76% |
| | - Wrists | : | 29–81% |
| | - Ankles | : | 41–68% |
| | - Axial skeleton | : | 34–52% |
| Severity | Pain may be intense & disabling, leading to immobilization | | |

Post-acute phase: Most patient shows transitory improvement in their clinical condition. Relapse occurs among 50-90% cases 2-3 weeks after transitory recovery. The percentages of persistent polyarthralgia are more common among people over 40 years[28].

Chronic phase: About 40-80% of acute CHIKV infection progresses to chronic infection persist for months to years[28]. Two hypotheses has been postulated for this persistence infection firstly, presence of viral RNA or antigens in the muscle and joint tissue. Second, infection triggers persistence immune response[29]. 25% of the cases have been reported to progress to chronic inflammatory rheumatism[30]. Joint manifestations or self discomfort among patient infected with CHIKV also vary among different genotypes. Of them who are infected with ECSA genotype or Asian strain have fewer complications or discomfort whereas ECSA-diverge/IOL and overlapping Asian genotype may produce more discomfort.

**Diagnosis:**

Laboratory diagnosis is principally based on three different techniques, e.g., molecular detection, serology and isolation of virus in cell culture. However use of appropriate specimen at the appropriate course of the illness is also critical in accurate diagnosis of CHIKV in the laboratory. Center for disease control (CDC), USA has developed an algorithm based on CHIKV infection and timing of specimen collection (Figer1).

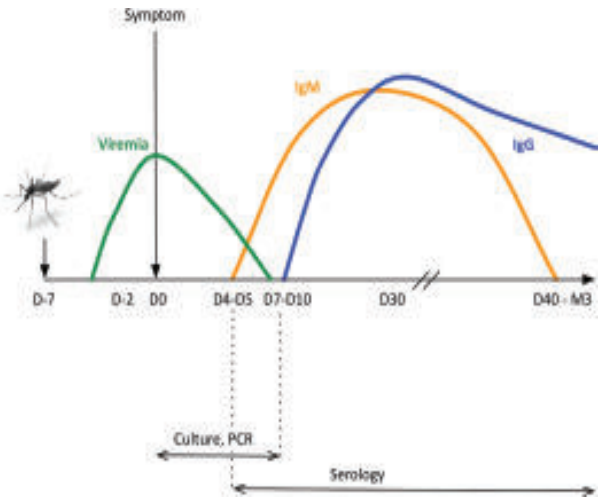


**Figure 1:** *Infection and immune response against Chikungunya virus*

The virus replicates rapidly in the first few days of infection. It also takes few days to form specific antibodies to become detectable level. So, molecular methods which particularly detects viral RNA is preferred within first week of onset of infection. While antibody particularly anti-IgM antibody is tested between 5-7 days of onset of infection[31]. Selection of a diagnostic tool depends on kinetics of viral replication and host immune response[32]. Several molecular tests are available which include RT-PCR, RT-LAMP, reverse transcription PCR[33]. CDC, USA follows testing of a suspected Chikungunya specimen received within 6 days of onset of symptoms by RT-PCR. CDC recommends for sample received on or six days after onset of symptoms or sample which are RT-PCR negative should be confirmed by CHIKV IgM antibody capture ELISA (MAC-ELISA) or fluorescence antibody test (IFA) or plaque assay[34-35].

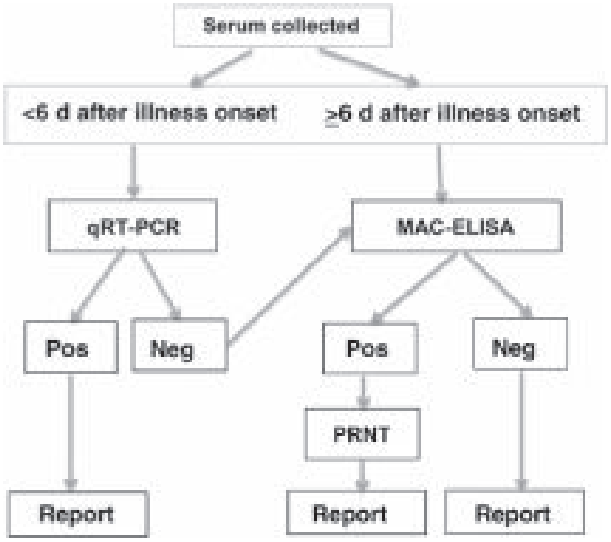


**Figure 2:** *Center for disease control and prevention diagnostic algorithm of Chikungunya fever*

**Type of samples for diagnostic tests**

Depends on patients (e.g, adult male or female, pregnant women, fetus), time lapses from the onset of symptoms, sites of infection e.g., CNS, GIT or cells of reticuloendothelial system. Collected sample should be sent to laboratory as soon as possible. For antibody testing blood should be collected in EDTA or heparin free tubes[36]. The same type of tubes can be used for PCR. Blood and plasma is preferred over serum for molecular tests like PCR.

**Viral replication kinetics and laboratory tests:**

The replicating viruses are found in the blood, plasma or serum samples between 2-6 days of appearance of clinical symptoms[37]. Viral load rapidly rises to $10^8$-$10^9$ copies/ml at this time which rationalizes the use of viral isolation techniques in cell culture, viral RNA or antigen detection[38].

**Viral Isolation:**

Viral isolation is gold standard and it is a robust research tool[39]. Although the viral load gradually drops down by day 5 of infection, still the method is very useful to detect viral strains. This is not commonly practice in regular CHIKV testing as it is costly, needs more time and expertise and BSL3 laboratory set up. Several cell lines are used to culture the virus, among them culture in HELA cells is more sensitive and the viral cytopathic effects are observed more earlier in that cell line than Vero cells[40].

**Antigen detection:**

Immunochromatography test (ICT) for viral antigen detection is a sensitive test in the early phase between 4-5 days of onset of infection. However the test sensitivity varies according to the geographical distribution of viral strains[41].

**Detection of viral genome:**

Reverse transcriptase PCR (RT-PCR) is a highly sensitive test in the acute phase of CHIKV infection. It is performed from plasma or serum. The test detects genes encoding nsP1, nsP2 and also E3, E2,E1 viral envelope proteins[42]. Viral quantification is done by label probe or SYBR green during the process of amplification[43]. The PCR assay can detect both positive and negative strands DNA particularly by strand-specific RT-PCR for nsP1 and nsP3 genes[44-45]. But the lower limit of detection (LOD) of viral copies is slightly decreased in detection of negative strand than the positive strand[46]. The limitations of conventional or RT-PCR particularly lack of financial and human resource have been overcome by an alternative molecular technique Loop-mediated-isothermal amplification (LAMP) which do not need a thermal cycler, easy and convenient to performed but with less sensitivity than PCR[47]. This may be effective in a resource limited set-up for molecular detection of CHIKV. Beside this a combining multiplex molecular amplification with DNA microarray hybridization has been designed to simultaneously detect CHIKV, Dengue virus (DENV) and other arboviruses[48]. Because CHIKV and DENV show serological cross-reactivity between their serocomplexes, they are difficult to distinguish using serology alone, making molecular detection methods essential for accurate differentiation[49]. A one-step duplex conventional RT-PCR assay has been developed for differentiating between DENV and CHIKV[50].

**Serology:**

CHIKV-specific IgM and IgG antibodies can be identified in serum through enzyme-linked immunosorbent assay (ELISA). IgM is the earliest antibody detected in chikungunya patients, typically appearing 3–8 days after infection and remaining for several months to up to 2 years. IgG antibodies usually become detectable 4–10 days after infection onset and can persist for many years[38]. The primary diagnostic methods for CHIKV infection are IgM antibody-capture ELISA (MAC-ELISA) and indirect ELISA (i-ELISA), which is used to detect immunoglobulin M (IgM) and immunoglobulin G (IgG), respectively[51]. Advancements in test design and procedures have reduced the likelihood of IgG cross-reactivity with other viruses compared to earlier methods. However, IgG seroconversion (a greater than fourfold rise) is still advised for a dependable diagnosis. However, ELISA has certain limitations: it may yield false positives due to cross-reactivity with other alphaviruses and its sensitivity drops significantly (to 4–20%) when testing serum samples collected during the acute phase[52].

**Conclusion:**

Chikungunya is a mosquito borne arboviral disease primarily affects joints and muscle. It also has CNS effect which could complicate the course of illness. The viral genome characteristically gives rise different Phylogenetic lineage resulting in three major strains. Although the virus does not have any life threatening complications like other arbovirus but it complicates to chronic debilitating arthritis that leads to morbidity. Early diagnosis is important for appropriate intervention and treatment. Among several test methods most early detection could be made by RT-PCR. A multiplex PCR can detect Dengue and Chikungunya virus

simultaneously. Although RT-PCR is more sensitive but it depends on viral load and on strain specific primers. An alternate to PCR could be LAMP that is cost effective and can be utilized in a resource constraint set-up. Serology test result depends on time lapse between sample collection and onset of infection. Anti-Chikungunya IgM ELISA (MAC-ELISA) is a sensitive method to detect virus specific IgM while Anti-Chikungunya IgG need to test two times in three weeks apart to conclude as recent chikungunya infection.

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