# Study of Molecular Diagnosis and Analysis of Chikungunya Virus by RT-PCR and Serological test

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## ABSTRACT

Chikungunya is caused by an arbovirus and spread by the mosquito Aedesaegypti. Chikungunya is a viral disease with symptoms and pathogenesis that are strikingly similar to dengue fever. Chikungunya is caused by the alpha virus, which is spread by the aedes aegypti mosquito, which is only active during the day.For CHIKV, a real-time RT-PCR assay was developed to detect currently circulating virus strains as well as other genotypes.Its sensitivity was compared to a previously published real-time assay and a standard RT-PCR assay.ELISA was used to identify CHIKV immunoglobulin (Ig) G and M antibodies in serum or blood absorbed on filter paper.

**Keywords:** Chikungunya, Filter paper; Real time RT PCR, IGM Test.

## INTRODUCTION:

The Chikungunya virus (CHIKV) belongs to the Togaviridae family and belongs to the Alphavirus genus. On the basis of serological cross-reactivity, the 29 Alphavirus species are divided into antigenic complexes, with CHIKV belonging to the Semliki Forest virus complex [1].It was responsible for extensive outbreaks on these two continents from the 1960s to the 1980s, before a period of relative quiet over the next 20 years. It was first described in Africa (Tanzania) in 1954 and later found in Asia. In the year 2000, there was a massive outbreak in the Congo [2].The CHIKV genome is an 11.8-kilobyte linear, single-stranded, positive sense RNA molecule [3].The Comoros were the source of the first case of chikungunya sickness in La Réunion (Indian Ocean) in March 2005 [4].This triggered a large-scale outbreak that lasted until mid-2006. In the second week of February 2006, the outbreak peaked with 47 000 cases [5].After the outbreak phase (August to October 2006), a serological survey revealed that chikungunya had afflicted roughly 300 000 people out of a total population of 787 000. (38.2 percent )[6].Symptoms of the outbreak included fever (with abrupt onset) and arthralgia, which were often coupled with asthenia, myalgia, skin rash, and digestive and neurological issues[7].New or unusual clinical forms, such as neurological symptoms, heart and liver problems, and materno-foetal transfer, have also been observed [5].Our understanding of the disease has substantially evolved as a result of the recent global outbreak, which has resulted in millions of infected patients. From an epidemiological and clinical standpoint, it was previously considered a minor arboviral disease.Chikungunya was once thought to be a harmless, uncomfortable fever, and was frequently confused with dengue fever. It was first described as having a high fever and a persistent peripheral polyarthritis that was occasionally accompanied by a rash. The disease's name comes from the severity of the pain and disability: in Makonde, "chikungunya" means "that which bends up." Recent outbreak studies have described the characteristics of the acute stage, including unusual, severe, and deadly complications, as well as the chronic stage's burden.[8,9,10].Because of a serious ongoing outbreak in the Western Hemisphere, CHIKV-induced sickness was recently designated as a nationally notifiable condition in the United States [11].As of March 2016, the outbreak had spread over the Western Hemisphere, affecting 45 nations and territories and caused around 1.8 million suspected cases[12].In the United States in 2014, 2,811 instances of CHIKV-induced illness were documented, including 12 locally acquired cases in Florida[11].In 2015, there were 896 instances of CHIKV-induced disease and 1 locally acquired case in Texas, resulting in a total of 896 cases of CHIKV-induced disease and 1 locally acquired case[13].Because of the continuous pandemic in the Americas and the extensive availability of competent mosquito vectors, this indicates a considerable risk of breakout in the United States[14].Despite the fact that millions of individuals have been infected with CHIKV in the previous decade, there are currently no effective prevention or treatment options. The mosquito vector is the focus of preventive actions, which include wearing long sleeves, using mosquito nets, eliminating mosquito breeding areas, and using insecticides [15].Treatments are mostly symptomatic and supportive, and the efficacy of potentially more specialised medications is uncertain at this time [16,17].

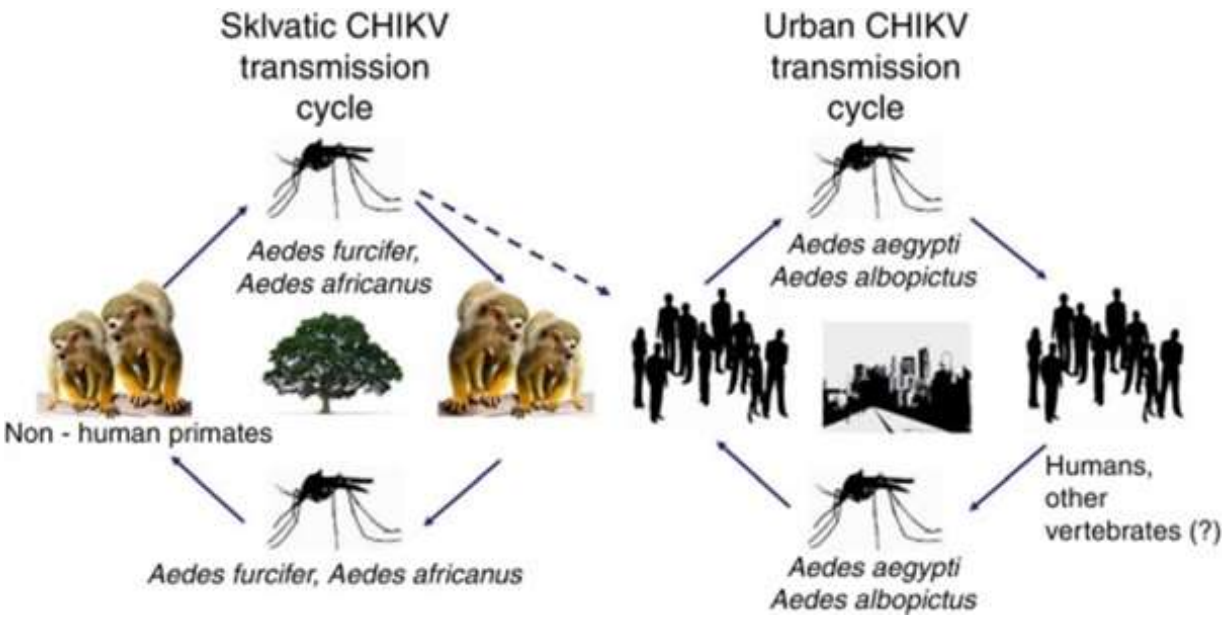


Fig:1 Life cycle of chikungunya

**METHODS**

## laboratory diagnosis

Several laboratory tests that use serum or plasma to detect virus, viral nucleic acid, or virus-specific immunoglobulin (Ig) IgM and neutralising antibodies are available to diagnose CHIK. CHIKV viral RNA can be detected during viremia due to the high viral load.commercial diagnostics with good sensitivity and specificity during the first 5-8 days of illness.The time of the sampling in relation to the onset of the symptomatology and the amount of the samples available determine the sort of test to use.

## SEROLOGICAL TESTS: IGM and ELISA

Antibodies to CHIKV IGM emerge at the end of the first week of sickness, and convalescent-phase samples from individuals whose acute-phase samples test negative should be acquired to definitively rule out the diagnosis. CHIK is diagnosed serologically by detecting CHIKV-specific IgM in serum samples for 5-7 days after symptom onset, or by demonstrating a four-fold increase (or seroconversion) of CHIK-specific IgG antibody titers in two serum samples taken at least 15 days apart (acute and convalescent phases of the disease)[18].IgM antibodies to CHIKV can last up to a year, especially in patients with long-term arthralgia, but they usually only last 3-4 months. For several years after infection, the particular CHIKV-IgG can be found. Infection with closely related alphaviruses belonging to the Semliki forest virus has been linked to serological cross-reactions and false positive testing[19].The existence of IgM and IgG anti-chikungunya antibodies can be confirmed using an enzyme-linked immunosorbent test (ELISA). IgM antibody levels peak three to five weeks after the onset of sickness and last about two months. Serological and virological tests should be performed on samples collected within the first week after the onset of symptoms (RT-PCR)[20].

## METHOD

Advantage chikungunya IgM card is the one step immunochromatographic assay.The test sample is delivered to an absorbent pad and passes laterally through it, mixing with the conjugate. If the sample contains Chikungunya-specific IgM antibodies, they form a combination with the gold colloid conjugate of the Chikungunya antigen. Capillary action causes the compound to move across the nitrocellulose membrane. The complex creates a pink purple line when it comes into contact with the line of immobilised Anti human IgM antibodies (Test line, 'T'), showing that the sample is reactive. An additional control line 'C' has been placed at a distance from the test line on the strip to act as a

procedural control. If the test is done correctly, the control region will create a pinkish-purple line when it comes into touch with the conjugate.

## MOLECULAR TESTS

A real-time PCR/nested PCR combination amplifying a segment of the envelope E-2 gene can be used to identify CHIKV [21]. The non-structural nsP1 and envelope E-1 genes have been targeted for CHIKV detection and genotyping [22].Recently, one-step taqman has become more sensitive and specific. By measuring viral load in clinical samples and cell culture supernatant, the RT-PCR test has been established as a tool for diagnosing CHIKV and as a fast indicator [23].The real-time loop-mediated isothermal amplification (RT-LAMP) assay has also been discovered to be an effective molecular diagnostic tool [24].RT-PCR can detect unique CHIKV viral RNA in serum, plasma, or EDTA samples collected from patients during the acute phase of infection (usually 7 days following onset of symptoms). CHIKV infection results in high levels of viremia, which lasts for 4-6 days after symptoms appear. This is a fantastic opportunity for a diagnosis. In the acute phase of infection, real-time RT-PCR is the best diagnostic for diagnosing CHIKV infections [21].To confirm CHIKV infection, an RT-PCR test should be performed within the first 7 days of symptom onset. During the first several days after infection, the virus can be isolated from the blood. There are several reverse transcriptase–polymerase chain reaction (RT–PCR) procedures available, but their sensitivity varies[25]. Some are better suited to clinical diagnosis than others .RT–PCR results from clinical samples can be used to genotype the virus, allowing for comparisons with virus samples from different geographical locations[26].In the first three days of illness, viral culture may detect the virus; nonetheless, CHIKV should be treated under BSL 3 conditions[27].

## METHOD

The Truenat KFD system (Molbio Diagnostics Pvt.Ltd.) is made up of three parts: (1) an AutoPrep sample prep kit; (2) a Trueprep AUTO RNA extraction system; and (3) a Truelab UnoDx PCR analyser system[28]. KFDV clinical specimens were handled with normal personal protective equipment and procedures, as well as BSL-3/BSL-2 biosafety measures. For inactivation, a 50-liter extraction volume from suspicious specimens (mostly human blood, serum, homogenised monkey necropsy organs, and homogenised tick pools) was treated with lysis buffer[29].The treated material was placed into an RNA extraction cartridge and subsequently into the Trueprep AUTO RNA extraction equipment. Each sample received 150 l of RNA elute, of which 6 l was added to a reagent tube containing lyophilized master mix. For 30 seconds, the reaction mixture was incubated[30]. 6 l of the reaction mixture was added to the microchip after it was placed on the PCR analyser tray. The cycle threshold (Ct) value and optical graph were viewed at the end of the 40-minute run[31].

## CONCLUSION:

The current spread of previously unnoticed CHIKV is an example of infectious disease globalisation.As a result, it's critical to comprehend the dynamics of CHIKV infection, its immune response, and the type and quality of antigen employed in diagnostic test design. Because CHIKV is a new focus of focused research, breakthroughs in diagnosis are expected to help all persons who are at risk, particularly those in underdeveloped nations.

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## Conflicts of interest

The authors declare no conflicts of interest.

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