



Diagnostic Limitation of Chikungunya Fever and its Future Prospect: A Review Update

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Abstract

Chikungunya is a febrile illness which is usually self-limiting caused by Chikungunya virus (CHIKV). It is transmitted by the bites of infected adult female mosquitoes mainly *Aedes aegypti* and *Aedes albopictus* from human to human; these vectors also transmit other viral diseases including dengue, zika virus and yellow fever. These viral diseases presented in a similar manner in their early stage of infection specially dengue and chikungunya since neither of them possesses any specific feature to be distinguished clinically. Their outcome and treatment strategies are distinct so early and accurate diagnosis is mandatory for better management and taking appropriate measures to prevent or reduce severity of complications. An early confirmation of any infection demands diagnostic tools that are highly specific and cost effective. Currently no diagnostic tool is available for CHIKV detection which can fulfill these criteria. Moreover effective surveillance, outbreak control, vaccine design and drug development all this demand proper diagnosis. In this review we focus on limitation of available laboratory tests related to diagnosis of chikungunya virus and discuss priorities for further studies needed for disease diagnosis in early stage to control the outbreaks. [*Bangladesh Journal of Infectious Diseases, December 2018;5(2):65-70*]

Keywords: Diagnostic Limitation; Chikungunya Fever; Future Prospect

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1.0. Introduction

Chikungunya is a mosquito borne viral disease characterized by high grade fever and debilitating joint pain mainly involving small joints of upper

and lower extremities. Other symptoms may include headache, myalgia, nausea, vomiting, skin rash and joint swelling¹. Though most of the patient recovers completely within few weeks without any residual effect, some patient may experience severe

arthralgia and arthritis which may persist for months, even years. Incubation period is usually 3-7 days but it may vary from 2-12 days².

Chikungunya was first detected in the serum of the patients who were suffering from fever during an outbreak in southern Tanzania in 1953³. The name "chikungunya" is derived from Makonde word that describes the characteristic posture due to joint pain⁴. Since then scattered cases have been identified in different countries of Africa and Asia. In 2004 CHIKV re-emerged in Kenya and spread rapidly to countries nearby the Indian Ocean. It was first reported in Italy by the end of 2007 and in France in 2010⁵. It has just recently drawn worldwide attention due to its emergence in the western countries and temperate zones at the end of 2013⁶.

As Chikungunya and dengue both are transmitted by the same *Aedes* mosquito and presented in the same manner so it is burdensome to characterize them clinically but it is pivotal to differentiate them because their management and prognosis is quite different⁷. Co-infection with chikungunya and dengue virus has been reported in the recent outbreak⁸⁻⁹. Complications due to chikungunya are rare whereas dengue fever may lead to severe complications like hemorrhagic fever and dengue shock syndrome. Early differential diagnosis and supportive measures can reduce the chance of mortality due to severe complications¹⁰. However advanced laboratory tests may be unavailable or costly in resource limited settings to distinguish them separately, necessitating epidemiological and symptom based approaches to diagnose them¹¹.

Accurate and rapid detection of chikungunya infection by reliable assays is the current challenge world is facing to combat the recent emergence and changing epidemiology of this viral disease¹². Despite the fact that CHIKV infection is now considered as a public health problem, only few specific serological and molecular tests are available. Currently available laboratory tests used for diagnosing CHIKV infection are virus isolation by cell culture, serological test for detection of viral specific antibody and genome detection by PCR based methods¹³. However these tests are not widely performed.

Choice of laboratory tests based on the time elapsed between the onset of symptoms and collection of sample. Period of viremia lasts for 5 to 6 days. During this period diagnosis is rest on detection of viral genome by PCR and virus isolation by culture.

So after 5 to 6 days of illness diagnostic strategy is detection of IgM or four fold rise of IgG compared in paired sample (acute and convalescent stage) to establish recent infection¹⁴.

Serological tests are comparatively quick and easy to perform, convenient and affordable than molecular test for majority of the people of under-developed country as it doesn't need costly instrument and skilled personnel¹⁵. In present time only antibody based serological test is widely used for diagnostic purpose. But one of the major drawbacks of this test is that early detection of acute infection is not possible through this test. Antibody against CHICKV is detected in this test usually developed after 5 days of symptoms onset and onward¹⁶. Therefore development of an antigen based serological test is critical demand of current situation to support the diagnosis in an early stage for proper management¹⁷.

2.0. Serological Test

IgM is developed first in recent infection followed by IgG which is lag behind 1 to 4 days. Pattern of antibody response differ between primary and secondary immune response. The first and major antibody that is produced during primary response is IgM, though low level of IgG is detectable. But in case of secondary immune response IgG is the major antibody that appears more early and in higher concentration within 2 to 3 days. Presence of IgM in a single serum is consistent with recent infection and IgG in single sera indicates recent or past infection⁹.

2.1. Immunochromatographic Test

It is the most rapid test for easy detection of CHIKV infection which takes only 10 to 15 minutes to give the results. Commercially available ICT kits are IgM: SD Biotec Chikungunya IgM (Standard Diagnostics Inc., Yongin-si, South Korea) and OnSite Chikungunya IgM Combo Rapid Test (CTK Biotech Inc., San Diego, CA, USA).

An IgM immunoassay is the most practical lateral flow ICT for qualitative detection of antibody against CHIKV that can be done from human serum, plasma or whole blood in those countries where limited laboratory facilities are available. It is used for screening purpose and any reactive result in this test must be co related with appropriate clinical findings and need to be confirmed with alternative test. At the field level it can be carried out to diagnosis chikungunya infection during an

outbreak as it does not need skilled personnel to carry out this test. Kit is stable at 1 to 30°C for 24 months. Test should be done as soon as possible after collection of sample or stored at 2 to 8°C if test is not done immediately. Stored sample can be kept for 5 days. It needs to be frozen at -20°C for longer storage¹⁷.

2.2. ELISA

It is an immunoassay designed for the qualitative detection of IgM or IgG from either acute or convalescent phase in patient’s serum or plasma targeting antigen of chikungunya virus. This test is done worldwide as a presumptive laboratory test in patients with consistent clinical features of chikungunya infection. Positive result must be confirmed by following guidelines recommended by the CDC for this disease. There are several Elisa kit has been manufactured by different company for detection of CHIKV IgM and IgG. However their performance is quite different. List of commercially available ELISA kit is given below¹⁴.

Table 1: List of RDT for CHIKV

Manufacture	Location	Assay Name and Format
IBL international	Germany	CHIK IgM micro capture Elisa
CTK biotech	USA/China	RecombiLISA CHIK IgM test
Genway	Germany	CHIKV IgM μ capture ELISA
Abcam	Germany	Anti-CHIKV IgM human ELISA KIT
SD diagnostic	Korea	CHIKA IgM ELISA
Euroimmun	Germany	Anti-CHIKV ELISA (IgM)
Inbios	USA	CHIKjj Detect MAC ELISA

It is useful to distinguish CHIKV from Dengue infection. Sensitivity of this test is improved after 5 days of illness. It is simple and easy to use but cross reaction to other alpha virus antibody such as ONNO and SFV usually limits its application as a confirmatory test. Kit is stable at 2 to 8°C for 12 months.

3.0. Indirect Immunofluorescence

It is quite sensitive and specific test but lack the

ability to quantify antibodies against CHIKV. Anti-Chikungunya virus IIFT- EUROIMMUN is the commercially available kit for detection of chikungunya antibody by immunofluorescence. The IgM IIFT shows a specificity of 98.3% and a sensitivity of 96.9%. The specificity is 100.0% and sensitivity is 95.4% for IgG IIFT. In spite of having reliable test results it is not suitable for resource limited country, because it demands technically skilled personnel and costly instrument to run the test. As test results may vary from person to person so expertise needed for accurate interpretation of the results¹⁸.

4.0. Plaque Reduction Neutralization Test (PRNT)

It is developed by Henderson and Taylor in 1959 for detection of arbovirus plaques and to measure the serum antibody neutralization titer and highly specific and sensitive test. According to CDC guidelines, this test is considered as a confirmatory test for diagnosis of chikungunya infection. Samples collected at or after day 6 of infection and those samples that are found to be positive by ELISA will be considered as a confirm if the PRNT test of that samples yields positive results also.

As virus concentration is usually fixed, this technique detects and quantifies neutralizing antibodies in samples by determining the percentage of reduction of virus activity. Test sample is diluted and mixed with a CHIKV suspension. This is incubated and allows the formation of virus- antibody complexes to occur. Virus susceptible cells are incubated for 3-5 days with this complex and later on covered with a semisolidmedium then plaque formation is observed. Plaque forming units are measured by microscopic observation, fluorescent antibody or adding specific dyes that react with the infected cells. In samples with CHIKV specific neutralizing antibody plaque forming units will be less than that of control wells, because infection of host cell is prevented by neutralizing antibody. Plaque usually develops between day 2 and day 4 after CHIKV infection. Simian kidney cell lines (Vero and LLC-MK2) are suitable for this test because these cells are susceptible upon CHIKV infection¹⁹.

Although this test is gold standard, the assay is time consuming and laborious which is available only in few reference laboratories. Another major drawback of this test is that it requires biosafety level 3 laboratories as it handles live virus²⁰. So this test is not widely used due to lack of laboratory facilities.

5.0. Haemagglutination Inhibition Test

Another way of diagnosing CHIKV infection is haemagglutination test. This test cannot distinguish between IgM and IgG thus required to compare between paired samples to get four fold rise of haemagglutination inhibition antibody to establish recent infection.

Test becomes positive within 5 to 8 days of infection. When this test is found to be positive in a patient with characteristic features and who had a recent traveling history to endemic area it is considered as a confirm case. It is simple to perform, less time consuming and requires inexpensive equipment and reagent but it is less specific and sensitive²¹.

6.0. Virus Isolation

Virus isolation is the most definitive and considered to be gold standard for detection of virus in early phase of infection within 7 days²². Detection of most of the alphavirus is currently based on cell culture. Isolation of CHIKV can be performed via mosquito isolation or cell culture. It can also be done by intracerebral inoculation of mice. Mosquito isolation is the most sensitive method though it is not suitable for routine diagnostic purpose due to its high maintenance cost and specialized requirements.

It can be done by obtaining sample from infected patients serum, whole blood or blood feeding arthropods. Due to its high cytopathic effect and fast growing nature of CHIKV in host cell it is comparatively easy and effective to culture. It will grow to very high titers in vitro cell culture medium. CHIKV can be grown in various cell line including insect cell line for example, C6/36 (*Aedes albopictus* cell line), nonhuman viz, Vero (African green monkey kidney cell line), Chick embryo fibroblast like cell, BHK-21 (baby hamster kidney fibroblast), L929 (subcutaneous adipose tissue of mouse), Hep-2 cells and human cell lines, for example epithelial cell line HeLa (human cervical cell line), 293T (human embryonic kidney cell) and BEAS-2B (human bronchial epithelium) as well as primary fibroblast (Hs 789.Sk skin cells and MRC5 lung cells)²³.

In vitro cell culture C6/36 cell line and mammalian cell line including HeLa, Vero and BHK-21 are commonly used because they produce cytopathic effect comparable to in vivo method. For confirmation of cytopathic effect produced in cell

culture CHIK-specific antiserum must be added and it takes one to two weeks to give positive result. So it is time consuming and not suitable for early detection of infection. Though it is considered to be gold standard due its high specificity it also requires highly trained operators and integrity of samples and it should be handled under biosafety level 3. Another drawback is virus isolation only early stage of infection thus providing narrow window of opportunity from illness onset. So despite of its advantages it is not used worldwide as a routine laboratory test¹⁵.

7.0. Genome Detection

Viral nucleic acid detection in acute phase of infection using PCR techniques is more sensitive and less time consuming compared to virus isolation. CHIKV causes high level of viremia (up to $1 \times 10^{6.8}$ plaque forming units/ml) which usually persists for 4-6 days but sometimes it can last for up to 12 days from onset of illness. While virus isolation is limited only first 7 days of illness, viral RNA detection can be accomplished by real time RT-PCR for a few additional days²⁴.

Detection of viral nucleic acid can be done by reverse transcriptase polymerase chain reaction (RT-PCR), real time RT-PCR, or isothermal amplification methods. All of these methods involve three basic steps: viral RNA extraction, amplification, and detection and characterization of amplified product. Samples that are used for PCR are blood, serum, plasma and fresh or formalin fixed paraffin embedded tissues.

RT-PCR using primer designed for structural and nonstructural protein found to be effective for rapid diagnosis of CHIKV. Conventional RT PCR was based on amplifying specific region of three structural gene regions, Capsid (C), Envelope E1 and E2 and part of nonstructural protein NSP1. Combination of RT-PCR and nested PCR for specific detection of CHIKV RNA is more specific and sensitive. It can amplify a specific viral amplicon from a sample containing as few as ten genome equivalents. The existing RT-PCR is qualitative, time consuming and high risk of contamination due to post PCR handling. Therefore a specific rapid test is necessary to quantify the viral load in clinical samples and culture supernatants²⁵.

Real time PCR holds a great deal of promise for highly specific and sensitive virus detection assays. Here fluorescently labelled virus specific probe is

used with PCR amplification so that it can detect amplified DNA during the reaction. It is rapid, quantitative assays with minimum contamination rate and ease of standardization. It is based on E1 immunodominant gene of CHIKV employing both TaqMan and SYBR Green I chemistry have been reported²⁶.

Nucleotide sequence of 2 sets of primers and probes are used in CDC for detection of viral genome by real time PCR. The 3855 primer/probe set is broadly reactive and designed to detect the East Central South African (ECSA) geno-types and Asian genotype in travelers returning from India. The 856 primer/probe is for detection of Asian genotype that is currently circulating in the Caribbean and it is slightly more sensitive for Asian lineage. The threshold of detection of both sets of primer/probes is approximately 1 RNA transcript²⁷.

Two sets of commercial PCR kit are available for detection of CHIKV genome. These are RealStar Chikungunya RT PCR kit (Altona Diagnostics, Hamburg, Germany) was assessed by Panning et al in the premarket format and was found to have a sensitivity (approximately 5.3 RNA copies/reaction) and specificity comparable to that of the in-house assay and genisig Chikungunya Nonstructural protein 2 standard (nsp2) RT-PCR kit (Primerdesign, United Kingdom) states that test is sensitive to < 100 RNA copies of target; however no validity has been published with the literature²⁸.

Collectively real time RT-PCR amplification methods have several disadvantages requiring high precision instrument, costly reagents and elaborate, complicated method for detection of amplified product which is not suitable for developing country and widespread use during outbreak. In this regard, the loop-mediated isothermal amplification (LAMP) method developed by Eiken Chemical Co. Ltd (Japan) has the potential to replace PCR as a result of its simplicity, rapidity, specificity and cost-effectiveness. It does not need thermal cycler and thus can be performed in any peripheral health care center easily with a heating block and/water bath. Because of high amplification efficiency it takes around 1 hour under isothermal condition (60-65°C) and sophisticated instrument is not required which makes it adaptive for field diagnosis²⁹.

Sensitivity and specificity of LAMP for detection of CHIKV has been evaluated by comparing with RT-PCR and cDNA sequencing and result showed that it could identify CHIKV RNA in all antibodies positive serum samples within 1 hour without any

cross reaction. The sensitivity was 100% and specificity was 95.25%. In fact it is convenient to detect CHIK virus quantitatively by monitoring the turbidity of the RT-LAMP or visually judging the reaction by using the SYBR Green I dye. This data indicates that RT LAMP is an effective diagnostic tool for rapid detection of CHIKV with higher sensitivity and specificity²⁹.

8.0. Conclusion

Limitation of available diagnostic tools makes it difficult to diagnose CHIKV in early stage and to take appropriate measures to control its sudden outbreak around the world. It is high time to develop a diagnostic tool for easy and early detection of CHIKV which can differentiate it from other arbo viral disease so that appropriate management strategies can be developed at an early stage to reduce its life threatening complication reported in the recent outbreak. An antigen based assay using both Elisa and ICT can play a significant role in this regard; especially ICT is more suitable for economically weak countries where outbreaks usually occur. These tests can be done on screening purpose. To confirm the infection more specific test is needed and promising role of RT LAMP in this context is considerable as it doesn't need expensive instrument and detection is easy which makes it an excellent tool for early detection of CHIKV infection even in the peripheral settings.

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