

## Brief Communication



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### Corresponding Author:

Eun-Jee Oh, MD, PhD

Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222, Banpo-daero, Seocho-gu, Seoul 06591, Korea.

Tel: +82-2-2258-1641

Fax: +82-2-2258-1719

E-mail: ejoh@catholic.ac.kr

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### ORCID iDs

Hyeyoung Lee

<https://orcid.org/0000-0001-8871-5091>

Ji Hyeong Ryu

<https://orcid.org/0000-0002-7223-4012>

Sojeong Yun

<https://orcid.org/0000-0002-7784-9673>

Joo Hee Jang

<https://orcid.org/0000-0001-9209-6844>

Ae-Ran Choi

<https://orcid.org/0000-0001-6150-1800>

Sung-Yeon Cho

<https://orcid.org/0000-0001-5392-3405>

Chulmin Park

<https://orcid.org/0000-0001-9147-0478>

# Evaluation of a Newly Developed Rapid Automated Fluorescent Lateral Flow Immunoassay to Detect IgG and IgM Antibodies to Chikungunya Virus

Hyeyoung Lee <sup>1</sup>, Ji Hyeong Ryu <sup>2</sup>, Sojeong Yun <sup>3</sup>, Joo Hee Jang <sup>3</sup>, Ae-Ran Choi <sup>2</sup>, Sung-Yeon Cho <sup>4,5</sup>, Chulmin Park <sup>5</sup>, Dong-Gun Lee <sup>4,5</sup>, and Eun-Jee Oh <sup>2</sup>

<sup>1</sup>Department of Laboratory Medicine, Catholic Kwandong University International St. Mary's Hospital, Incheon, Korea

<sup>2</sup>Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

<sup>3</sup>Department of Biomedicine & Health, Graduated School, The Catholic University of Korea, Seoul, Korea

<sup>4</sup>Division of Infectious Diseases, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea

<sup>5</sup>Vaccine Bio Research Institute, College of Medicine, The Catholic University of Korea, Seoul, Korea

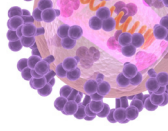
## ABSTRACT

The ichroma Chikungunya virus (CHIKV) IgG/IgM (Boditech Med Inc., Chuncheon, Korea) is a newly developed rapid lateral flow immunoassay for detection of anti-CHIKV-IgG/IgM. This study conducted with thirty-six anti-CHIKV IgG positive sera, 57 anti-CHIKV IgM positive sera and 163 anti-CHIKV IgG/IgM negative sera which were confirmed by commercial enzyme-linked immunosorbent assays (ELISAs) (Inbios CHIKjj Detect™ IgM Capture ELISA, Inbios CHIKjj Detect™ IgG ELISA (InBios International Inc., Seattle, WA, USA), Anti-CHIKV ELISA (IgM), Anti-CHIKV ELISA (IgG) (Euroimmun, Lübeck, Germany)). The ichroma detected all 36 anti-CHIKV IgG and 57 anti-CHIKV IgM positivity (100% sensitivity). For 163 anti-CHIKV IgG/IgM negative sera, the ichroma showed one false positive for IgM (99.4% specificity). The ichroma showed no cross-reactivity and no interference. The ichroma demonstrated good diagnostic performance compared to the current ELISAs.

**Keywords:** Chikungunya; Antibody; Infection; Lateral flow immunoassay; Diagnostic performance

Chikungunya virus (CHIKV) is a mosquito-borne virus belongs to the alphavirus genus of the family *Togaviridae* [1]. The virus was first isolated in 1952 in southern Tanzania [2] and restricted small outbreaks were occurred in Africa and Southeast Asia [3]. Since early 2000s, the virus transmitted in India and Southeast Asia. In 2013, a local transmission of virus on Caribbean island was reported and, to date, more than 20 countries are infected [4].

The CHIKV is primarily transmitted by female mosquitoes, *Aedes aegypti* and *Aedes albopictus* [3]. Unlike dengue fever, in which only humans or primates serve as reservoirs, non-primates such as rodents, birds and small mammals can act as reservoirs [5]. And several other mosquito vectors, including the *Aedes furcifer-taylori* group and *Aedes luteocephalus*, were involved in disease transmission in Africa [5]. Therefore, it is considered to be one of the diseases with high risk of



Dong-Gun Lee   
<https://orcid.org/0000-0003-4655-0641>  
Eun-Jee Oh   
<https://orcid.org/0000-0001-5870-915X>

#### Conflict of Interest

DGL is editor-in-chief of *Infect Chemother*; however, he did not involve in the peer reviewer selection, evaluation, and decision process of this article. Otherwise, no potential conflicts of interest relevant to this article was reported.

#### Author Contributions

Conceptualization: EJO. Data curation: SYC. Funding acquisition: DGL. Investigation: JHR, ARC, CP. Methodology: SY, JHJ. Project administration: DGL. Resources: SYC. Validation: EJO, HL. Writing - original draft: EJO, HL. Writing - review & editing: EJO, HL.

temporary epidemic and enculturation when introduced [6]. Total 23 cases of CHIKV infection were officially reported in Korea from 2013 to 2018, and all cases were from infected travelers [6].

Clinical manifestation of CHIKV infection is characterized by acute onset of fever, intense asthenia and arthralgia, affecting bilateral small joints of extremities [3]. Clinical course is usually mild and self-limited [7]. But as symptoms are not specific and difficult to distinguish from other similar viral infection (such as dengue and Zika virus), the diagnosis cannot be solely confirmed on clinical findings and laboratory diagnosis is essential [8].

Laboratory diagnosis of CHIKV infection includes virus isolation, reverse-transcription polymerase chain reaction (RT-PCR), or serologic assays [9]. Serologic confirmation requires either demonstration of IgM antibody or fourfold rise in IgG antibodies between acute and convalescent phase [3]. RT-PCR is most sensitive assay for early phase infection, but limited due to short viremia. For sera collected after 5 days of onset, serodiagnosis is more accurate indicator of disease [8]. Serum IgM detection is available from 5 days to several months after symptom onset and IgG antibodies are detected after 4 - 10 days after symptom onset and persisted for years [10]. Serologic detection is important since there may be a certain proportion of patients visiting the hospital when RT-PCR is no longer effective for diagnosis [11].

Point-of-care (POC) assays are now available for detecting anti-CHIKV antibodies and recommended to facilitate outbreak control [8]. Especially, travelers returning from endemic areas changes the epidemiology of CHIKV infection nowadays [10]. Prompt diagnosis and surveillance is essential for disease transmission. Therefore, implementation of easy-to-use, rapid and accurate rapid diagnostic test (RDT) is required to improve patient management and infection control. But previously reported sensitivities of RDTs for anti-CHIKV IgM detection were poor as from 20.0% to 50.8% [11-14].

The ichroma CHIKV IgG/IgM (Boditech Med Inc., Chuncheon, Korea) is a newly developed automated fluorescence immunochromatography based lateral flow assay using a small bench-top fluorescence reader for detection of anti-CHIKV IgG and IgM. The assay could detect antibodies from serum, plasma and whole blood. We evaluated the diagnostic performance of the ichroma CHIKV IgG/IgM in comparison with commercial enzyme-linked immunosorbent assay (ELISA) tests.

The test cartridge of the ichroma was coated with dried fluorescence conjugated recombinant CHIKV specific antigens and monoclonal anti-chicken IgY for detecting target antibodies and internal control, respectively. According to the manufacturer's instruction, 30  $\mu$ L of serum or plasma sample was mixed with 75  $\mu$ L of a sample mixture and dispensed into the cartridge, and then incubated at room temperature for 12 minutes. Target antibodies present in the sample would react with antigen and form antigen-antibody complex. Antigen-antibody complexes migrate onto nitrocellulose matrix, are captured by the antigen immobilized on test line, and form a sandwich complex. The fluorescence labeled anti-chicken IgY binds with chicken IgY fixed in control line. Fluorescence intensities of the target and control were detected using a portable reader (ichroma™ II, Boditech Med Inc., Korea) by inducing a laser. As there is no international reference serum exists for anti-CHIKV, the calibration was performed with commercial positive samples including sample at the cut-off ratio. Internal calibrators traceable to the commercial CHIKV positive plasma were used for in-house calibration. The result interpreted as positive when the cut-off index  $\geq 1.1$ , negative for cut-off index less than 0.9, and indeterminate between 0.9 and 1.1 ( $\geq 0.9$ ,  $< 1.1$ ) for both IgG and IgM.

**Table 1.** Characteristics of tests evaluated in this study

Company	Assay principle	Product name	Analyte	Sample type	Sample volume (µL)	Incubation time (minutes)
Inbios	ELISA	Inbios CHIKjj Detect™ IgM Capture ELISA	IgM	S	10	100
		Inbios CHIKjj Detect™ IgG ELISA	IgG	S	10	100
Euroimmun	ELISA	Anti-Chikungunya virus ELISA (IgM)	IgM	S, P	10	135
		Anti-Chikungunya virus ELISA (IgG)	IgG	S, P	10	135
Boditech Med	Lateral flow	ichroma CHIKV IgG/IgM	IgM, IgG	S, P, W	30	12

ELISA, enzyme-linked immunosorbent assay; S,serum; P,plasma; W,whole blood.

Confirmatory and comparative studies were performed with two ELISA kits (1) Inbios CHIKjj Detect™ IgM Capture ELISA, Inbios CHIKjj Detect™ IgG ELISA (InBios International Inc., Seattle, WA, USA), (2) Anti- CHIKV ELISA (IgM), Anti- CHIKV ELISA (IgG) (Euroimmun, Lübeck, Germany). The characteristics of the test are summarized in **Table 1**. Assays were performed according to the manufacturer's instructions. Reference positivity was defined when the both ELISAs give positive results.

Thirty-six anti-CHIKV IgG positive sera and 57 anti-CHIKV IgM positive sera from patients with confirmed CHIKV infection (purchased from Trina Bioreactives AG, Zurich, Switzerland) and 163 anti-CHIKV IgG/ IgM negative sera from healthy Korean individuals were included. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC19DDSE0737). Informed consent was waived as this study was performed using purchased of leftover samples. Cross-reactivity with other viral infections were tested with serum samples with antibodies against hepatitis A virus (HAV), hepatitis B virus (HBV) hepatitis C virus (HCV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), zika virus and dengue virus. Cross-reactivity for dengue virus was tested with serotype 2 and serotype 4. For interference tests, sera from icterus (bilirubin <500 µmol/L), hemolysis (Hb <0.12 mmol/L), lipemia (triglyceride <1.69 mmol/L, cholesterol <20 mmol/L), and anticoagulants (EDTA, heparin, and sodium citrate) were tested. Statistical analyses were done with MedCalc Statistical Software version 19.1 (MedCalc Software bv, Ostend, Belgium). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated based on ELISA results.

None of the HAV, HBV, HCV, EBV, CMV, zika virus and dengue virus positive serum showed cross-reactivity with the ichroma anti-CHIKV IgM. None of the HAV, HBV, HCV, EBV and CMV positive samples showed positivity with the ichroma anti-CHIKV IgG. There was no interference from icteric, hemolytic or lipemic serum samples, albumin and anticoagulants.

All positive sera (36 anti-CHIKV IgG positive sera and 57 anti-CHIKV IgM positive sera) from patients with CHIKV infection were positive with both Euroimmun and Inbios ELISAs. Overall, the ichroma detected all 36 anti-CHIKV IgG positive and 57 anti-CHIKV IgM (100.0% sensitivities) (**Table 2**). For 163 anti-CHIKV IgG/IgM negative sera, the ichroma showed false positive for anti-CHIKV IgM in one sample (99.4% [95% confidence interval (CI) 97.5 - 99.4] specificity). The index value (1.3) of a sample with false positive showed near the cut off index for positivity (1.1).

**Table 2.** Diagnostic performances of the ichroma CHIKV IgG/IgM compared with commercial ELISAs

Analyte	TP	FP	FN	TN	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)
CHIKV-IgG	36	0	0	163	100 (92.4 - 100.0)	100 (98.3 - 100.0)	100 (92.4 - 100.0)	100 (98.3 - 100.0)
CHIKV-IgM	57	1	0	162	100 (94.7 - 100.0)	99.4 (97.5 - 99.4)	98.3 (93.1 - 98.3)	100 (98.1 - 100.0)

CHIKV, Chikungunya virus; TP, true positive; FP, false positive; FN, false negative; TN, true negative; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

For IgG antibody detection, the correlation coefficient ( $\gamma$ ) of optimal density (OD) value of ichroma showed 0.847 (95% CI 0.802 - 0.882,  $P < 0.001$ ) with Inbios and 0.955 (0.941 - 0.966,  $P < 0.001$ ) with Euroimmun IgG ELISA. For IgM, the OD value of ichroma showed  $\gamma = 0.651$  (0.568 - 0.721,  $P < 0.001$ ) with Inbios and 0.706 (0.633 - 0.767,  $P < 0.001$ ) with Euroimmun IgM ELISA. The median index ratio of the ichroma for positive IgG was 2.2 (95% CI 2.1 - 2.5) and positive IgM was 7.5 (6.7 - 9.5).

In previous study, overall accuracy of the InBios IgG ELISA was 91.7% with 92.8% sensitivity and 90.9% specificity compared with Centers for Disease Control and Prevention (CDC) reference result [15]. The Euroimmun IgG ELISA showed 88.8% of accuracy with 100% sensitivity and 81.8% specificity [15]. For IgM detection, Euroimmun ELISA showed 94.0 - 100.0% sensitivity and 96.0 - 100.0% specificity with 95.0-100.0% accuracy compared with RT-PCR or CDC in-house MAC-ELISA [16]. Inbios ELISA showed 100% sensitivity and 93.0 - 100.0% specificity with 98.0 - 100.0% accuracy compared with RT-PCR or CDC in-house MAC-ELISA [16]. However, RDT from CTK Biotech (San Diego, CA, USA) showed sensitivity of 20.0 - 37.5% and specificity of 92.3 - 100.0% in comparison to in-house capture ELISA or RT-PCR [8, 12]. RDT from SD Bioline (Standard Diagnostics Inc., Yongin-si, Gyeonggi-do, Korea) reported sensitivities of 30.0 - 50.8% and specificities of 69.2 - 89.2% [11-13]. The specificity of RDTs was comparable with commercial ELISA, but the sensitivity still need improvement.

This study has some limitations. Due to limited resources and difficulties in obtaining, positive sera for other alphaviruses (O'nyong'nyong virus, Mayaro viruses, Venezuelan equine encephalitis virus and eastern equine encephalitis virus) were not included in evaluation. It has been reported that the CHIKV-E2 antigen has a high level of (more than 50%) amino acid sequence identity with other alpha viruses (O'nyong'nyong virus, Semliki Forest virus, Ross River virus, Mayaro virus) [17]. In previous study, sera from patients infected with Mayaro virus or O'nyong'nyong virus showed cross-reactivity with commercial ELISA [11]. This assay was developed for use with serum, plasma and whole blood. But we used only serum samples because collecting large quantities of fresh whole blood samples from patients was not feasible. In addition, the onset time of the disease for purchased positive specimen was unknown, therefore, we could not compare the diagnostic performance of assay between acute and convalescent phase. Previous study reported about 29.9% of the CHIKV positive specimens were positive for dengue virus antibodies. It was suspected due to coinfection or cross-reactivity of ELISA kit [18]. But we did not tested for dengue virus co-infection in this study.

Nevertheless, the ichroma showed comparable results with ELISA on the confirmed positive samples. Assay has advantages including short turnaround time, easy to use and cost effectiveness. It could be used as a screening tool for prompt diagnosis of CHIKV infection and early infection control.

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