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CHIKUNGUNYA VIRUS INFECTION EVALUATION USING RAPID DIAGNOSTIC TESTS (RDTS) BASED ON ANTI-CHIKUNGUNYA IGM/IGG

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ABSTRACT

Chikungunya virus (CHIKV) is a single-stranded RNA alphavirus. Recently, CHIKV began an unprecedented global expansion. Thus, it is necessary to develop commercial rapid diagnostic kits for CHIKV infection. We assessed the utility of the commercially available blood tests approved by the rapid diagnostic tests (RDTs) of GenBody Company (Korea) for the detection of CHIKV. In addition, to detect the presence of viral genomic RNA in human samples, RT-PCR of CHIKV was done. RT-PCR of CHIKV was shown the typical banding profile of the rapid diagnostic test for 30 cases of CHIKV negative sera with primers targeting a 557-bp region of the E2 gene. 29 sera were distinct bands (96.6% sensitivity). In this study, the 470 samples were submitted to the Focus Diagnostics Reference Laboratory for CHIKV RNA and/or CHIKV antibody (IgG and IgM). Sensitivity of the GenBody Company RDT was 87.5% (105/120) for IgG and its specificity was 98.8% (316/320). 40 samples were IgM-antibody positive. Sensitivity of the GenBody Company RDT was 74.1% (40/54) for IgM and its specificity was 97.5% (312/320). Most IgM-positive sera were also IgG positive (145/174 = 83.3%). 16.7% were positive for IgM-antibody but negative for ELISA/RT-PCR. It needs to make a new analysis kit such as RNA composition for the detection of anti-chikungunya IgM.

KEYWORDS: Chikungunya fever (CHIKV), IgG, IgM, rapid diagnostic tests (RDTs), RT-PCR of CHIKV.

INTRODUCTION

Chikungunya virus (CHIKV) belongs to the genus *Alphaviruses* and an emerging mosquito-borne alphavirus. CHIKV is an enveloped virus with a single-stranded positive-sense RNA genomic alphavirus.[1] Although primarily African and zoonotic, it is known chiefly for its non-African large urban outbreaks during which it is transmitted by the same vectors as those of Dengue viruses.[2] CHIKV causes a major public health problem. Recently, CHIKV began an unprecedented global expansion and has been responsible for epidemics in Africa, Asia, islands in the Indian Ocean region, and surprisingly, in temperate regions, such as Europe.[3]

It is mainly caused by two types of mosquitos: *Aedes albopictus* and *Aedes aegypti*. Other species potentially able to transmit the chikungunya virus include *Ae. furcifer-taylori*, *Ae. africanus*, and *Ae. Luteocephalus*.[4] The symptoms of chikungunya are similar to those of dengue and Zika, diseases spread by the same mosquitoes that transmit chikungunya.[5] Most people infected with CHIKV have a fever that may be accompanied by joint pain or swelling in multiple joints. Although most symptoms resolve, some patients have joint pain that can continue for years and can be so severe that they adopt a bent or stooping posture.[6]

Laboratory diagnosis of CHIKV infection is accomplished by serologic methods, virus isolation, and viral RNA detection by reverse transcription–polymerase chain reaction (RT-PCR).[7] An acute onset of fever and severe arthralgia or arthritis that is not explained by other medical disorders is considered as a possible CHIKV case.[4] RT-PCR using nested primer pairs is used to amplify several chikungunya-specific genes from whole blood, confirming of the diagnosis in the acute phase of illness.[8]

Indirect immunofluorescence and ELISA are rapid and sensitive techniques for detection of an immune response to chikungunya and can distinguish between IgG and IgM antibodies. A specific IgM antibody response is usually detectable between 2 days and 7 days after onset of fever with ELISA and immunofluorescence, although it has been reported as early as day 1 with a lateral flow rapid test.[9] Similarly, an IgG antibody response has

been reported as early as day 2 after onset, although it is more frequently detected from days 5 to 6.[10]

The increasing threat of CHIKV emergence in temperate regions and the need to anticipate possible outbreaks of CHIKV infection are presenting a challenge to the current level of diagnostic preparedness.[11]

Enzyme-linked immunosorbent assay (ELISA), real-time PCR (RT-PCR), and virus isolation can be performed to arrive at a definitive diagnosis or to clarify the immune response, but these methods are not widely performed in hospitals because they require specialist equipment and laboratory skills.[12] Virus isolation and nucleic acid detection are more accurate than antigen detection, but these tests are not widely available due to their greater cost. In the present study, we assessed the utility of the commercially available blood tests approved by the rapid diagnostic tests (RDTs) of GenBody Company (Korea) for the detection of chikungunya virus. Namely, we conducted the usefulness of antibody (IgM and IgG) detection by ELISA. Our evaluation was a pilot study using a small number of samples, but the findings show the importance of evaluating commercial diagnostic kits and published protocols before using such tools in clinical settings.

MATERIALS AND METHODS

Clinical specimens and clinical evaluation of RDT

The study population was composed of 770 patients for whom the history of CHIKV infection was recorded, as previously described. Samples were collected from Wama Laboratories of San carlosin Brasil and D. University in Korea. To detect the presence of viral genomic RNA in human samples, RT-PCR of CHIKV was done. Total RNA was extracted directly from the viral culture supernatant (140µL) and sera (140µL) of CHIKV-suspected patients. RNA was extracted using the QIAamp viral RNA minikit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. The Quanti-Tect SYBR green RT-PCR kit (Qiagen) was used for quantitative RT-PCR (qRT-PCR) with the primers CHIKV-E1-F (CTCATACCGCATCCGCATCAG) and CHIKV-E1-R (ACATTGGCCCCACAATGATATTG) for CHIKV samples from Brazil and Korea.[12] In addition, RT-PCR was performed with primers targeting a 557-bp region of the E2 gene, namely, CHIK_F1 (GAAACTCTGACCGGTGGGATTCAC) and CHIK_R2 (GAGTGTTGGGTGGTCAGGATACAG) for CHIKV, as described previously.[13] This fragment was cloned in pET21b expression vector that added a His tag at the N terminus. E2 protein that was expressed in *E. coli* BL21 (DE3) strain and purified with Ni nTA affinity chromatography. The purified rHis E2 protein was characterized by SDS - PAGE and western blotting using an anti-His monoclonal antibody.[14]

The 470 samples were submitted to the Focus Diagnostics Reference Laboratory for CHIKV RNA and/or CHIKV antibody (IgG and IgM) testing between 5 Febuary and 25 September 2014. Assays were performed according to the manufacturers' instructions. In brief, 5 µL of whole blood or serum sample was transfered by pipette into the sample well of the freshly unpackaged test device. 100 µL reaction buffer was added to reaction fields of a reagent tray. At this time, anti-human IgG coated on Test 1 line is reacted and bound to anti-human IgM coated on Test 2 line. Recombinant CHIKV E2 protein attached to colloidal gold particles binds to two lines and develops color. When IgG antibody of goat, which is a common antibody, is reacted on the control line, all of the test line 3 lines are positive if the mucus moves along the nitrocellulose membrane by the immunochromatographic principle and Chikungunya IgM/IgG is all positive. When only the test 1 line is developed, only IgG is positive. When the test 2 line is developed, only IgM is positive. The reason for distinguishing IgM from IgG is that IgM represents a previous infection of Chikungunya, IgG represents Chikungunyaemia, and IgM/IgG represents about 15 days after infection with Chikungunya. The appearance of the test and control lines after a specified migration time (15-20 minutes) indicated a positive result. For each RDT involving the interpretation of the presence of a line, two people read the results independently and concurred on a given call.

Statistical analysis

The sensitivity, specificity, efficiency, positive predictive value (PPV), and negative predictive value (NPV) for the assays were calculated based on true positive chikungunya samples (virus isolation/PCR positives, sero-negative acute sera, acute primary, acute secondary).

Statistical analysis was performed with Statistica version 18 (StatSoft, Inc., Tulsa, OK).[15] Significance was assigned at $p<0.05$ for all parameters and were two-sided unless otherwise indicated. Uncertainty was expressed by 95% confidence intervals. Categorical variables between groups were compared by Fisher's exact test. The t-test was used for continuous variables.

RESULTS

For analytical specificity for the interfering substances testing, relevant performance characteristics were summarized in Table 1. Positive serum and plasma were none. Smearing and/or negative interferences due to each material test were not observed. Figure 1 was the pattern of expression in *E. coli*. The E2 protein of the Chikv virus was successfully cloned and characterized as a 37KD protein.

The characteristics of the study population (n = 814 cases) that contributed acute plasma to the test panel is shown in Table 1. Thee panel of dengue cases (n = 170) were consecutively enrolled. A total of 770 prospective serum samples submitted for chikungunya virus (CHIKV) IgM and IgG testing by the Focus Diagnostics CHIKV IgM and IgG EIAs were also tested by the IgM and IgG CHIKV assays (Figure 2).

Figure 3 was shown the typical banding profile of the rapid diagnostic test for 30 cases of CHIKV positive sera with primers targeting a 557-bp region of the E2 gene. The rE1 was showed no reactive to 30 cases of CHIKV patient's sera. The rE2 was showed 83.3% (25/30) sensitivity in IB to 30 cases of CHIKV patient's sera with distinct bands (triangular allow). Five sera (No. 4, No. 7, No. 25, No. 27, and No. 29) were absent the band. Figure 4 was shown the typical banding profile of the rapid diagnostic test for 30 cases of CHIKV negative sera with primers targeting a 557-bp region of the E2 gene. 29 sera were not a distinct band and only one (sera: No. 2) has the unique band. Thus the specificity of rE2 was 96.6%.

The appearance of the control line alone indicated a negative result. The results were compared and the data summarized in Table 1. 105 samples were IgG-antibody positive. Sensitivity of the GenBody Company RDT was 87.5% (105/120) for IgG and its specificity was 98.8% (316/320) (Table 2). 40 samples were IgM-antibody positive. Sensitivity of the GenBody Company RDT was 74.1% (40/54) for IgM and its specificity was 97.5% (312/320). Most IgM-positive sera were also IgG positive (145/174=83.3%). Very few samples (<3.7%) were positive for IgG-antibody but negative for ELISA/RT-PCR. Whereas, some samples (<16.7%) were positive for IgM-antibody but negative for ELISA/RT-PCR. The specificity of IgM and IgG tests alone was not significantly different among test samples. However, the sensitivity of their tests alone was significantly different among test samples.

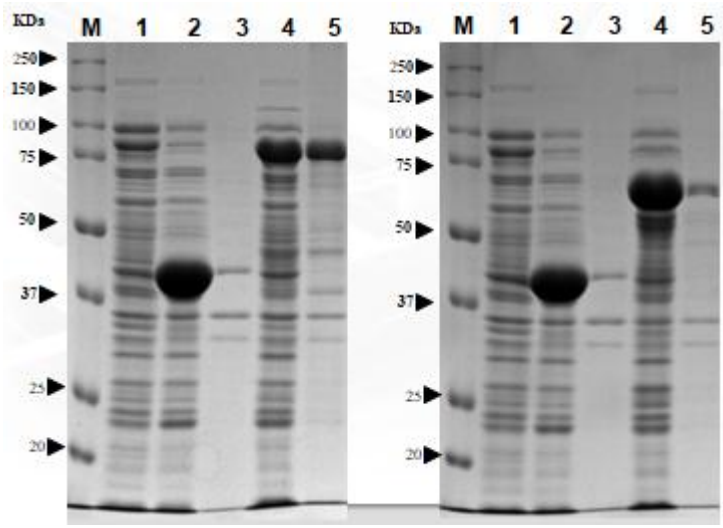

Figure 1: Expression in *E. coli*. Left is expression using pET21a and right is pET21a pMAL-c5x.

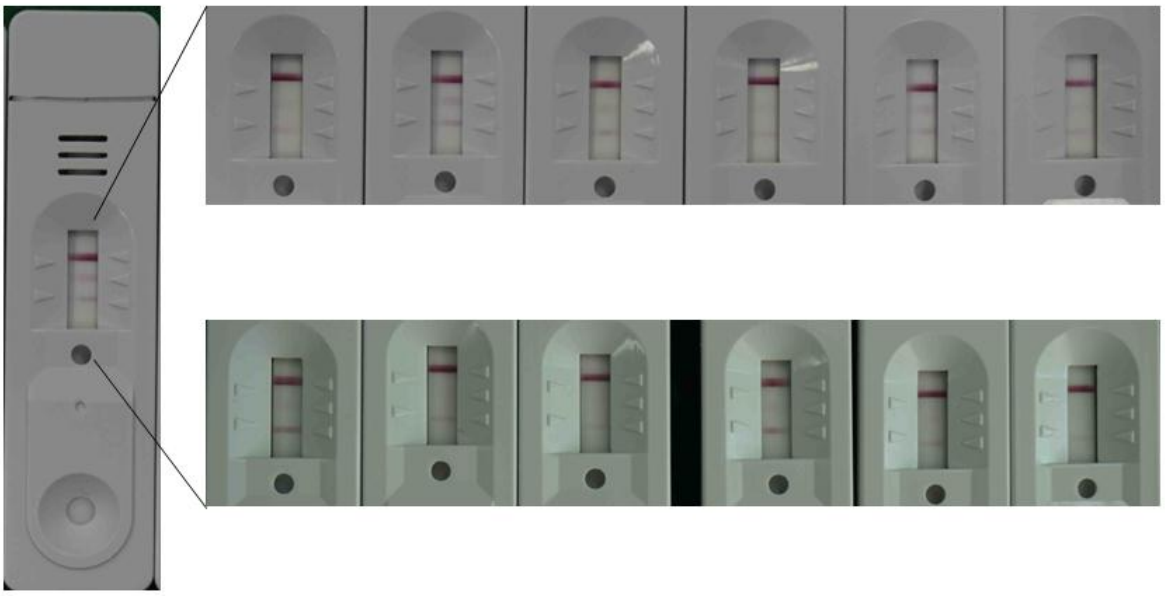

Figure 2: The EasyTest showed Chikungunya IgG/IgM rapid strip in a plastic cassette.

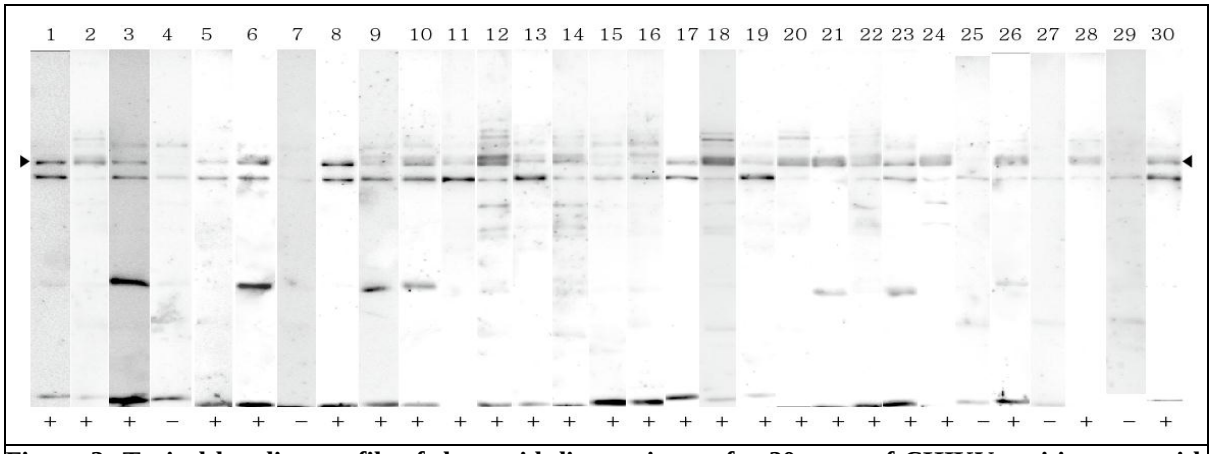

Figure 3: Typical banding profile of the rapid diagnostic test for 30 cases of CHIKV positive sera with primers targeting a 557-bp region of the E2 gene.

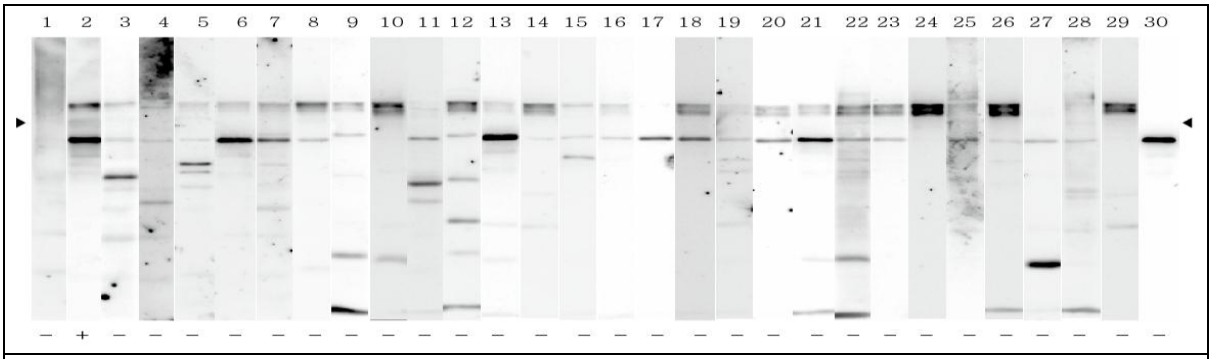

Figure 4: Typical banding profile of the rapid diagnostic test for 30 cases of CHIKV negative sera with primers targeting a 557-bp region of the E2 gene.

Table 1: Analytical specificity for interfering substances testing.

Compound	Concentration	Only positive sample		Positive sample + Material		Negative sample + Material	
		S	P	S	P	S	P
K_2EDTA	540 mg/dL	+w	+w	+w	+w	-	-
Citrate	327 mg/dL	+w	+w	+w	+w	-	-
Heparin	3 KU/dL	+w	+w	+w	+w	-	-
Hemoglobin	200 mg/dL	+w	+w	+w	+w	-	-
Cholesterol	500 mg/dL	+w	+w	+w	+w	-	-
Albumin	14.7 g/dL	+w	+w	+w	+w	-	-
Bilirubin	25 mg/dL	+w	+w	+w	+w	-	-

S: serum P: Plasma +w: weak -: No signal.

Table 2: The evaluation of chikungunya diagnosis using the CHIKV IgG/IgM rapid test kits in this study.

CHIKV IgG/IgM (N=470)		ELISA/RT-PCR			
		Positive		Negative	
		IgG	IgM	IgG	IgM
Positive	IgG	105		4	
	IgM		40		8
Negative	IgG	15		316	
	IgM		14		312
Total		120	54	320	320

Table 3: Clinical evaluation of sensitivity and specificity using the chikungunya IgG/IgM rapid test kits.

RDTs	Positive	Sensitivity (%)	Negative	Specificity (%)
IgG	105/120	87.5	316/320	98.8
IgM	40/54	74.1	312/320	97.5

DISCUSSION

16.7% were positive for IgM-antibody on CHIKV but negative for ELISA/RT-PCR (Table 1). It need to make a new analysis kit for the detection of anti-chikungunya IgM. Of course, an IgM-positive, IgG-negative patient, with a 63% chance of being viremic, is more likely to transmit the infection if bitten by a mosquito than an IgM-positive patient with an IgG titer of 1:280, who has only a 15% chance of being viremic.[16] Since CHIKV RNA positive (viremic) patients are the source of CHIKV transmission to another individual via transfer by a mosquito bite.[17]

These time-related increases in the numbers of samples submitted for CHIKV RNA and/or antibody testing and in the proportion of submitted samples positive for CHIKV RNA and IgM reflect the timeline for increasing numbers of suspected CHIKV infections among residents of the Caribbean basin.[18-19]

Enzyme-linked immunosorbent assays (ELISAs) and indirect immunofluorescence assays (IFAs) are the most frequently used serological tests for the diagnosis of CHIKV infection. The most common ELISAs used are the IgM antibody-capture ELISA (MAC-ELISA) and the indirect ELISA (i-ELISA) for the detection of IgM and IgG immunoglobulin, respectively.[20]

As demonstrated in the studies summarized above, neither the SD BIOLINE Chikungunya IgM nor the OnSite Chikungunya IgM Combo Rapid Test demonstrated good sensitivity, ranging from 0 to 50.8% and 12.1% to 37.5%, respectively. The results of GenBody Company product in this study had greater overall sensitivity than SD BIOLINE (Table 1). The specificity of each of these tests was better, ranging from 71% to 95% for the SD BIOLINE and from 93% to 100% for the OnSite Chikungunya rapid test. In the evaluation by Johnson et al, the performance was considered so poor that additional testing was not done to consider specificity or accuracy.[3] In a study of the SD BIOLINE rapid test, Rianthavorn et al found overall sensitivity and specificity to be 37% and 85%, respectively.[21] However, the sensitivity of the assay increased significantly when tested on patients having symptoms for more than seven days, rising to 83% from 22% for patients having symptoms for less than one week. The specificity of the assay declined, however, from 88% to 71%.[14] Immunoglobulin M (IgM) antibodies elicited in the immune response are normally detectable in serum by days 5-7 after onset of illness.[22] Thus accuracy evaluations and precision of CHIKV infections were relative to the determined time periods.

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