BMC Infectious Diseases

RESEARCH

Open Access

Facile quantitative diagnostic testing for neutralizing antibodies against Chikungunya virus

Hui-Chung Lin[1,2†], Shu-Fen Chang[3†], Chien-Ling Su[3], Huai-Chin Hu[3], Der-Jiang Chiao[1], Yu-Lin Hsu[1], Hsuan-ying Lu[1], Chang-Chi Lin[1,2], Pei-Yun Shu[3*] and Szu-Cheng Kuo[1,2*]

Abstract

Background Viral neutralization (NT) assays can be used to determine the immune status of patients or assess the potency of candidate vaccines or therapeutic monoclonal antibodies (mAbs). Focus reduction neutralization test (FRNT) is a conventional neutralization test (cVNT) with superior specificity for measurement of neutralizing antibodies against a specific virus. Unfortunately, the application of FRNT to the chikungunya virus (CHIKV) involves a highly pathogenic bio-agent requiring biosafety level 3 (BSL3) facilities, which inevitably imposes high costs and limits accessibility. In this study, we evaluated a safe surrogate virus neutralization test (sVNT) that uses novel CHIKV replicon particles (VRPs) expressing eGFP and luciferase (Luc) to enable the rapid detection and quantification of neutralizing activity in clinical human serum samples.

Methods This unmatched case-control validation study used serum samples from laboratory-confirmed cases of CHIKV ($n=19$), dengue virus (DENV; $n=9$), Japanese encephalitis virus (JEV; $n=5$), and normal individuals ($n=20$). We evaluated the effectiveness of sVNT, based on mosquito cell-derived CHIK VRPs (mos-CHIK VRPs), in detecting (eGFP) and quantifying (Luc) neutralizing activity, considering specificity, sensitivity, and reproducibility. We conducted correlation analysis between the proposed rapid method (20 h) versus FRNT assay (72 h). We also investigated the correlation between sVNT and FRNT in NT titrations in terms of Pearson's correlation coefficient (r) and sigmoidal curve fitting.

Results In NT screening assays, sVNT-eGFP screening achieved sensitivity and specificity of 100%. In quantitative neutralization assays, we observed a Pearson's correlation coefficient of 0.83 for NT50 values between sVNT-Luc and FRNT.

Conclusions Facile VRP-based sVNT within 24 h proved highly reliable in the identification and quantification of neutralizing activity against CHIKV in clinical serum samples.

†Hui-Chung Lin, Shu-Fen Chang contributed equally as first authors.

*Correspondence:
Pei-Yun Shu
bilitislin@gmail.com
Szu-Cheng Kuo
szucheng1234@gmail.com

Full list of author information is available at the end of the article

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Keywords Chikungunya virus, Virus-like replicon particle, Neutralizing antibodies, Surrogate virus neutralization test, Surveillance, Diagnostics, Vaccine

Background

The transmission of chikungunya virus (CHIKV) by infected Aedes mosquitoes [1] results in periodic outbreaks of a febrile disease characterized in humans by fever, rash, myalgia, and severe arthralgia [2]. Due to similarities in clinical manifestation, CHIKV is commonly misdiagnosed as dengue fever, particularly in regions where both diseases are co-endemic [3]. There are at present no approved antiviral therapies for CHIKV. The effective management of CHIKV infection depends on the ability to obtain an accurate diagnosis as early as possible.

Nucleic acid detection can only be used during the brief viremia phase (~7 days). Enzyme-linked immunosorbent assay (ELISA) is a serological test applicable to the detection of CHIKV-specific IgM antibodies after roughly 5 days of illness and the detection of CHIKV-specific IgG antibodies in later stages [4, 5]. Note however that ELISA results must be confirmed via NT assay to determine the immune status of the patient [6, 7]. Neutralization assays are highly practical in assessing the potency of candidate vaccines [8, 9] and therapeutic mAbs [10]. Unfortunately, applying the conventional neutralization test (cVNT) to CHIKV detection involves a highly pathogenic bio-agent requiring biosafety level 3 (BSL3) facilities, which inevitably imposes low throughput and long turnaround times [11]. The development of sVNTs from mammalian cell-derived VRP [12–18] and pseudotyped viruses [19–24] has lowered the safety margin (BSL2) and reduced turnaround times (6–72 h) for the measurement of neutralizing antibodies (nAbs).

There is a pressing need for a reliable and versatile CHIKV NT assay to estimate the seroprevalence [25, 26] and subclinical infection rates [27], identify associated diseases [28], assess humoral protective immunity in convalescent patients and vaccine candidates [24, 29], and search for potential reservoir hosts [30]. Researchers have recently generated a mosquito cell-derived CHIK VRP (mos-CHIK VRP) as an alternative to rapid nAbs detection [31]. In the current study, we evaluated the efficacy of mos-CHIK VRP VNT (20 h) in the detection (via eGFP) and quantification (via Luc) of neutralization activity (Fig. 1). We then compared our analysis results with those obtained using standard FRNT. In NT screening assays ($n=52$), the sensitivity and specificity of sVNT-eGFP screening were both 100%. In 19 serum samples from CHIKV patients, we observed a strong correlation between the quantitative NT50 titers of sVNT-Luc and corresponding titers obtained from FRNT50.

Methods

Cells and viruses

Sf21 cells (Gibco™ Sf21 cells) were grown at 28 °C in Sf-900 II insect medium containing 10% heat-inactivated fetal bovine serum (FBS) (Gibco) and 1% antibiotic-antimycotic (Gibco). AP-61 cells (kindly provide by Dr. Cheng-Chen Chen) were cultured at 28 °C in Leibovitz L-15 medium (Gibco) with 10% FBS and 1% antibiotic-antimycotic. C6/36 cells (ATCC® CRL-1660™) were cultured in RPMI 1640 medium (Gibco) with 10% FBS and 1% penicillin/streptomycin (Gibco) at 28 °C under 5% CO_2. Vero cells (BCRC, no.60013) were cultured under 5% CO_2 at 37 °C in minimum essential media (MEM) (Gibco) containing 10% FBS, 1% GlutaMAX (Gibco), 1% MEM NEAA (Gibco), 1% sodium pyruvate (Gibco), and 1% penicillin/streptomycin (Gibco). Baculovirus propagation and viral titrations were performed using Sf21 cells. CHIKV (CHIKV/ECSA/Malaysia/2008) propagation and viral titrations were performed using C6/36 and Vero cells, respectively.

Transfer vector and recombinant baculovirus

The modified two-in-one transfer vector (without VSVG) was constructed by initially subcloning a 9.5-kb PCR fragment of CHIKV replicon-GFP from the pFastBac1-VSVG-CHIKV replicon-GFP [32] as a template. This subcloning was accomplished using a pair of primers (forward: 5'--CGCGGATCCCGGTCCGAA -GCGCGC -ACTCAAATCCTGCGCGATC −3'; reverse: 5'- AAG CTTGGTACCGCATGC-CTCGAG-GTACCGCATGC TGTTTAAAC −3') inserted into the *BssH*II (*Pte*I) and *Xho*I sites of the pFastBac1 vector (Invitrogen, Carlsbad, CA, USA), which resulted in the creation of a plasmid, designated as pFastBac1-CHIKV replicon-GFP. We subsequently subcloned a 4.4-kb PCR fragment of hr1pag1-CHIKV 26 S (originating from pFastBac1-VSVG-CHIKV replicon-GFP-hr1pag1-CHIKV 26 S [31]) into pFastBac1-CHIKV replicon-GFP using *Pme*I. This was achieved with the aid of a pair of primers (forward: 5'- CTAAGGG AGGGCGGTTTGTGTTTTACAAGTAGAATTCTACC −3'; reverse: 5'-AGGTACCGCATGCTGTTTAAACTT AGTGCCTGCTGAACGACAC-3') using the NEBuilder HiFi DNA assembly system. The resulting plasmid is referred to as pFastBac1-CHIKV replicon-GFP-hr1pag1-CHIKV 26 S. We then replaced the eGFP gene in pFastBac1-CHIKV replicon-GFP-hr1pag1-CHIKV 26 S with eGFP-T2A-Luc in accordance with a published protocol [31]. Recombinant baculovirus was generated using the Bac-to-Bac expression system (Invitrogen, Carlsbad, CA, USA) in accordance with the standard protocol.

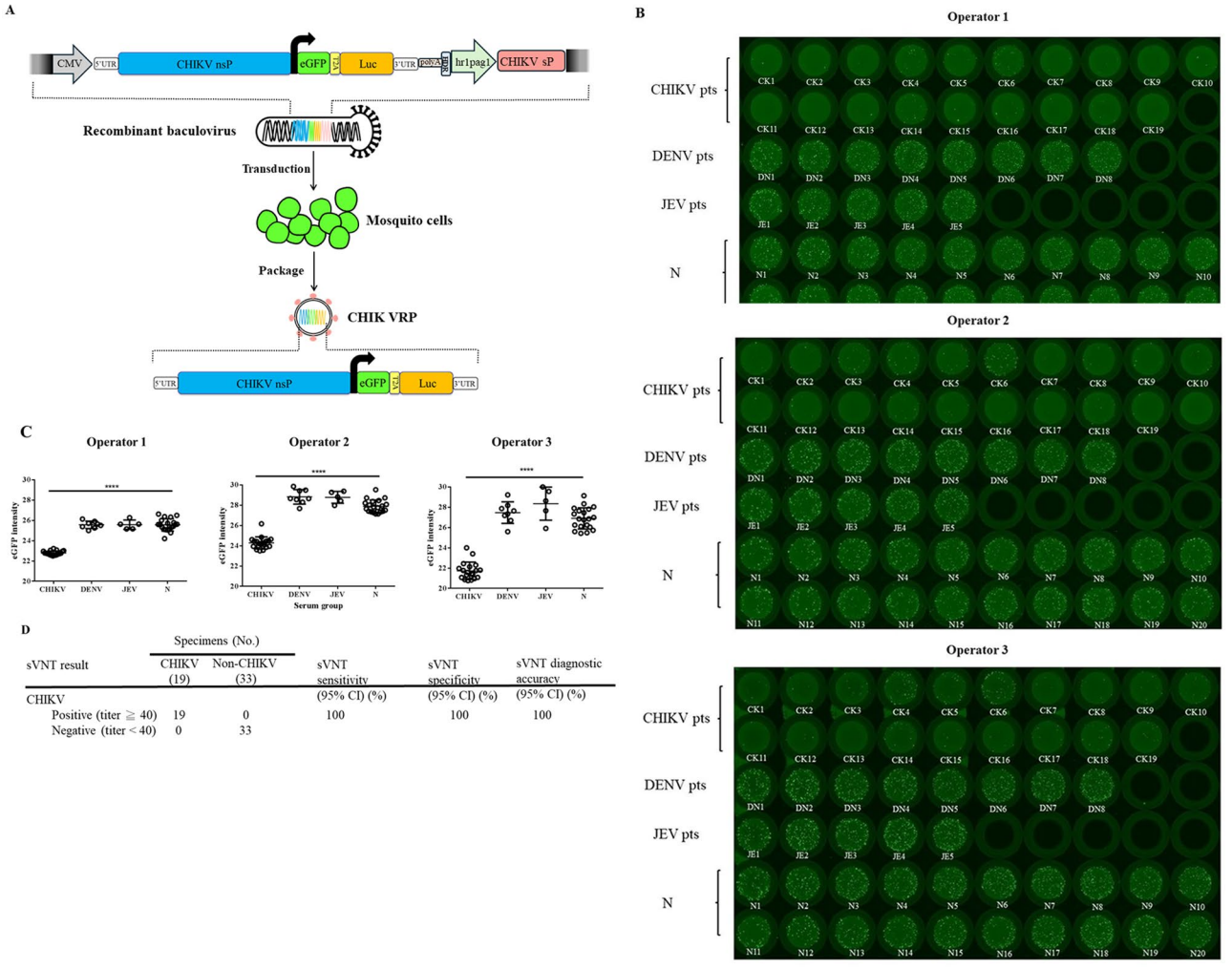


Fig. 1 Qualitative NT evaluation. (**A**) A schematic diagram of VRP production is illustrated. A recombinant baculovirus was designed to carry two DNA cassettes: one containing the CHIKV replicon with dual reporters—enhanced green fluorescent protein (eGFP) and luciferase (Luc)—under the control of a cytomegalovirus (CMV) promoter, and another encoding the CHIKV structural proteins (sP) driven by a mosquito-specific hr1pag1 promoter. Upon transduction, the recombinant baculovirus introduces these DNA cassettes into mosquito cells, enabling the expression of all necessary components for VRP packaging. CHIK VRP contains a defective genome with dual reporters (eGFP and Luc) under the control of subgenomic RNA promoter, where nsP refers to nonstructural proteins, T2A refers to Thosea asigna virus 2 A self-cleaving peptides, and UTR indicates the untranslated region of the CHIKV genome; (**B**) Reproducibility assessment of mos-CHIK VRP-based sVNT-eGFP screening results obtained by three operators (1-3) on 52 serum samples from CHIKV patients (n =19), DENV patients (n =8), JEV patients (n =5), and 20 normal individuals (N). Sample numbers (corresponding eGFP intensities) are indicated beneath each well in the left panel; (**C**) Statistical analysis comparing eGFP intensity data from (Fig. 1B) between CHIKV patients and unrelated groups. The significance levels are ($P < 0.0001$), with a significance level set at $p < 0.0001$ and (****) denoting extreme significance; (**D**) Overall performance of sVNT-eGFP screening

VRP production and titration

VRP production (Fig. 1A) and titration were performed using a modified 2-in-1 recombinant baculovirus (with VSVG deletion) as described in [31]. In brief, AP-61 cells were transduced with recombinant baculovirus at a multiplicity of infection (MOI) of 20. The culture supernatant containing VRPs was harvested between days 7 and 14 post-transduction. The supernatant was centrifuged, filtered using a 0.22-μm filter, aliquoted, and stored at -80 °C. Vero cells (1.5×10^4 cells per well) were seeded in a 96-well plate and incubated overnight. The VRP stock was serially diluted ten-fold (from 1:10 to 1:1,000) in MEM medium. Vero cells were then infected with the diluted VRP mixture and incubated at 28 °C for 1 h. After infection, the medium was replaced, and the cells were incubated for 20 h at 28 °C under 5% CO_2. The number of eGFP-positive cells was counted to calculate the VRP titer (infectious units, IU).

Ethics statement

This study on clinical human serum samples was reviewed and approved by the Institutional Review Board

(IRB 109105) under the auspices of the Taiwan Center for Disease Control.

Patient details

Assessments were performed on 52 clinical serum samples collected from a hospital-based reporting system and a fever screening program at an airport. The status of all samples was confirmed by the Center for Disease Control in Taiwan. Informed consent waivers were obtained for the use of all samples. The specimens were categorized as follows: positive for CHIKV ($n=19$), positive for DENV ($n=8$), positive for JEV ($n=5$), and normal control ($n=20$). Note that positive detections were confirmed in-house via capture IgM and IgG ELISA and/or real-time RT-PCR [33, 34].

VRP screening for NT activity

Vero cells (1.5×10^4 cells / well) were seeded in a 96-well plate and incubated overnight. VRPs were pre-incubated with heat-inactivated serum samples (1:40 dilution with MEM) at 37 °C for 1 h. Cells were infected with mixtures of sera and VRP (500 IU/well) and then incubated at 28 °C for 1 h, whereupon the medium was refreshed to continue incubation at 28 °C for another 20 h. Images were then captured using a Sapphire biomolecular imager (Azure Biosystems). The eGFP intensity was quantified using Image J software (version 1.53e). To extract the specific green signal, we adjusted the color threshold to a hue tone range of 79 to 90. Quantification was performed in accordance with the instructions in Image J documentation: Image → Adjust → Color Threshold → Measure. The level of eGFP intensity was calculated as the product of the area and mean fluorescence in the readings. Statistical analysis was conducted to compare the eGFP intensity data between the group of CHIKV patients and unrelated groups, including DENV patients, JEV patients, and normal individuals. A two-tailed, unpaired t-test was used to determine the presence of statistically significant differences, with a significance level set at $p<0.0001$ and (****) denoting extreme significance.

VRP quantitative NT assay

VRP-based quantitative NT assays were performed in accordance with the methods outlined in [31] with slight modifications, including a decrease in the infectious component (2,500 to 500 IU/well), an increase in incubation time (5 to 20 h), and a decrease in incubation temperature (34 to 28 °C). In summary, Vero cells (1.5×10^4 cells per well) were seeded in a 96-well plate and incubated overnight. Equal volumes of VRPs (500 IU/well) were pre-incubated with serial two-fold dilutions of patient sera (ranging from 1:100 to 1:12,800) or CHK265 mAb (ranging from 0.078 to 10 μg/ml) as a positive control in triplicate at 37 °C for 1 h. The Vero cells were then infected with the VRP/serum mixture at 28 °C for 1 h. After infection, the medium was replaced, and the cells were incubated for 20 h at 28 °C under 5% CO_2, followed by a luciferase assay.

Focus reduction neutralization tests (FRNT)

Vero cells (8×10^4 cells/well) were seeded in a 12-well plate and incubated at 37 °C for 48 h. Heat-inactivated human sera were serially diluted and then incubated with 100 PFU of CHIKV (CHIKV/ECSA/Malaysia/2008) in an incubator under CO_2 at 37 °C for 1 h. Mixtures of sera and virus were transferred to a monolayer of Vero cells for infection. After infection at 37 °C for 1 h, the cells were overlaid with 1% (wt/vol) methylcellulose in 10% FBS and 1× MEM, and then incubated at 37 °C for an additional 36 ~ 48 h. The cells were fixed using 10% formalin and permeabilized with 0.1% Triton-100 in PBS (PBST). Staining involved incubating the cells in PBS with primary anti-CHIKV E2 monoclonal antibodies (diluted at 2.5μg/ml), followed by biotinylated anti-Ig secondary antibodies with antibody diluent at a ratio of 1:50 prior to the addition of streptavidin-HRP solution. Focus-forming units (brown color) were developed by treating the cells with DAB chromogen (BD Pharmingen) at room temperature for 30 min. FRNT50 (50% focus reduction neutralization titer) values were obtained by identifying the highest serum dilution that exhibited a reduction of 50% or more in the number of virus plaques, compared to the values of the negative control.

Statistical analysis

The correlation between sVNT50 and FRNT50 titers was examined using Pearson's correlation coefficient (r) and sigmoidal curve fitting. NT50, the half-maximal inhibitory concentration (or dilution), was calculated using nonlinear regression analysis. Data were analyzed using GraphPad Prism 6.01 software.

Results

Qualitative NT evaluation

sVNT-eGFP neutralization activity was assessed qualitatively by screening serum samples from patients with CHIKV ($n=9$), DENV ($n=8$), or JEV ($n=5$) as well as normal patients ($n=20$) at a serum dilution fold of 1:40. As shown in Fig. 1B, we observed a blockade of VRP infection in the sera from CHIKV patients, as evidenced by the absence of eGFP expression; however, no inhibitory effects were observed in the unrelated sera. sVNT-eGFP achieved sensitivity of 100% and specificity of 100%.

As shown in Fig. 1C, subsequent statistical analysis revealed a substantial disparity in eGFP intensities among the various groups, with a striking divergence between the CHIKV patients and all other groups. This robust differentiation reaffirms the reliability and effectiveness

of the sVNT-eGFP assay in distinguishing CHIKV infection. The reproducibility of the sVNT-eGFP assay was rigorously evaluated across three different operators with varying levels of laboratory experience. The aim of this meticulous evaluation was to determine whether operator bias played a role in the results. Encouragingly, the consistent outcomes across these operators underscored the robustness and operator-independence of the sVNT-eGFP technique. As shown in Fig. 1D, these results collectively confirm the exceptional sensitivity and specificity of the sVNT-eGFP method, marking it as a valuable tool for CHIKV neutralization testing.

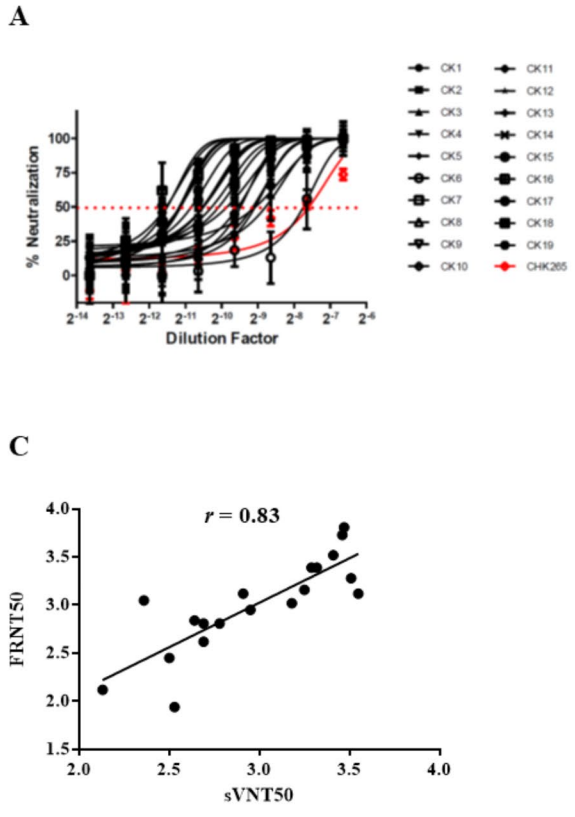
Quantitative NT evaluation

Neutralization performance was also evaluated quantitatively by deliberately selecting serum samples from 19 CHIKV-infected patients with well-established neutralizing antibody (nAb) titers, as determined using the sVNT50 (Luc) method (Fig. 2A). This panel was used to perform meticulous comparative and correlational analysis using the conventional FRNT50 method. Figure 2B lists the NT50 values obtained using sVNT-Luc and FRNT. As shown in Fig. 2C, we obtained a Pearson correlation coefficient of 0.83 for NT50 values obtained using sVNT-Luc vs. FRNT. We also observed a strong correlation between the results obtained using sVNT50 vs. FRNT50.

Our findings support that mos-CHIK VRP sVNT, as shown in Fig. 3, is a reliable tool for the rapid screening and quantification of CHIKV-neutralizing activity in clinical serum samples.

Discussion

Viral neutralizing antibodies are a valuable marker for determining the immune status of patients and assessing the potency of candidate vaccines or therapeutic mAbs [35]. At present, IgG and NT detection are the only available methods to confirm suspected chronic cases of CHIKV and survey the seroprevalence of CHIKV infection. Moreover, commercial CHIKV IgG detection assays have a high false-positive rate (12.5 – 22%) [36]. IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) is currently the preferred approach to the serological diagnosis of CHIKV infection; however,



B

Sample No. (ID)	sVNT50(Log)	FRNT50(Log)
CK1 (62022)	2.95	2.95
CK2 (62156)	2.78	2.81
CK3 (62174)	2.64	2.84
CK4 (62261)	2.36	3.05
CK5 (62280)	2.50	2.45
CK6 (62349)	2.13	2.12
CK7 (62410)	3.55	3.12
CK8 (62609)	2.69	2.81
CK9 (62660)	3.25	3.16
CK10 (62769)	2.69	2.62
CK11 (62908)	3.51	3.28
CK12 (S10801300)	3.46	3.73
CK13 (62335)	3.29	3.39
CK14 (62509)	2.53	1.94
CK15 (62718)	3.18	3.02
CK16 (62729)	3.41	3.52
CK17 (62972)	3.32	3.39
CK18 (63030)	2.91	3.12
CK19 (S10802992)	3.47	3.81

Fig. 2 Quantitative NT evaluation results (A) Titration curves of neutralizing antibodies for the sera of 19 CHIKV patients. CHK265 mAb serves as a positive control. Data represent means ± standard deviation (triplicate in 1 experiment). (B) Mean neutralizing titers from the sera of 19 CHIKV patients, as determined by mos-CHIK VRP-based sVNT50 (sVNT-Luc) and FRNT50; (C) Performance of sVNT-Luc based on the correlation between sVNT50 and FRNT50 values for the sera of 19 CHIKV patients

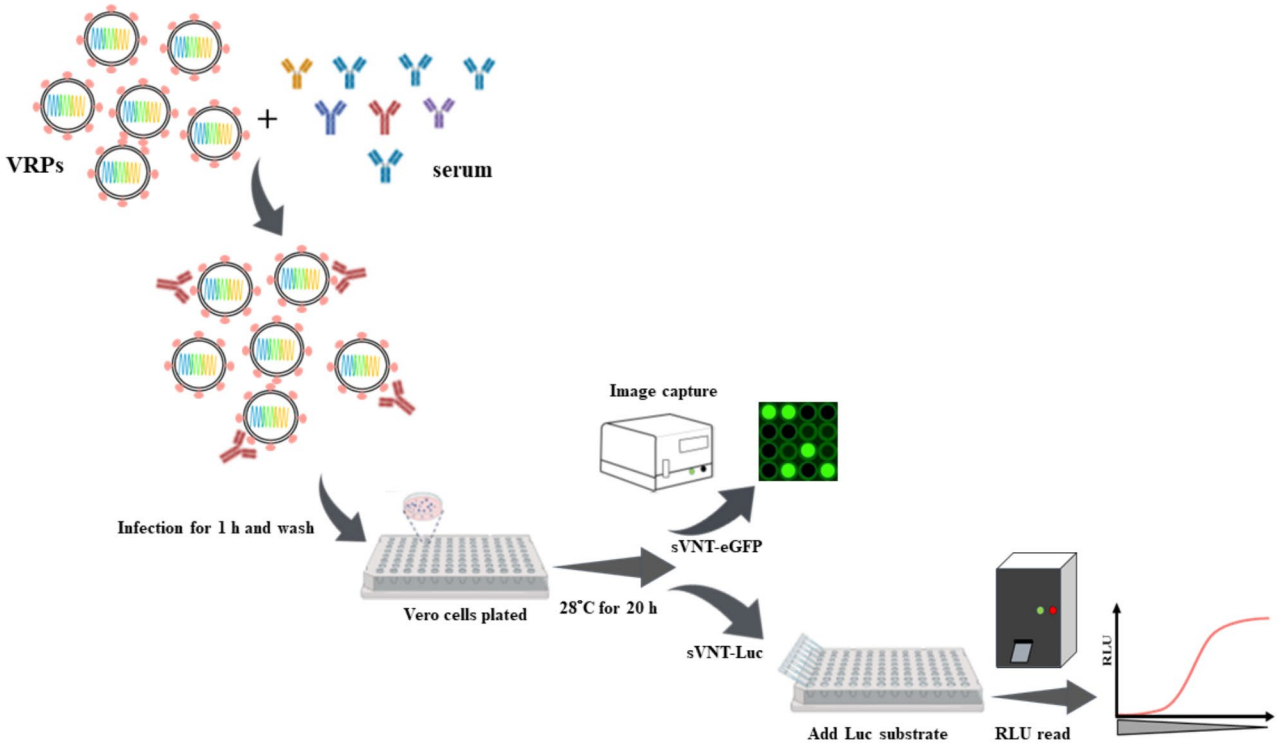


Fig. 3 Flowchart showing the application of mos-CHIK VRP-based sVNT as a tool for the rapid measurement of CHIKV NT activity. After pre-incubating VRPs with test serum for 1 h, Vero cells are infected with the pre-incubated VRPs for 1 h and washed. After incubation at 28 °C for 20 h, the cells are subjected to either sVNT-eGFP to qualify NT activity by inhibiting eGFP expression or sVNT-luc to quantify NT activity by inhibiting luciferase activity

positive detections require further confirmation via cVNT [4], which is arduous and time-consuming, particularly in low-resource settings. There is a pressing need for advanced sVNTs to facilitate the assessment of vaccine efficacy and improve the accuracy and capacity of diagnostic methods for CHIKV infection. The mos-CHIK VRPs engineered with dual reporter expression (eGPF and Luc) in the current study are close-to-perfect mimics of CHIKV in terms of viral structure and function, but with genomic deviations. This enables VRP-infected cells to express the reporters of eGPF and Luc without conferring the ability to produce virus progeny, thereby allowing the high-throughput measurement of nAbs within 20 h [31]. sVNT-eGFP neutralization activity presented excellent specificity and sensitivity in unmatched case-control validation tests (Fig. 1D). Importantly, the reproducibility of sVNT-eGFP remained consistent across operators with various degrees of laboratory experience (see Fig. 1B and C). These findings underscore the efficacy of sVNT-eGFP as an operator-independent technique to obtain results of high reliability. The ability to confirm the infectious status of ELISA-positive sera within just 20 h makes sVNT-eGFP as a valuable diagnostic tool for efficient, high-throughput screening, even in extensive human sero-epidemiology investigations. The results in Fig. 2C reveal a strong correlation (r=0.83) between sVNT50 and the gold standard FRNT50 in human serum samples. It should be possible to further reduce the turnaround time of sVNT-Luc by increasing the MOI (from 500 to 2,500 IU VRPs/well), potentially reducing NT quantification time from 20 to 5 h [31]. The high fluorescent background of Vero cells (Fig. 1C) limits the sensitivity and specificity of eGFP intensity, making it challenging to accurately distinguish between positive and negative signals. As a result, measuring NT50 using the eGFP intensity becomes difficult, especially under low VRP input conditions (500 IU/well). In contrast, luciferase, which exhibits low background noise in cell lysates, provides a broad and linear dynamic range [37], allowing for more sensitive NT50 quantitation. A comparison of surrogate neutralization assays (Table 1) revealed that all surrogate NT tests, including those using pseudotyped virus and VRPs, involve luciferase reporters for quantitative assessment. However, the mos-CHIK VRP expressing an eGFP reporter provides an image-based assay for real-time NT screening. The eGFP-based NT assay is cost-effective as it does not require expensive reagents. Moreover, the mos-CHIK VRP sVNT also has high throughput potential to screen eGFP signal under a high-content setting. Large quantities of mosquito cell-derived VRPs are easily obtained from baculovirus-transduced mosquito cells, whereas the transfection procedures in producing

Table 1 Comparison of surrogate neutralization assays for CHIKV

sVNT	Reporter (s)	Quantitative NT assay	Image-based NT screening	Incubation time (hours)	Reference
Pseudotype virus	Luciferase	yes	no	48	19–21, 23–24
	ZsGreen1 and Luciferase	yes	yes	72	22
Mammalian cells derived-VRP	Gaussia luciferase	yes	no	6–24	12–18
Mosquito cells derived -VRP	eGFP and Luciferase	yes	yes	5–20	31 and this study

mammalian cell-derived CHIKV VRPs or pseudotyped lentivirus is labor-intensive and technically challenging.

In summary, the proposed versatile mos-CHIK VRP sVNT (Fig. 3) could be used for the direct screening of seroprevalence, the development of therapeutic NT mAb, the monitoring of neutralizing titers after mass vaccination, and the assessment of vaccine efficacy in preclinical and clinical trials.

Conclusions

The findings in this study are summarized as follows:

- Virus neutralization assays are essential to assessing specific antibodies against CHIKV.
- The mos-CHIK VRP-based sVNT-eGFP enables CHIKV neutralization screening with excellent specificity and sensitivity.
- The operator-independence of the mos-CHIK VRP-based sVNT-eGFP technique ensures highly reliable (reproducible) results.
- There is a strong correlation ($r = 0.83$) between the quantitative NT50 titers obtained from mos-CHIK VRP-based sVNT-Luc and those obtained using the conventional FRNT assay.
- The proposed mos-CHIK VRPs provides a safe and efficient means of detecting and quantifying neutralizing antibodies associated with CHIKV infection.

Abbreviations

BSL3	Biosafety level 3
CHIKV	Chikungunya virus
cVNT	Conventional neutralization test
ELISA	Enzyme-linked immunosorbent assay
FRNT	Focus reduction neutralization test
IU	Infectious unit
Luc	Luciferase
mAb	Monoclonal antibody
MOI	Multiplicity of infection
mos-CHIK VRP	Mosquito cell-derived CHIK VRP
nAb	Neutralizing antibody
NT	Neutralization
NT50	50% reduction neutralization titer
sVNT	Surrogate virus neutralization test
VRP	Virus replicon particle

Acknowledgements

We are grateful to Dr. Cheng-Chen Chen (from National Yang-Ming Chiao-Tung University) for providing the AP-61 cell line.

Author contributions

HCL: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation. SFC: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology. CLS: Methodology. HCH: Methodology. DJC: Methodology, Investigation. YLH: Methodology, Investigation. HYL: Methodology, Investigation. CCL: Project administration, Supervision. PYS: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing.SCK: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing.

Funding

Financial support was received from the NHRI (NHRI-11A1-MRCO-08222201 and NHRI-12A1-MRCO-08232301 to SCK), MOST (NSCT 111-2327-B-016-002 and NSTC 113-2327-B-016-001 to SCK), and Medical Affairs Bureau (MND-MAB-D-111081, MND-MAB-D-112079, 111-P-12 and 112-G4-4 to SCK) (IPM-110-G7-4 and 111-G4-2 to HCL) of Taiwan.

Data availability

Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate

This study on clinical human serum samples was reviewed and approved by the Institutional Review Board (IRB 109105) under the auspices of the Taiwan Center for Disease Control. Patient consent was waived due to dis-linkage of all data from patients.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Clinical trial number

not applicable.

Author details

[1]Institute of Preventive Medicine, National Defense Medical Center, 237010 No. 172, Dapu Rd., Sanxia Dist, Taipei 11490, Taiwan
[2]Department and Graduate Institute of Microbiology and Immunology, National Defense Medical Center, Taipei 11490, Taiwan
[3]Center for Diagnostics and Vaccine Development, Centers for Disease Control, Ministry of Health and Welfare, Taipei 11561, Taiwan

Received: 23 July 2024 / Accepted: 20 September 2024
Published online: 30 September 2024

References

1. Bartholomeeusen K, Daniel M, LaBeaud DA, Gasque P, Peeling RW, Stephenson KE, Ng LFP, Ariën KK. Chikungunya fever. Nat Rev Dis Primers. 2023;9(1):17.

2. Silva LA, Dermody TS. Chikungunya virus: epidemiology, replication, disease mechanisms, and prospective intervention strategies. J Clin Invest. 2017;127(3):737–49.
3. Velasco JM, Valderama MT, Lopez MN, Chua D Jr., Latog R 2nd, Roque V Jr., Corpuz J, Klungthong C, Rodpradit P, Hussem K, et al. Chikungunya Virus infections among patients with Dengue-Like illness at a Tertiary Care Hospital in the Philippines, 2012–2013. Am J Trop Med Hyg. 2015;93(6):1318–24.
4. Johnson BW, Russell BJ, Goodman CH. Laboratory diagnosis of Chikungunya Virus infections and Commercial sources for diagnostic assays. J Infect Dis. 2016;214(suppl 5):S471–4.
5. Panning M, Grywna K, van Esbroeck M, Emmerich P, Drosten C. Chikungunya fever in travelers returning to Europe from the Indian Ocean region, 2006. Emerg Infect Dis. 2008;14(3):416–22.
6. Piantadosi A, Kanjilal S. Diagnostic Approach for Arboviral Infections in the United States. J Clin Microbiol 2020, 58(12).
7. Allen SW, Ribeiro Dos Santos G, Paul KK, Paul R, Rahman MZ, Alam MS, Rahman M, Al-Amin HM, Vanhomwegen J, Weaver SC et al. Results of a nationally representative seroprevalence survey of Chikungunya virus in Bangladesh. J Infect Dis 2024.
8. Schneider M, Narciso-Abraham M, Hadl S, McMahon R, Toepfer S, Fuchs U, Hochreiter R, Bitzer A, Kosulin K, Larcher-Senn J, et al. Safety and immunogenicity of a single-shot live-attenuated chikungunya vaccine: a double-blind, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet. 2023;401(10394):2138–47.
9. Milligan GN, Schnierle BS, McAuley AJ, Beasley DWC. Defining a correlate of protection for Chikungunya virus vaccines. Vaccine. 2019;37(50):7427–36.
10. Smith SA, Silva LA, Fox JM, Flyak AI, Kose N, Sapparapu G, Khomandiak S, Ashbrook AW, Kahle KM, Fong RH, et al. Isolation and characterization of Broad and Ultrapotent Human Monoclonal Antibodies with therapeutic activity against Chikungunya Virus. Cell Host Microbe. 2015;18(1):86–95.
11. Azami NA, Moi ML, Takasaki T. Neutralization assay for Chikungunya Virus infection: Plaque reduction neutralization test. Methods Mol Biol. 2016;1426:273–82.
12. Gläsker S, Lulla A, Lulla V, Couderc T, Drexler JF, Liljeström P, Lecuit M, Drosten C, Merits A, Kümmerer BM. Virus replicon particle based Chikungunya virus neutralization assay using Gaussia luciferase as readout. Virol J. 2013;10:235.
13. Roques P, Ljungberg K, Kümmerer BM, Gosse L, Dereuddre-Bosquet N, Tchitchek N, Hallengärd D, García-Arriaza J, Meinke A, Esteban M, et al. Attenuated and vectored vaccines protect nonhuman primates against Chikungunya virus. JCI Insight. 2017;2(6):e83527.
14. Mugabe VA, Ali S, Chelene I, Monteiro VO, Guiliche O, Muianga AF, Mula F, António V, Chongo I, Oludele J, et al. Evidence for chikungunya and dengue transmission in Quelimane, Mozambique: results from an investigation of a potential outbreak of Chikungunya virus. PLoS ONE. 2018;13(2):e0192110.
15. Hallengärd D, Kakoulidou M, Lulla A, Kümmerer BM, Johansson DX, Mutso M, Lulla V, Fazakerley JK, Roques P, Le Grand R, et al. Novel attenuated Chikungunya vaccine candidates elicit protective immunity in C57BL/6 mice. J Virol. 2014;88(5):2858–66.
16. García-Arriaza J, Cepeda V, Hallengärd D, Sorzano C, Kümmerer BM, Liljeström P, Esteban M. A novel poxvirus-based vaccine, MVA-CHIKV, is highly immunogenic and protects mice against chikungunya infection. J Virol. 2014;88(6):3527–47.
17. López-Camacho C, Kim YC, Blight J, Lazaro Moreli M, Montoya-Diaz E, Huiskonen JT, Kümmerer BM, Reyes-Sandoval A. Assessment of Immunogenicity and Neutralisation Efficacy of viral-vectored vaccines against Chikungunya Virus. Viruses 2019, 11(4).
18. Szurgot I, Ljungberg K, Kümmerer BM, Liljeström P. Infectious RNA vaccine protects mice against Chikungunya virus infection. Sci Rep. 2020;10(1):21076.
19. Chung WC, Hwang KY, Kang SJ, Kim JO, Song MJ. Development of a neutralization assay based on the pseudotyped Chikungunya virus of a Korean isolate. J Microbiol. 2020;58(1):46–53.
20. Weber C, König R, Niedrig M, Emmerich P, Schnierle BS. A neutralization assay for Chikungunya virus infections in a multiplex format. J Virol Methods. 2014;201:7–12.
21. Tsai SK, Hsu YL, Chiao DJ, Shu PY, Lin HT, Chang SF, Lin HC, Huang SH, Wang CH, Hsiung CC, et al. Antigenicity and immunogenicity of Chikungunya virus-like particles from mosquito cells. Appl Microbiol Biotechnol. 2023;107(1):219–32.
22. Su C, Ding K, Xu J, Wu J, Liu J, Shen J, Zhou H, Liu H. Preparation and application of chikungunya pseudovirus containing double reporter genes. Sci Rep. 2022;12(1):9844.
23. Henss L, Yue C, Kandler J, Faddy HM, Simmons G, Panning M, Lewis-Ximenez LL, Baylis SA, Schnierle BS. Establishment of an Alphavirus-Specific neutralization assay to Distinguish infections with different members of the Semliki Forest complex. Viruses 2019, 11(1).
24. Henss L, Yue C, Von Rhein C, Tschismarov R, Lewis-Ximenez LL, Dölle A, Baylis SA, Schnierle BS. Analysis of Humoral Immune responses in Chikungunya Virus (CHIKV)-Infected patients and individuals vaccinated with a candidate CHIKV Vaccine. J Infect Dis. 2020;221(10):1713–23.
25. Ayu SM, Lai LR, Chan YF, Hatim A, Hairi NN, Ayob A, Sam IC. Seroprevalence survey of Chikungunya virus in Bagan Panchor, Malaysia. Am J Trop Med Hyg. 2010;83(6):1245–8.
26. Ang LW, Kam YW, Lin C, Krishnan PU, Tay J, Ng LC, James L, Lee VJM, Goh KT, Ng LFP, et al. Seroprevalence of antibodies against Chikungunya virus in Singapore resident adult population. PLoS Negl Trop Dis. 2017;11(12):e0006163.
27. Yoon IK, Alera MT, Lago CB, Tac-An IA, Villa D, Fernandez S, Thaisomboonsuk B, Klungthong C, Levy JW, Velasco JM, et al. High rate of subclinical Chikungunya virus infection and association of neutralizing antibody with protection in a prospective cohort in the Philippines. PLoS Negl Trop Dis. 2015;9(5):e0003764.
28. Barr KL, Khan E, Farooqi JQ, Imtiaz K, Prakoso D, Malik F, Lednicky JA, Long MT. Evidence of Chikungunya Virus Disease in Pakistan since 2015 with patients demonstrating involvement of the Central Nervous System. Front Public Health. 2018;6:186.
29. Ramsauer K, Schwameis M, Firbas C, Müllner M, Putnak RJ, Thomas SJ, Desprès P, Tauber E, Jilma B, Tangy F. Immunogenicity, safety, and tolerability of a recombinant measles-virus-based Chikungunya vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial. Lancet Infect Dis. 2015;15(5):519–27.
30. Bosco-Lauth AM, Nemeth NM, Kohler DJ, Bowen RA. Viremia in north American mammals and birds after experimental infection with Chikungunya viruses. Am J Trop Med Hyg. 2016;94(3):504–6.
31. Lin HC, Chiao DJ, Shu PY, Lin HT, Hsiung CC, Lin CC, Kuo SC. Development of a Novel Chikungunya Virus-Like Replicon Particle for Rapid Quantification and Screening of Neutralizing Antibodies and antivirals. Microbiol Spectr. 2023;11(2):e0485422.
32. Lin HC, Chiao DJ, Lin CC, Kuo SC. Facile method for delivering chikungunya viral replicons into mosquitoes and mammalian cells. Sci Rep. 2021;11(1):12321.
33. Shu PY, Yang CF, Su CL, Chen CY, Chang SF, Tsai KH, Cheng CH, Huang JH. Two imported chikungunya cases, Taiwan. Emerg Infect Dis. 2008;14(8):1326–7.
34. Shu PY, Chang SF, Kuo YC, Yueh YY, Chien LJ, Sue CL, Lin TH, Huang JH. Development of group- and serotype-specific one-step SYBR green I-based real-time reverse transcription-PCR assay for dengue virus. J Clin Microbiol. 2003;41(6):2408–16.
35. Klasse PJ. Neutralization of Virus Infectivity by Antibodies: Old Problems in New Perspectives. *Adv Biol* 2014, 2014.
36. De Salazar PM, Valadere AM, Goodman CH, Johnson BW. Evaluation of three commercially-available Chikungunya virus immunoglobulin G immunoassays. Rev Panam Salud Publica. 2017;41:e62.
37. Honda T, Gomi S, Yamane D, Yasui F, Yamamoto T, Munakata T, Itoh Y, Ogasawara K, Sanada T, Yamaji K et al. Development and Characterization of a Highly Sensitive NanoLuciferase-Based Immunoprecipitation System for the Detection of Anti-Influenza Virus HA Antibodies. *mSphere* 2021, 6(3).

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.