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RESEARCH ARTICLE

Analytical and Clinical Evaluation of a TaqMan Real-Time PCR Assay for the Detection of Chikungunya Virus

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ABSTRACT Due to the general symptoms presented by the Chikungunya virus (CHIKV)-infected patients, a laboratory test is needed to differentiate CHIKV from other viral infections. The reverse transcription-quantitative real-time PCR (RT-qPCR) is a rapid and sensitive diagnostic tool, and several assays have been developed for detecting and quantifying CHIKV. Since real-time amplification efficiency varies within and between laboratories, an assay must be validated before being used on patient samples. In this study, the diagnostic performance of a TaqMan RT-qPCR assay was evaluated using synthetic RNA and archived patient samples. The cutoff quantification cycle (C_q) value for the assay was determined by experimental evidence. We found the in-house assay was highly sensitive, with a detection limit of 3.95 RNA copies/reaction. The analytical specificity of the assay was 100%. The analytical cutoff C_q value was 37, corresponding to the mean C_q value of the detection limit. Using archived samples characterized previously, the sensitivity and specificity of the assay were 76% and 100%, respectively. The in-house assay was also compared with a commercial assay, and we found that the in-house assay had higher sensitivity. Although further evaluation with prospective patient samples is needed in the future, this validated RT-qPCR was sensitive and specific, which shows its potential to detect CHIKV in clinical samples.

IMPORTANCE Chikungunya virus causes chikungunya fever, a disease characterized by fever, rash, and joint pain. In the early phase of infection, chikungunya fever is always misdiagnosed as other arbovirus infections, such as dengue. Laboratory tests such as RT-qPCR are therefore necessary to confirm CHIKV infection. We evaluated the performance of an in-house RT-qPCR assay, and our study shows that the assay could detect CHIKV in clinical samples. We also show the cutoff determination of the assay, which provides important guidance to scientists or researchers when implementing a new RT-qPCR assay in a laboratory.

KEYWORDS Chikungunya virus, reverse transcription-quantitative real-time PCR, in-house assay, cutoff quantification cycle

Chikungunya virus (CHIKV) is an enveloped virus that belongs to the family *Togaviridae* and the genus *Alphavirus*. The CHIKV genome is approximately 12 kb and has two open reading frames (ORFs); the 5′ end has a 7-methylguanosine cap and a polyadenylation signal at the 3′ end. The 5′ ORF is translated from genomic RNA and encodes four nonstructural proteins (nsP1 to nsP4) essential for viral replication and processing. The 3′ ORF is translated from the 26S subgenomic RNA as a single polypeptide, which undergoes cleavage and posttranslational modification to form capsid protein (C), two surface envelope glycoproteins (E1 and E2), and two minor proteins, E3 and 6K (1–3).

Based on the glycoprotein E1 gene phylogenetic analysis, three genotypes of CHIKV were identified, West African, East/Central/South African (ECSA), and Asian (4). A massive chikungunya outbreak caused by the ECSA genotype in the Indian Ocean islands,

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