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# Expression and evaluation of a 24-kDa recombinant protein of the N-terminal E2 glycoprotein of chikungunya virus

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## ABSTRACT

Chikungunya is an acute febrile illness caused by chikungunya virus (CHIKV). In this study, a short 24-kDa N-terminal of E2 glycoprotein of chikungunya virus was cloned and expressed in *E.coli* expression system. The E2 recombinant protein was expressed as a fusion protein to 6-Histidine for ease of purification. The expression of the 24-kDa recombinant protein was detected by SDS-PAGE and the protein reactivity was evaluated by western blot analysis. The immunogenicity of the 24 kDa protein was further tested against human positive and negative sera for chikungunya and dengue. The results showed that the recombinant antigen was able to detect CHIKV positive sera and no cross reactivity was observed with dengue virus positive serum. 

## KEYWORDS

Chikungunya virus;
Glycoprotein;
Recombinant antigen;
Immunoblot assay;
*E.coli* expression system.

## INTRODUCTION

Chikungunya fever is an acute illness caused by chikungunya virus (CHIKV), an alphavirus of the family *Togaviridae*. CHIKV is transmitted to humans by mosquitoes of the genus *Aedes*, particularly *Aedes aegypti* and *Aedes albopictus*. The hallmark of CHIKV infection is a long lasting polyalthralgia, which may persist for months or even years[1]. Though generally a non-fatal condition, CHIKV infections may rarely be associated with complications such as encephalopathy and hepatic failure[2] and occasional deaths have been reported over the last decade[3]. The clinical illness is often associated with prolonged morbidity, which can impose enormous social and economic disadvantages on affected communities[4]. The first formal description of the disease was during an outbreak of chikungunya fever in 1952 in Tanzania[5] and the subsequent isolation of CHIKV[6]. The first outbreak in Asia was documented in Bangkok, Thailand in 1958 and since then, outbreaks have been reported in Cambodia, Vietnam, Laos, Myanmar, Malaysia, Singapore, the Philippines, and Indonesia[7]. Malaysia reported its first outbreak between December 1998 and February 1999[8]. There are three distinct lineages of CHIKV, a West African, an East Central and South African (ECSA) and an Asian lineage[9]. In 2004-2005, CHIKV of the ECSA lineage caused massive outbreaks in the

Indian Ocean island of La Reunion[10]. The single mutation in the E1 (A226V) of the CHIKV during the Indian Ocean outbreaks enhanced the ability of the virus to replicate in *Aedes albopictus* mosquitoes that greatly facilitated the transmission of the disease[11]. Currently there are no specific treatments for CHIKV infections and no licensed vaccine for any alphavirus is available for human use.

The CHIKV genome consists of a linear, positive-sense, single-stranded RNA of approximately 11.8kb, and encodes four non-structural proteins (nsP1, nsP2, nsP3 and nsP4) at the 5'end and five structural proteins (C, E3, E2, 6k and E1) at the 3' end. The non-structural proteins are required for viral replication whereas the structural proteins are produced by translation of an mRNA that is generated from an internal, sub-genomic promoter immediately downstream of the non-structural open reading frame[12]. The 5' end of the genome has a 7-methylguanosine cap, while the 3' end is polyadenylated. Two important viral glycoproteins E1 and E2 are conserved among alphaviruses[13]. The E1 glycoprotein of CHIKV mediates fusion of the viral and host cell membranes during virus entry and the E2 glycoprotein is responsible for receptor binding to host cells[14]. Therefore the glycoproteins E1 and E2 serve as the major targets for diagnostic and vaccine development.

Along with the clinical diagnosis based on symptoms, laboratory confirmation of CHIKV infection is critical, especially in dengue endemic areas, as clinical symptoms of the two diseases are similar. The ability to distinguish CHIKV infection from dengue virus infection is important to launch different control strategies. Enzyme-linked immunosorbent assays (ELISAs) and reverse transcriptase-polymerase chain reactions (RT-PCR) are among the recognized serological and molecular tools for the specific detection of CHIKV in patient samples[15]. RT-PCR is an excellent tool for the early phase confirmation of CHIKV infections, and many protocols have been established for this purpose[16]. Unfortunately, this viral detection method is limited to the viraemic phase, which is usually one to five days after fever onset[17]. Thereafter, confirmation of CHIKV infection requires serological tests. The serological tests include hemagglutination inhibition (HI) and ELISAs detecting IgM antibodies of CHIKV. HI test is a simple and rapid test, however the results can be difficult to interpret due to cross-reactivity with other viruses[18]. ELISA is a more preferable method to detect viral antigen specific antibodies because of its high sensitivity and specificity. Currently, reported serological methods for CHIKV infection commonly use whole virus or crude extracts for the target antigen[19]. The use of such materials presents a potential health hazard through exposure to infectious virus particles. In addition, production costs associated with the cultivation of virus for live or inactivated viral antigen are generally high. As a result, utilization of recombinant proteins, which can be produced more cheaply and present little or no health hazard, is an attractive alternative.

Hence in this study we aimed to develop an effective diagnostic method for serological detection of anti-chikungunya antibodies in chikungunya patients using recombinant protein expressed in *E.coli* expression system as an alternative antigen. In this paper we are reporting the expression of a 24-kDa of the N-terminal of CHIKV E2 protein, and the immunogenicity evaluation of the recombinant antigen in immunoblot assay.

## EXPERIMENTAL

### Propagation of CHIKV in cell culture

The virus, named BS5 was isolated from a local outbreak of CHIKV in Sarawak in 2009. The virus was propagated in Vero cells, which was maintained in Dulbecco's Modified Eagle's Medium, DMEM (Gibco, South America), supplemented with 5% Fetal Calf Serum (FCS), 100 U/ml Penicillin G and 100 µg/ml Streptomycin Sulfate. Vero cells were maintained in a humidified incubator at 37°C supplemented with 5% $CO_2$.

### RNA isolation and RT-PCR

The N-terminal of E2 gene was amplified using primer pairs as listed in TABLE 1. Prior to the amplification, RNA was extracted using High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany) according to the manufacturer's manual. The RNA was subjected to reverse transcription PCR (RT-PCR). For this purpose 6 µl of the extracted RNA was mixed with 1 µl of downstream primer for 10 minutes at 70°C and immediately chilled on ice afterwards. A master mix containing 0.5 µl of 10 mM dNTPS, 2.0 µl l of 5X RT