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Development of recombinant proteins as diagnostic intermediates for chikungunya infection

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Chikungunya is an important disease with explosive outbreaks occurring in many South East Asian countries. As the clinical symptoms associated with chikungunya viral infections are often indistinguishable from those of many other viral, bacterial and parasitic infections confirmation of chikungunya outbreaks is important for clinicians for proper management of patients and for vector control programmers. Laboratory diagnosis of chikungunya in Sri Lanka is hindered by the non-availability of reliable commercial diagnostic kits and inaccessibility to reagents. There is a need to develop an assay that can confirm chikungunya, produced at low cost and easily standardized for the use in field settings. Currently available laboratory diagnostic kits depend on ELISA based on whole viral antigens which cause biohazard risk, high production cost and cross reactivity with other organisms of the same genus/family. Therefore, a diagnostic intermediate using a single recombinant protein antigen to overcome problems associated with whole viral antigen/lysate is important. The objective of this study was to assist laboratory confirmation of outbreaks through developing competencies for a rapid laboratory diagnostic method using recombinant protein antigens for chikungunya infection.

We have designed 2 novel recombinant protein antigens based on Envelope domain (E), a critical antigenic region of the major structural protein of chikungunya virus. They were expressed in *Escherichia coli* separately, and resultant proteins were affinity purified and obtained ~5mg and ~10mg respectively and protein of >95% purity per liter of culture. These 2 proteins were evaluated as potential diagnostic intermediates in ELISA separately for the detection of anti-chikungunya Immunoglobulin M (IgM) antibody using a panel of well characterized serum samples. E1 and E2 showed 60% and 67% positivity respectively. Specificity proteins were tested using serum from healthy volunteers and infected with other viral diseases. Two proteins could detect only anti-chikungunya IgM antibodies. We demonstrated that these 2 novel recombinant protein antigens can function as diagnostic intermediates.

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