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Development of recombinant protein antigens using a yeast expression system, for the detection of anti-Chikungunya (CHIK) antibodies in clinical samples

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Laboratory confirmation of Chikungunya (CHIK) virus is very useful as clinical symptoms of CHIK overlap with those of other diseases. Laboratory diagnosis depends on Enzyme-Linked Immunosorbent Assay (ELISA) based on whole viral antigens which cause a biohazard risk, have a high production cost and show cross reactivity with other organisms of the same genus / family. A diagnostic intermediate using a single recombinant protein antigen to detect both immunoglobulin (Ig) M (IgM) and IgG antibodies of CHIK is important to overcome problems associated with whole viral antigen / lysate. The objective of this study was to prepare recombinant protein antigens for detection of anti-CHIK antibodies. Recombinant CHIK protein antigens were prepared using two Envelope (E) gene regions of the CHIK virus which were custom designed and chemically synthesized with 6X His tag. Yeast expression systems; *Pichia pastoris* (*P.pastoris*) were used to clone and express the recombinant proteins. Purification of proteins was performed using Ni-NTA columns under denature conditions. Novel in-house IgM and IgG ELISAs were developed using recombinant protein antigens. The two antigens were evaluated for detection of both IgM and IgG CHIK antibodies using a panel of well characterized serum samples. A total of 55 serum samples confirmed as positives and 186 confirmed as negatives were used to evaluate the antigens using novel IgM ELISA. A total of 78 serum samples confirmed as positives and 227 (148 and 79) confirmed as negatives were used to evaluate the antigens using novel IgG ELISA. These samples were tested by HAI test, IgM capture ELISA and indirect IgG ELISA using the purified CHIK antigen and Infected Culture Fluid (ICF). The E1 recombinant protein showed 15% (8/55) sensitivity and 97% (181/186) specificity for IgM ELISA and 86% (67/78) sensitivity and 61% (90/ 148) specificity for IgG ELISA. The E2 recombinant protein showed 49% (27/55) sensitivity and 78% (146/186) specificity for IgM ELISA and 76% (59/78) sensitivity and 81% (183/227) specificity for IgG ELISA. Recombinant protein antigens expressed in yeast showed lower specificity and sensitivity than expected for detection of both IgM and IgG anti-CHIK antibodies, but E2 recombinant proteins performed better than E1recombinant antigen as it is exposed more to the human immune system than E1 protein.

Keywords: Chikungunya, recombinant protein antigens, E1, E2, ELISA

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