IVD_10 - DiagSyn-Design of synthetic proteins for the serological diagnosis of dengue virus infection

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Introduction: Dengue virus infection is still a major health problem. Immunodiagnostic tests that do not cross- react with other flavivirus are on demand, as dengue virus infection can cause serious problems if not treated properly, such as dengue hemorrhagic fever.

Objectives: The present work aims to develop synthetic proteins for the correct diagnoses of Dengue infection.

Methodology: Two multiepitope synthetic proteins of DENV were designed *in silico*, using Alphafold, based on epitope sequences for the consensus Dengue genome and for the DENV genotypes circulating in Brazil during the last 10 years. The recombinant genes were synthesized commercially in expression vector pET28a^{+}. *E.coli BL21 (DE3)* cells were transformed and protein expression was induced with 1 mM IPTG. The soluble fractions were purified by Ni^{2+}-affinity chromatography and their purity and solubility were evaluated by SDS-PAGE and Western Blot assays. Afterward, indirect Elisa was used for evaluation of the immunogenicity against mouse serum infected with Zika virus and human-infected patients. The sensitivity of the assay was compared with that of commercial assays (Euroimmun).

Results: The two synthetic proteins DME-C and DME-BR were successfully purified in the soluble form and interacted with anti-6XHis antibody as shown by SDS-PAGE analysis and Western Blot, respectively. Indirect ELISA tests were standardized with varying concentrations of synthetic proteins, varying from 0.1 to 1.5 µg of protein per assay and different serum dilutions. The best results were obtained with 1.0 µg protein per assay and 50X dilution of human serum. Both IgG and IgM were recognized with both proteins. Indirect Elisa results show that mice serum from Zika-infected animals does not cross-react with either DME-C or DME-BR. Results were compared with Euroimmun Dengue Kit which show similar results.

Conclusion: DiagSyn synthetic proteins DME-C and DME-BR are specific for the diagnosis of DENV and do not cross-react with Zika-infected serum. These proteins show a potential to be further applied for the implementation of point-of-care DENV infection diagnosis.

Keywords: Dengue virus, synthetic proteins, serological assay