Aims and Objectives
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Dengue is an emerging infectious disease of great public health importance, and is caused by Dengue virus. Dengue viruses are members of the family *Flaviviridae*, genus flavivirus, and exist as four antigenically distinct serotypes. These are mosquito-borne human pathogens, causing a spectrum of illness ranging from mild fever to severe hemorrhagic fever and shock. The genome of the dengue virus is ~11 Kb single-stranded RNA of positive polarity, and serves as the viral mRNA. It encodes 10 viral proteins, of which three are structural (capsid C, membrane M and envelope E) proteins, and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins. The genomic RNA contains a single open reading frame (ORF), flanked by 5' and 3' non-translated regions. This ORF is translated into a single polyprotein precursor, which is co-translationally and post-translationally processed, by both host as well as virus-encoded proteolytic enzymes, to give rise to the individual structural and non-structural proteins. Dengue infections may be clinically inapparent or may result in non-specific febrile illness, DF or DHF\(^{34, 35}\). Severe plasma leakage can lead to fatal DSS and mortality rates for untreated patients can be as high as 40-50%. Early diagnosis, followed by supportive care, and symptomatic treatment through fluid replacement are the keys to survival in cases of severe dengue infection\(^{34, 37}\). Definitive diagnosis of dengue infection depends on the identification of the virus, virus-encoded antigens, viral genomic RNA or the virus-induced antibodies\(^{38, 39}\). Virus identification through its isolation can take several days and may not always be successful due to very small amounts of viable virus in the clinical samples. Viral antigens can be detected by immunohistochemistry or immunofluorescence. However, the complexity of these assays and their high cost preclude their routine use. Viral RNA can be detected with a high degree of sensitivity, using coupled reverse transcription and polymerase chain reaction (RT-PCR). Besides being expensive and requiring sophisticated equipment, the RT-PCR assay is subject to wide variability, as demonstrated in a recent study conducted by the European
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Network for Diagnostics of Imported Viral Disease\textsuperscript{40}. Further, a shortcoming common to all these methods is the narrow time window (~5 days), available for successful detection, which coincides with the febrile period during which patients are viremic\textsuperscript{30, 31}. Often, dengue infected patients do not seek immediate medical care as the initial manifestations are usually asymptomatic or mild fever. This precludes diagnostic tests based on the identification of the virus or its RNA genome because of the short duration of viremia. Thus, in a majority of cases the only feasible diagnostic test would have to be based on the identification of anti-dengue antibodies.

Numerous dengue diagnostic kits, in a multiplicity of formats, have become available recently. Most of these kits rely on the use of whole virus antigens for the detection of anti-dengue antibodies in patient sera. In one of these, the biohazard risk inherent in the use of whole virus antigens has been eliminated through the use of insect cell expressed dengue envelope protein as the antigen. Regardless of the antigen employed, the high production costs associated with antigen manufacture makes these kits unaffordable for use in the economically weaker countries where dengue is mostly prevalent. Thus, there is currently a need for developing cost-effective, safe and simple diagnostics that combine sensitivity and specificity. From this perspective, we believe that the use of recombinant antigens that can be produced in large amounts would not only provide an inexpensive, but also a safer alternative.

The main objective of this project is to design and develop two novel recombinant antigens; one of these will be designed to specifically detect dengue-specific IgM antibodies and the other to detect IgG. To this end, recombinant proteins that would satisfy these criteria, namely, (i) it should specifically detect anti-dengue IgG/IgM- antibodies, (ii) it should be safe and affordable, will be designed from IgG/IgM-specific immunodominant epitopes of dengue viruses. These multi-epitope proteins will be expressed to high levels in \textit{E. coli} and purified efficiently in a single step by affinity chromatography. The purified multi-epitope proteins will be evaluated as dengue diagnostic intermediates. In parallel we propose to scan two dengue proteins, namely, capsid and NS4a for identification of additional
immunodominant epitopes by pin-bound peptides. The newly identified epitopes will be included in designing the next generation dengue diagnostic antigen with higher degree of sensitivity.

The specific aims of this work are as follows:

- Design and assembly of anti-dengue IgG- and IgM-specific recombinant dengue multi-epitope (r-DME) proteins from dengue virus specific, linear and immunodominant epitopes.
- Generation of fusion constructs expressing these novel r-DME proteins.
- Purification and characterization of these r-DME proteins.
- Evaluation of the r-DME proteins as dengue diagnostic intermediates.
- Standardization of anti-dengue ELISA and rapid tests
- Identification of additional immunodominant linear epitopes of the Capsid and the Non-structural protein-4A (NS4A) of Dengue virus.