Performance assessment of a multi-epitope chimeric antigen for the serological diagnosis of acute Mayaro fever

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Abstract

Mayaro virus (MAYV) is endemic to limited regions of South America and the possible involvement of *Aedes spp.* mosquitoes in its transmission increase the risk that its geographical range will reach greater populations. MAYV shares clinical symptoms and structural similarities with other emerging members of the alphavirus family such as Chikungunya (CHIKV), Ross River (RRV), and O'nyong nyong (ONNV) viruses, which complicate diagnosis and is currently restricted to nucleic acid-based tests. Effective control of the spread of these diseases will require the identification of infected individuals as well as the development of preventative and prophylactic therapies. Serological tests have a greater potential to expand testing at a reasonable cost than molecular approaches. Their performance largely reflects that of the antigen utilized to capture pathogen-specific antibodies. Here, we describe the assembly of a synthetic gene coding multiple copies of antigenic determinants mapped from the nsP1, nsP2, E1, and E2 proteins of MAYV that readily produced a stable chimeric protein in bacteria. The serological performance (AUC, sensitivity, specificity, and confidence intervals) of this multi-epitope protein as the target in ELISAs revealed a high diagnostic accuracy for the detection of specific anti-MAYV IgM antibodies. No cross-reactivity was observed with serum from seropositive individuals for CHIKV, yellow fever (vaccinated), dengue, Zika, and other infectious diseases as well as healthy individuals. Our data suggest that this bioengineered antigen could be used to develop high-performance serological tests for MAYV infections.

Introduction

Mayaro Virus (MAYV) is an emerging zoonotic circulating arbovirus that is part of the Alphavirus genus and Togaviridae Family. Due to its antigenic nature, it is included in the Semliki Forest group. First isolated in Trinidad and Tobago in 1954 [1,2], its geographical distribution exclusively encompasses Central and South American countries, mainly those containing extensive areas of tropical forests [3–8]. MAYV was isolated in Brazil in 1955 and since then it has been the cause of several epidemics in the North and Central-West regions, where it can be considered endemic [9–12].

Its main vector is the *Haemagogus janthinomys* mosquito, however, other mosquito genera are also known as vectors, such as *Culex* and *Psophor* [3,13]. Some studies suggest that *Aedes aegypti* is another potential vector, which could be considerably troublesome to public health, not only because this mosquito is well adapted to the urban environment, there would be a possibility to associate MAYV dissemination to the spread of dengue virus (DENV) [14, 15].

The MAYV genome is composed of a single positive RNA chain consisting of 11,429 nucleotides. Viral particles are enveloped with a diameter of 69 ± 2.3 nm and an icosahedral nucleocapsid [16]. The first Operating Reading Frame (ORF) starting from the 5' end encodes a polyprotein that undergoes cleavage during the translation process and generates four nonstructural proteins (nsP1, nsP2, nsP3, and nsP4) [17,18]. A second ORF region is contained in the 3' end that when translated produces subgenomic RNA 26S and, sequentially, into a structural polyprotein that yields E1, E2, E3, 6K proteins, and capsid (C) proteins [19-21].

MAYV is an arbovirus of great concern due to the high rate of morbidity observed with infection [22], which can cause a highly debilitating clinical condition, Mayaro fever, characterized by high fever, headaches, diarrhea, vomiting, skin eruptions, myalgia, and persistent arthralgia [23,24]. Its symptoms are similar to those from other arboviruses that co-circulate in areas endemic for MAYV that include Chikungunya, Dengue, Yellow fever, and Oropouche, which can lead to a clinical misdiagnosis of MAYV infections for one of them [25-27]. Besides, MAYV infections display a short viremic phase [15], which compounds the difficulty of a molecular diagnosis through the direct detection of viral nucleic acid by reducing the capture of the virus in biological samples obtained from clinically symptomatic patients. Lastly, the laboratory techniques developed for molecular diagnosis are complicated, which has limited the implementation of routine diagnostic assays.

The Global Research Collaboration for Infectious Disease Preparedness has highlighted the need for the urgent development of a specific diagnostic assay for the widespread surveillance of MAYV, CHYV, and ONNV has been pointed and discussed by the international GloboPid-R group [28]. A variety of diagnostic tests have been proposed based on molecular assays [11,29], ELISAs using viral proteins [30,31], and ELISAs based on chimeric alphavirus proteins [32]. For serological diagnostic assays, the cross-reactivity of antibodies generated from related alphavirus antigens, in CHYV and others, is a major concern [33-35]. Consequently, new high throughput methodologies must be applied to identify and exclude these cross-reactive sequences during the development of new immunological diagnostic tests.

Beginning with the search for IgM-specific epitopes in the B-cell epitome of MAYV, four epitopes that originated from the proteins nsP1, nsP2, E1, and E2 from MAYV were identified. Two copies of each epitope were fused *in silico* into specific positions of a synthetic gene encoding for a core platform protein that can support the insertion of extraneous sequences without compromising its structure (Rx). Termed Dx-MAYV-M, the chimeric multi-epitope protein was expressed in the *Escherichia coli* system, purified, and evaluated as an antigen in an ELISA to detect IgM antibodies in patient serum that specifically bound MAYV. A panel of patient sera infected with MAYV, CHYV, DENV, and ZIKAV, as well as other infectious diseases, such as C-hepatitis, leptospirosis, leishmaniasis, and Chagas disease were analyzed.

Results

Epitope selection-The complete Spot-synthesis analysis identified 134 linear B-cell IgM epitopes that were recognized by a pool of patient sera in the five structural (C, E1, E2, E3, and 6K) and four non-structural (nsP1, nsP2, nsP3, nsP4) MAYV proteins. As a primary goal of this work was to identify antigenic determinants that could differentiate MAYV infections from closely related pathogens, bioinformatics was used to BLAST the sequence of each of the 134 epitopes. Four epitopes that originated from proteins nsP1, nsP2, E1 and E2 were determined to meet the criteria for a high potential to avoid cross-reactivity based on the absence of multiple sequential amino acids that were identical to segments in other pathogens (Table 1). Figure 1A-1D shows the image of the four-peptide spots that encompass the individual epitopes along with a graphical representation of their relative intensities. The minimal motifs

were chosen for this study comprising the epitope nsP1-20 (⁴⁰⁶HRIRLLLQS⁴¹⁴), nsP2-10 (²⁴⁰NGVKQTVDV²⁴⁸), E2-11 (⁶¹⁹SYRTFGAERV⁶²⁸), and E1-8 (¹⁰¹⁰QSRTLDS¹⁰¹⁶).

Table 1: Selected epitopes mapped in MAYV proteins using a pool of sera from patients with mayaro fever.

aa	Epitope Sequence	Code	Insertion site of eGFP (aa)
466-474	HRIRLLLQS	MAYV/nsP1-20huM	1,2; 186,187; 253,254
240-248	NGVKQTVDV	MAYV/nsP2-10huM	127,128; 225,226
619-628	YRTFGAER	MAYV/E2-11huM	112,113; 202,203
1010-1018	SRTLDSR	MAYV/E1-8huM	148,149; 165,166

Dx-MAYV-M production- Each antigenic region was bioengineered into the backbone of eGFP into two independents sites, except HRIRLLLQS; three copies were inserted. Table 1 lists the amino numbers of the insertion sites that was performed *in silico* to create the multi-epitope chimeric recombinant protein herein referred to as Dx-MAYV-M. It was produced as a synthetic gene for cloning into pET-28a that maintained an amino terminus six-histidine affinity tag while excluding the tag available on the carboxy terminus. The final protein had a deduced molecular mass of 40.6 kDa with an isoelectric point of 8.35. Within the pET expression system, the recombinant protein was readily overexpressed in *E. coli* (~250 µg/ml) and was localized to inclusion bodies when the expression temperature was 37 °C (Fig. 1E). An increased proportion of soluble protein was observed at lower expression temperature, albeit at lower levels. In addition to the higher production levels, the use of inclusion bodies provided an initial purification step resulting in >90% purity that also avoided degradation of Dx-MAYV-M during bacteria lysis and avoided the need for protease inhibitors (Fig. 1F).

A western blot of the soluble and insoluble recombinant protein probed with a pool of serum from patients with Mayaro fever confirmed that Dx-MAYV-M was recognized by anti-MAYV antibodies (Fig. 1G). Some other bands could be observed that can be attributed to the general reactivity of human sera against bacterial proteins. The purification of Dx-MAYV-M by immobilized metal affinity chromatography (IMAC) was feasible in the presence of urea and soluble protein could be purified after removal of urea on-column, before elution (Fig. 1H).

ELISA-peptide assay and performance comparison- To verify the expected individual diagnostic performance of the epitopes, two were chosen to generate synthetic bispecific MAPS8 and used in an in-house peptide ELISA. Sera from eighteen MAYV infected patients were tested for sensitivity and a variety

of sera from patients infected with CHYV, YFV vaccinated, DENV, ZIKAV, C-hepatitis, leptospirosis, leishmaniasis, and Chagas disease patients along with fifty, non-symptomatic healthy donors were tested for cross-reactivity. Excellent performance was measured for both MAYV/nsP1-20 and MAYV/E2-11 with all MAYV patient serum giving signals above the cut-off (Fig. 2A). Besides, no cross-reactivity was observed and the results were statistically significant ($p < 0,001$).

Performance of Dx-MAYV-M in the serodiagnosis of Mayaro fever -To determine whether the multi-epitope chimeric protein could be considered as an effective tool for the serodiagnosis of acute MAYV infections, we analyzed its reactivity against the same panel of sera as used to screen the peptide library (Fig. 2A). The positive reactivity indices for MAYV patient serum increased more than 2-fold in comparison to the MAPs. As importantly, the reactivity indices for the negative controls were lower, which gave a large statistically significant difference, $p < 0.0001$, between positive and negative signals that could greatly reduce borderline results (Fig. 2B). Based on a Receiver Operating Characteristics curve (Fig. 2C), the area under ROC (AUC) for MAYV-M protein varied from 0.9839 to 0.9998 ($p < 0.0001$) as detected by ELISA and interval confidence of 95%, demonstrating high diagnostic accuracy for the Dx-MAYV-M recombinant chimeric proteins. Considering the high number of infectious agents that co-circulate in endemic regions of MAYV, serum from another eighty individuals with other diseases were evaluated and the reactivity against each of the pathogens was grouped (Fig. 2 D).

Spatial localization of the epitopes in Dx-MAYV-M. The I-Tasser server was utilized to generate a predicted structure of the entire Dx-MAYV-M protein that presented a confidence score equal to -1.41 and a TM-score of 0.47 ± 0.15 (borderline quality). To confirm that the localization of the epitope sequences was on the surface of the chimeric protein, a space-filling model was generated with the individual epitopes colored (Fig. 3). All four of the selected linear epitopes were located to surface regions in Dx-MAYV-M. A hydropathy plot of the protein also suggested that all of the epitopes were present on the surface of the proteins (data not shown).

Discussion

Over the past 10 years, the diagnosis of a MAYV infection has principally depended on either the identification of the virus by the molecular detection of its genome or a hemagglutination inhibition (IH) assay [36,37], but have been mostly abandoned due to the low sensitivity of the assays. More recently, attempts have been made to improve serodiagnostic assays by using recombinant proteins representing viral proteins as antigens for the capture of IgG antibodies in in-house ELISAs [34,36]. However, the results of these assays have displayed considerable variations in their reproducibility, reliability, feasibility and specificity with notable cross-reactivity being described, which is a major challenge in the field for the differential diagnosis of highly similar pathogens. Here, we focused on the detection of MAYV specific IgM antibodies as their production can begin between 3-8 days after an infection [38,39]. This timing

overlaps with the onset of clinical symptoms (3-5 day post infection) and could be detectable over the course of a hospitalization, which can last more than 5 days. This is consistent with the observations of alphaviruses in general as the levels of IgM antibodies generated against them often reach detectable levels during the course of the associated illness.

The capacity of the target in a diagnostic test to bind pathogen-specific antibodies for detection by the secondary antibody depends on the presence of the actual epitope sequences that interact with the antibodies. To develop a diagnostic assay for the detection of anti-MAYV IgM, we began with the identification of the antigenic determinants within the proteome of MAYV that was based on the identification of its linear B-cell epitopes for IgM. An understanding of the biochemical and antigenic characteristics of the epitopes that interact with the antibodies is crucial to control factors during the assay that can affect the antigen-antibody interaction, such as ionic strength and pH as well as the formation of aggregates of the target in the assay solvent [40,41]. As successful binding of antibodies to proteins has been correlated with hydrophilicity and the surface exposure of epitopes [41,42], these characteristics were used to reduce the primary list of epitopes. Next, we restricted the epitopes based on the highest affinity according to their relative signal strength in the Spot Synthesis analysis. To minimize the potential for cross-reactivity, the epitope sequences were subjected to a BLAST homology analysis to identify those that were strictly associated to the MAYV proteome and no others (data not shown). Ultimately, four epitopes were selected.

As the availability and spatial distribution of the epitopes in a target on the solid phase of the test can influence performance, linearized epitopes as peptides have the advantage of presenting individual fragments separately for their interaction with polyclonal antisera that avoids overlapping to minimize steric hindrance competition. Using the multiple antigen peptide, two epitopes of the four epitopes were evaluated as shown in Figure 2. The results showed excellent specificity, as expected from the sequence analysis that showed that peptides nsP1-20 and E2-11 are not found in any other pathogen. While a separation in the signal pattern between positive samples and the negative controls was observed, the use of a diagnostic assay at the earliest times of IgM production required the highest possible sensitivity. We chose to incorporate the four different IgM linear B-cell epitopes into a proteinaceous core.

During the construction of the chimera multi-epitope protein, not only was the selection of highly immunogenic sequences reconsidered, but also the most hydrophilic epitope sequences for the location of the sequence replacement. Before generating the synthetic gene, the coding sequence of the bioengineered Dx-MAYV-M multi-epitope protein was subjected to a computer simulation to predict the spatial positioning of the introduced epitope sequences to consider that the substitution/ insertion of peptide sequences into the protein matrix could lead to the concealment or impediment of the accessibility of these sequences to the surface of the molecule and hence to the antibodies [43]. In addition to being localized on the surface, each epitope was depicted as a random coil structure, which is an excellent structure for interacting with its cognizant antibody and should improve the potential for high diagnostic accuracy.

The chimeric protein maintained the excellent specificity of the MAPs for the detection of IgM antibodies only in the sera from patients confirmed to be infected by MAYV. The ELISA results using the Dx-MAYV-M recombinant chimeric multi-epitope protein revealed that all epitopes appropriately discriminated between negative and positive samples ($p < 0.0001$). No cross-reactivity was observed as anticipated from the BLAST analysis criteria. In comparison to the individual epitope MAPs, the use of the multi-epitope protein improved the performance of our in-house ELISA by generating a higher signal that showed a greater difference between positive and negative samples. From the ROC analysis, the sensitivity of the ELISA was 99.6%. This increase in signal most likely reflects the presence of multiple copies of each epitope as well as their incorporation into a protein environment as opposed to being peptides. Another benefit of the multi-epitope protein design was to increase the specific activity of each molecule to bind antibodies; with each containing up to nine sites.

Phase I studies are typically conducted using a group of seropositive individuals and a group of seronegative individuals, to assess whether the test can differentiate between them [44]. For MAYV, the sample size of the positive samples is still small, mostly due to the difficulties for its diagnosis, which limits the scope of the likelihood score of the ROC analysis that relates to the probability for an increase in sample size to alter the sensitivity and specificity of the assay. A much larger and diverse panel of negative controls was available to support our conclusion that Dx-MAYV-M has 100% specificity. The performance to date strongly support continued use of Dx-MAYV-M as a target in diagnostic tests and suggest that the antigen may be eligible to enter phase II studies.

Methods

Serum samples and ethical considerations. This investigation was approved by the Ethical Committee of FIOCRUZ (CEP/IOC-CAAE:52892216.8.0000.5248 and 384/07) following the principles of the Declaration of Helsinki. MAYV serum samples used in this study were obtained from 18 patients with mayaro fever and diagnosed as positive when tested by a PCR test that was provided by the Leônidas and Maria Deane Institute of FIOCRUZ (Manaus, AM, Brazil) and Evandro Chagas Hospital (Manaus, AM, Brazil). The voluntary donor group was composed of residents of endemic areas that provided informed consent by a signed Free and Informed Consent Form (TCLE) for themselves or as the adult responsible for a minor without a definition of their health status, social group, age, sex, or race. Samples from healthy individuals were obtained from blood bank donors of Rio de Janeiro (HEMORIO). Biorepositories provided anonymous serum samples for dengue (Laboratory of Flavivirus, FIOCRUZ/IOC/RJ), chikungunya (LACEN/Ce), yellow fever (vaccinated individuals), and other laboratories of the Oswaldo Cruz Institute (Zika, C-hepatitis, Chagas disease, and leishmaniasis) that ensured the protection of patient privacy and negated the need for verbal or written consent.

Epitope mapping. The complete sequences of nonstructural (Q8QZ72) and structural (Q8QZ73) proteins of MAYV circulating in Brazil were obtained through access to the Uniprot database

[<http://www.uniprot.org/>]. Microarrays of peptides were used to perform the mapping of linear B-cell epitopes, as described previously [45]. One hundred thirty-four linear B-cell IgM epitopes were identified using a pool (n=5) of patient's sera infected with MAYV (non-published data).

Synthetic gene, protein expression, and purification. Two copies of three epitopes and three copies of one epitope sequences were inserted into the green fluorescent protein (GFP, *Aequorea victoria*) core protein sequence [46] as described in Table 1. The final chimeric amino acid sequence was back transcribed into an optimized nucleic acid for expression in bacteria with end restriction sites for cloning into pET28a designed to include an amino terminus 6xHis tag. A synthetic gene fragment (GeneArt, ThermoFisher) was cloned in a single step into the expression vector and the resulting clone was confirmed by sequencing. For the expression of recombinant protein, the plasmid was transformed into *Escherichia coli* BL21 (DE3) by standard techniques. Production was induced for 3h at 37 °C with 0.8 mM of isopropyl β -D-1-thiogalactopyranoside. Cells were collected by centrifugation and disrupted by sonification in lysis buffer (300 mM NaCl, 50 mM Tris, pH 8.0). Inclusion bodies were collected by centrifugation, washed in lysis buffer with 0.5% Triton-X100, and resuspended in lysis buffer with 6M urea and 20 mM imidazole. Proteins were solubilized overnight at 4 °C, clarified by centrifugation, and Dx-MAYV-M was purified by affinity chromatography on a HisTrap™ column (GE Healthcare, Piscataway, NJ) connected to an Äkta prime chromatography system (GE Healthcare, Piscataway, NJ). Urea was removed by a gradient wash followed by a two-step elution with 250 and 500 mM imidazole. Fractions with Dx-MAYV-M, as confirmed by SDS-PAGE, were diluted and applied to the HiTrap™ Q HP column (GE Healthcare, Piscataway, NJ) to remove imidazole and to concentrate the chimeric protein. The final Dx-MAYV-M preparation was quantified spectrophotometrically and confirmed by SDS-PAGE.

Synthesis of MAPs containing bispecific multi-antigen-peptides. For the preparation of dendrimeric bispecific multi-antigen peptides (MAP), was used a standard solid-phase synthesis protocol and the octameric Fmoc8-Lys4-Lys2-Lys-B-Ala Wang resin as described previously [47]. preparation of the dendrimer multi antigen peptides (MAPs), was used the protocol of synthesis in solid phase and the tetrameric Fmoc4-Lys2-Lys- β -Ala Wang resin as described previously. Each peptide epitope [nsP1-20 (RIRLLQLGGNGVKQTVD) and E2-11 (YRTFGAERGGSLTLDSR)] was linked in tandem with the addition of two extra Gly residues to improve the presentation of antigens to antibodies. Briefly, the construct was prepared in an automated peptide synthesizer (PSS8-model, Shimadzu, Kyoto, Japan) and the side chains of octafunctional Fmoc-amino acids were protected with TFA-labile protecting groups as required. Residues corresponding to the monovalent ('tail') part of the construct, up to the first (bis-Fmoc) Lys residue initiating the dendrimer structure, were incorporated via single couplings. Once sequence assembly was completed, the Fmoc groups were removed and the peptide-resin was cleaved and fully deprotected with TFA/H₂O/EDT/TIS (94/2.5/2.5/1.0 v/v, 90 min). The peptides were precipitated by the addition of chilled diethyl ether followed by centrifugation. The resulting pellet was taken up into aqueous

AcOH (10% v/v), dried, and stored as a lyophilized powder. When necessary, the MAP was dissolved in water, centrifuged (10,000 g, 60min, 15 °C) and the supernatant filtered by a centricon filter. Synthetic peptides were purified by high-performance liquid chromatography (HPLC) using a reverse-phase column (Vydac C18) and characterized by mass spectrometry at the National Institute of Quality Control on Health (INCQS) of FIOCRUZ, Rio de Janeiro, Brazil.

In-house ELISA optimization and procedure. Optimal target quantities along with dilution of patient serum and secondary antibody were determined by a checkerboard titration [48]. After optimization, Dx-MAYV-M chimera was used at 300 ng/well in coating buffer (0.05 M carbonate-bicarbonate, pH 9.6) to sensitize high-binding 96-well microplates (Jet Biofil®, Guangzhou, China) overnight at 4 °C. Microplates were rinsed and blocked with TBSM (TBS with 1% fat-free dry milk) for 1h at 37 °C and washed with TBST before the addition of patient serum samples (100 µl, diluted 1:150 in TBS). Plates were incubated for 90 min at 37 °C, washed in TBST, and then incubated with alkaline phosphatase-conjugated goat anti-human IgM (1:20,000) for 90 min at 37 °C. Next, plates were washed five times before the addition of 100 µl of PNPP (p-nitrophenyl phosphate, 1mg/ml, Thermo Fisher, USA). After a 15 min incubation at room temperature in the dark, the absorbance at 405 nm was measured in a FlexStation® 3 Multi-Model microplate reader (Molecular Devices, USA).

Molecular modeling. As three-dimensional structure data does not exist for Dx-MAYV-M, a theoretical model from the primary sequence of the chimeric protein was generated on the I-TASSER server (<http://zhanglab.ccmb.med.umich.edu/I-TASSER/>), which combines the methods of threading, ab initio modeling, and structural refinement [49]. A model with a TM-score value greater than 0.70 and a reasonable quality c-score was chosen for visualization using Visual Molecular Dynamic (VMD) 1.9 [50].

Statistical analysis. Data were encoded and analyzed using scatter computer graphic software (GraphPad Prism version 6, San Diego-CA, USA). Descriptive statistics were presented as geometric mean $\pm$ SD. Reactivity performance of the enzyme-linked immunosorbent assay was evaluated by receiver operating characteristic curve (ROC) curve analysis. The ROC curve also produces a table that relates the specificity, sensitivity, and cutoff (cutoff). The cutoff selection criteria were 100% specificity and 100% sensitivity. To test the normality of datasets, the D'Agostino & Pearson test and Kologorov-Smirnv test followed by Student's t-test was used, and the variance homogeneity assumption was confirmed. The ANOVA (Brown-Forsythe and Welch) parametric test was chosen for analysis of variance with confidence intervals. This index is referred to as reactivity index (RI) and all results <1.00 were considered negative. However, samples were deemed inconclusive (or in the gray zone) if the RI values fell into the undetermined zone, which was hypothesized as RI values of $1.0 \pm 10\%$.

Declarations

Data availability

The full dataset that supports the findings of this study is available from the authors upon reasonable request.

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Author contribution

S.G.S designed and ran experiments, analyzed and interpreted data, and drafted the manuscript, P.N.P., F.R.G., A.M.D. designed experiments, interpreted data, D.W.P drafted the manuscript, and C.M.M conceptualized the project.

Competing interests

There is no potential conflict of interest, within the contributing authors. The peptides and methodology described in this study are protected under Brazilian and U.S. provisional patent application BR 10.2019.017792.6 and PCT/BR2020/ 050341, respectively, filed by FIOCRUZ and may serve as a future source of funding. The funding agencies had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

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Figures

Figure 1

Epitope identification and production of Dx-MAYV-M. The entire IgM epitome of MAYV was revealed by Spot synthesis. The image of the reactivity from three consecutive peptides that define the minimal motif of the four epitopes that showed the lowest theoretical potential for cross-reactivity to other pathogens, along with a quantification of the relative intensity, is shown for nsP1-20 (A); nsP2-10 (B); E1-8 (C); and E2-11 (D). Each epitope was included twice in the engineered Dx-MAYV-M protein and its overexpression

in *E. coli* led to insoluble proteins. (E) SDS-PAGE with lane 1, molecular markers; lane 2, whole extract; lane 3, inclusion bodies; lane 4, soluble proteins. (F) An overloaded SDS-PAGE gel of soluble (lane 5) and insoluble (lane 6) protein. (G) A western blot of soluble (lane 7) and insoluble (lane 8) protein probed with Mayaro fever patient sera. (H) SDS-PAGE of Dx-MAYV-M before (lane 9) and after (lane 10 and 11) affinity purification by a HisTrap™ HP column (from insoluble fraction and soluble proteins, respectively).

A

B

C

Figure 2

Diagnostic performance of IgM epitopes in MAYV as peptides and in Dx-MAYV-M. In-house ELISAs were generated with peptides MAP8/nsP1-20 (RIRLLLQGGNGVKQT VD) and MAP8/E2-11 (YRTFGAERGGSRTLDSR), and the engineered Dx-MAYV-M. (A) Their reactivity index for each of 18 serum samples from Mayaro fever patients, 80 serum of other infectious diseases [CHYV, YFV, ZIKV, HBV, LEP, LEIS, CD]. (B) The median and 95% confidence interval along with maximum and minimum indices of IgM reactivity of Dx-MAYV-M was significant (Brown-Forsythe and Welch, $p = 0.0001$). (C) ROC curve analysis for Rx-MAYVM. (D) The absence of cross-reactivity by antibodies against a series of co-circulating pathogens for Dx-MAYV-M (NS, healthy individual sera; CHIKV, Chikungunya virus; YFV, Yellow fever virus; DENV, Dengue virus; ZIKAV, Zika virus; HEP, Hepatitis B; LEP, Leptospirosis; LEIS, Leishmaniosis; and CD, Chagas Disease).

Figure 3

Spatial localization of the MAYV IgM linear epitopes in Dx-MAYV-M. A space-filling model of a computer predicted structure for the multi-epitope chimeric protein with the core structure of the eGFP in gray along with the epitope of nsP1-20 in red, nsP2-10 in yellow, E1-8 in pink and E2-11 in green. Multiple angles are depicted.