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USING AN IMMUNOINFORMATIC APPROACH, IDENTIFICATION OF B-CELL ENVELOPE PROTEOME FOR MULTIPATHOGENIC DENGUE AND ZIKA VIRUSES

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ABSTRACT: Mosquitoes are carriers of the DENV and ZIKV diseases. DENV and ZIKV are epitopes. The current performance and technique of some of the most frequently used B-cell epitope predictors used for DV and ZV proteomes are reviewed in this paper. Our specific prediction methods, ABC pred, BC pred, and AAP Methods, as well as FBC pred, were utilized to predict 1529 B cell epitopes. For the B cell epitops, the stability index, aliphatic index, GRAVY, antigen city, allergen city, toxicity, clustering, and conservancy were investigated further. The anticipated immunogenic epitopes PLPWHAGADTGTPHWNNKEA, GGFGSLGLDCEPRTGLDFSD of ZIKV and IGVEPGQLKLSWFKKGSSIG, VEPGQLKLSWFKKGSSIGQM, IEAKLTNTTTASRCPTQGEP, LPLPWLPGADTQGSNW of DENV were 100 percent conserved. The goal of this study is Flaviviridae viruses that now affect 128 nations throughout the world. And they may both be fatal and life-threatening. A quicker and less expensive vaccine design procedure used to identify the B cell was to use computer simulations to find potential vaccination candidates and antiviral efficacy testing. The study's goal is to develop patient therapies that are both safe and effective.

INTRODUCTION: Mosquitoes carry the DEN and ZIK viruses, which are health risks and burdens that have lately attracted public attention¹. The principal vector for horizontal transfer of Viruses to humans is *Aedes aegypti*². As a result of population increase and mobility, urbanization and climate change, predictive models show that the global spread of *Aedes aegypti* will continue to expand³.

DENV and ZIKV replicate their DNA via viral protein receptor-mediated endocytosis in host cells. The extrinsic incubation period is the time it takes for a virus to enter the mosquito's midgut and then be secreted in mosquito saliva. The EIP of different viral species varies. Flaviviruses like DENV and ZIKV have an estimated extrinsic incubation period of 10 to 14 days⁴.

Infection of the salivary glands also changes the content of saliva, affecting blood acquisition and skin infection⁵. DENV ZIKV and CHIKV infections activate the c-jun N terminal kinase (JNK) pathway, activating complement and apoptotic effectors, resulting in a broad antiviral response⁶. The ZIKV pandemic was a "perfect storm" in which a novel American subclade arose

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from the virus's Asian origin and was introduced into a uniformly susceptible population that had never been exposed to ZIKV before⁷. In some areas, such as India and Southeast Asia, where substantial populations of pregnant women are vulnerable to the virus, outbreaks and infection clusters continue to occur⁸. Prior dengue infection and anti-DENV antibodies were found to diminish rather than increase the likelihood of ZIKV infection and illness in people in prospective investigations⁹.

With a non-segmented, single-stranded, positive-sense RNA genome of 10 794 kb in length and two flanked non-coding regions, ZIKV, like DENV, is encapsulated and icosahedral. (5' and 3' NCR) and a long single open reading frame encoding a polyprotein: 5'-(C, pr, M, E, NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5)-3'¹⁰. Dengue infection usually causes asymptomatic, flu-like symptoms; however, the severe and fatal form, called dengue hemorrhagic fever, can cause bleeding, shock, and death. ZIKV infections are usually asymptomatic or minimally symptomatic, with symptoms of self-limiting acute febrile sickness, rash, joint discomfort, myalgia, and conjunctivitis. Severe sickness consequences, such as Guillain-Barré syndrome in adults and congenital ZIKV syndrome in neonates, have posed significant public health challenges¹¹.

Controlled human challenge infection models are being investigated as an alternative method of acquiring efficacy proof for regulatory approval of a vaccine¹². There is currently no effective Zika vaccine available, including a ZIKV produced, and is presently being tested in clinical studies^{13, 14}. Dengvaxia is a licensed dengue vaccine that offers only limited protection and comes with a list of precautions^{15, 16, 17, 18} which Sanofi Pasteur^{19, 20}, developed. As a result, my research aimed to identify possible vaccine candidates and antiviral effectiveness tests in silico. The research objective is to find safe and effective treatments for patients^{21, 22, 23}. The endemic zone of vectors has grown as a result of unplanned urbanization, highlighting the need for a powerful vaccination. Because the E protein's two-layer membrane is made up of two antiparallel monomer units, it engages in membrane fusion and receptor binding as soon as the antigen enters.

The structure of E proteins categorizes them into several groups. They either recognize a quaternary epitope on the E protein dimer interface or detect a quaternary epitope on the E protein dimer interface to block ZIKV and DENV vertical transmission²⁴.

The E proteins of DV and ZV had 35, 51 and 29 percent protein similarity in EDI, EDII, and EDIII, respectively. Cross reactivation was rapid in domains I and II, while it was gradual in domain III. B cells are involved in cell activation, serological testing, and signal transmission. B cell peptides are made up of hydrophilic amino acids present in protein antigen molecules or other macromolecules, and they're made by soluble or membrane bound immunoglobulin²⁵. The epitopes were employed to develop diagnostic reagents as well as vaccines. Because B cells are antigenic, they have a high chance of attaching to antibodies. Therefore, it is proposed that soluble ED III be included as an antigen in DENV and ZIKV candidate vaccines to produce neutralizing antibodies that impede viral attachment or hinder post-entry membrane fusion.

The covalently stabilized E dimer has consistently elicited protective antibody responses against ZIKV and DENV infection. The prediction of B cell epitopes may be made using a variety of approaches. There are used in several B cell epitope prediction approaches^{26, 27}. When both B cell epitopes are present on the same molecule, and some of them are more immunogenic than others, the immunostimulatory one, referred to as immunodominant, would successfully limit productive capacity while inducing antibody responses. With the use of these technologies, I was able to identify immunogenic B cell epitopes, and they may improve prediction performance. These techniques are quick, simple, and affordable. Similar techniques have been used to develop vaccines against viruses including SARS-CoV, Ebola, and Chikungunya^{28, 29}.

MATERIALS AND METHODS:

Viral Protein Selection: The amino acid sequences of DV and ZV were retrieved from the virus pathogen resource sequence database. DENV and ZIKV E viral proteins were extracted using the FASTA format.

Prediction of B-cell Epitope: Using E protein, we predicted possible 12, 16, and 20 mer B cell epitopes from all zika and dengue virus strains. ABC pred produced continuous B-cell epitopes by using a constant length pattern³⁰. BC Preds is made up of two fixed-length algorithms (BC Pred and AAP methods) and one flexible length algorithm (FBC Pred algorithm). This study used two fixed length techniques (BC Pred and AAP methods) and one flexible length method (FBC Pred algorithm) to predict B-cell epitopes by the user-entered epitope sequence.

Predictions of Instability Index, Aliphatic Index and GRAVY Analysis: To estimation of stability, aliphatic index, and GRAVY for a protein, Prot param was used as TrEMBL³¹. The aliphatic index is the relative quantity in the form of aliphatic facet chains (alanine, valine, isoleucine, and leucine). The extracellular matrix is one of the calculated metrics for GRAVY analysis.

Consensus Epitope Prediction: When the anticipated Zika and dengue virus serotype epitopes (12, 16 and 20 mer) were analyze, the consensus epitope produce a better chance of eliciting an immune response to the virus.

Predictions of Antigenicity and Allergenicity: VaxiJen is the first tool for predicting viral user-defined origin defense antigens without using alignment with a threshold value of 0.4. Above 0.4 value the best immune response possible when the parental antigen is presented³². Allergen FP was used to determine allergenicity. Non-allergenic proteins were chosen based on a similarity index³³.

Predictions of Toxicity, Hydrophobicity, Hydropathicity, Charge, and Molecular Weight: Toxin Pred is used to assess epitope toxicity,

hydrophobicity, hydropathicity, and terms of epitope charge and molecular weight³⁴.

Epitope Cluster Predictions: Using the IEDB program, all epitopes were grouped into clusters based on sequence identity within a cluster and an 80 percent sequence similarity criterion³⁵. For conservation, we utilized the IEDB tool³⁶.

Conservancy Analysis: The conservancy study is used to assess epitope dispersion in a homologous protein sample. To help with the ideal degree of conservation in epitope collection, we used the IEDB tool for the conservancy.

RESULTS:

Selection of Viral Proteins for Vaccine Preparation: The virus pathogen resource sequence database was used to produce the multipathogenic vaccine. DENV and ZIKV E proteins developed b cellular response.

Prediction of B-cell Epitopes: The Zika and Dengue viral proteomes contained a total of 1529 B-cell epitopes. The ABC pred server predicted the most B-cell epitopes (669,628) for Zika and Dengue. The BC pred server predicted 32 and 44 epitopes for Zika and Dengue, respectively, whereas AAP methods predicted 34, 43 epitopes for Zika and Dengue. 1450 epitopes were studied with the use of a fixed-length pattern (12, 16, 20 mer). The FBC pred flexible length pattern predicted 33 and 46 epitopes for Zika and Dengue. 412 epitopes, 26.94% were 12 mer, 500 epitopes, 32.70% were 16 mer, and 617 epitopes, 40.35% were 20 mer developed, respectively. A total of 82,106 epitopes were chosen for Zika and Dengue further study based on a score value of 0.9 or above.

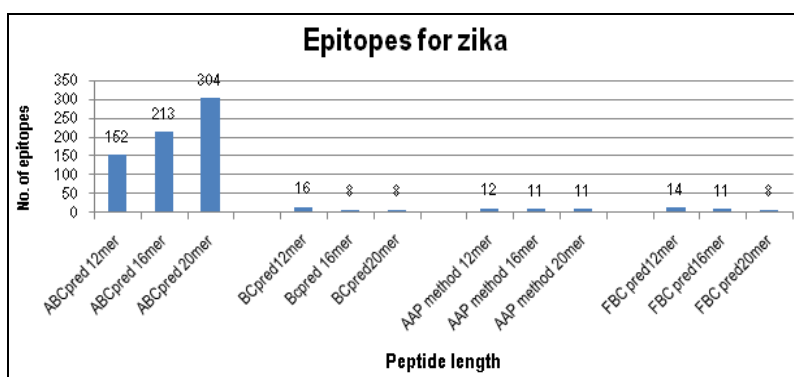


FIG. 1: EPITOPE PREDICTION BY ABCPRED, BCPRED, AAP METHODS AND FBCPRED FOR ZIKA

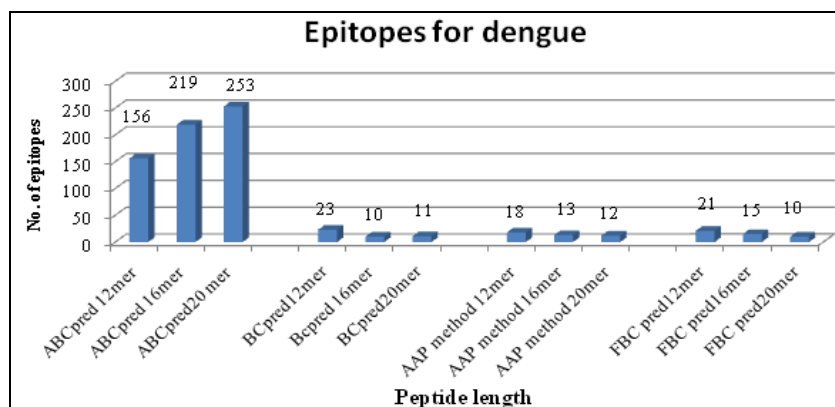


FIG. 2: EPIOTOPE PREDICTION BY ABCPRED FOR BCPRED, AAP METHODS AND FBCPRED FOR DENGUE

Predictions of Instability Index Aliphatic Index and GRAVY Analysis: For ZIKV, 66 stable epitopes were utilised, with 16 epitopes being removed. In addition, 73 stable epitopes were utilized, with 33 epitopes for DENV removed. The thermal stability of ZIKV and DENV will be

improved by all super Aliphatic index values safeguarded inside aspect by examination. GRAVY research revealed 58 and 89 B cell epitopes for ZIKV and DENV, respectively, with a greater terrible value and stability score 45, 57 B cell epitopes finalized for ZIKV and DENV.

TABLE 1: PREDICTIONS OF GRAVY, ALIPHATIC INDEX AND INSTABILITY INDEX VALUE FOR ZIKA VIRUS

Start position	Sequence	Score	Gravy	Aliphatic index	instability index	Result	Name of tool
327	TVEVQYAGTDGPKVVP	0.94	-0.263	60.62	16.18	Stable	ABC 16 mer
165	AKVEVTPNSPRAEATL	0.94	-0.419	79.38	21.51	Stable	ABC 16 mer
156	TGHETDENRAKVEVTP	0.93	-1.438	42.5	-6.54	Stable	ABC pred 16 mer
183	FGSLGLDCEPRTGLDF	0.91	0.013	73.12	6.68	Stable	ABC 16 mer
98	DRGWGNGCGLFG	0.999	-0.442	32.5	-22.15	Stable	BC Pred 12 mer
332	YAGTDGPKVPA	0.995	-0.258	40.83	40.57	Unstable	BC Pred 12 mer
177	EATLGGFGSLGL	0.984	0.783	105.83	19.45	Stable	BC Pred 12 mer
227	AGADTGTPHWNN	0.974	-1.233	16.67	-23.78	Stable	BC Pred 12 mer
74	CPTQGEAYLDKQ	0.968	-1.15	40.83	25.31	Stable	BC Pred 12 mer
348	DMQTLTPVGRLI	0.948	0.275	121.67	11.86	Stable	BC Pred 12 mer
164	RAKVEVTPNSPR	0.944	-1.2	56.67	25.35	Stable	BC Pred 12 mer
163	NRAKVEVTPNSPRAEA	0.998	-1.113	55	21.51	Stable	BC pred 16 mer
328	VEVQYAGTDGPKVPA	0.996	-0.106	66.88	28.21	Stable	BC pred 16 mer
96	LVDREGWNGCGLFGKG	0.991	-0.1	66.88	-34.12	Stable	BC pred 16 mer
227	AGADTGTPHWNNKEAL	0.99	-1.038	43.12	-0.53	Stable	BC pred 16 mer
72	SRCPTQGEAYLDKQSD	0.986	-1.462	30.63	60.98	Unstable	BC pred 16 mer
390	GVDKKITHHWHRSGS	0.97	-1.319	42.5	33.92	Stable	BC pred 16 mer
425	GDTAWDFGSGVGVFNS	0.947	0.031	42.5	-2.33	Stable	BC pred 16 mer
359	ITANPVITESTENSKM	0.922	-0.394	73.12	76.1	Unstable	BC pred 16 mer
220	DIPLPWHAGADTGTPHWNNK	0.988	-1.06	49	20.16	Stable	BC pred 20 mer
160	TDENRAKVEVTPNSPRAEAT	0.985	-1.31	44	19.21	Stable	BC pred 20 mer
328	VEVQYAGTDGPKVPAQMAV	0.98	0.135	73	30.74	Stable	BC pred 20 mer
378	LDPPFGDSYIVIGVGDKIT	0.962	0.15	107	21.41	Stable	BC pred 20 mer
118	VEVQYAGTDGPKVPAQMAV	0.962	-1.02	39	34.19	Stable	BC pred 20 mer
66	SDMASDSRCPTQGEAYLDKQ	0.947	-1.2	29.5	44.61	Unstable	BC pred 20 mer
30	CVTVMAQDKPTV	1	0.408	80.83	8.78	Stable	AAP methods 12 mer
252	RQTVVVLGSQEG	1	-0.075	105	57.32	Unstable	AAP methods 12 mer
67	DMASDSRCPTQG	1	-1.092	8.33	61.32	Unstable	AAP methods 12 mer
164	RAKVEVTPNSPR	1	-1.2	56.67	25.35	Stable	AAP methods 12 mer
94	RTLVDREGWNGC	1	-0.692	56.67	-34.68	Stable	AAP methods 12 mer

378	LDPPFGDSYIVI	1	0.592	121.67	50.24	Unstable	mer AAP methods 12
336	DGPCKVPAQMAV	1	0.158	65	38.19	Stable	mer AAP methods 12
360	TANPVITESTEN	1	-0.667	65	70.97	Unstable	mer AAP methods 12
13	EGMSGGTWVDVV	1	0.325	72.5	16.87	Stable	mer AAP methods 12
199	SDLYYLTMNNKH	1	-1.017	65	39.18	Stable	mer AAP methods 12
46	VTTTVSNMAEVR	1	0.158	80.83	12.38	Stable	mer AAP methods 12
452	AFKSLFGGMSWF	1	0.725	40.83	43.54	Unstable	mer AAP methods 12
328	VEVQYAGTDGPCKVPA	1	-0.106	66.88	28.21	Stable	mer AAP methods 16
353	TPVGRLITANPVITES	1	0.312	115.62	50.42	Unstable	mer AAP methods 16
433	SVGGVFNSLGKGIHQI	1	0.419	109.38	3.57	Stable	mer AAP methods 16
91	VCKRTLVDRGWNGCG	1	-0.369	60.62	-3.13	Stable	mer AAP methods 16
392	GDKKITHHWHRSGSTI	1	-1.319	48.75	39.23	Stable	mer AAP methods 16
70	SDSRCPTQGEAYLDKQ	1	-1.462	30.63	45.56	Unstable	mer AAP methods 16
9	RDFVEGMSGGTWVDVV	1	0.181	72.5	10.44	Stable	mer AAP methods 16
147	QHSGMIVNDTGHETDE	1	-1.238	42.5	-10.63	Stable	mer AAP methods 16
371	NSKMMLELDPPFGDSY	1	-0.637	48.75	64.06	Unstable	mer AAP methods 16
207	NNKHVLVHKEWFHDIP	1	-1.219	66.88	33.69	Stable	mer AAP methods 16
255	VVVLGSQEGAVHTALA	0.999	1.081	140	23.43	Stable	mer AAP methods 16
162	ENRAKVEVTPNSPRAEATLG	1	-0.93	63.5	19.21	Stable	mer AAP methods 20
346	AVDMQTLTPVGRLITANPVI	1	0.7	131.5	11.79	Stable	mer AAP methods 20
317	VPAETLHGTVTVEVQYAGTD	1	0.025	87.5	18.91	Stable	mer AAP methods 20
141	LSVHGSQHSGMIVNDTGHET	1	-0.5	68	-2.06	Stable	mer AAP methods 20
13	EGMSGGTWVDVVLEHGCVT	1	0.31	77.5	3.32	Stable	mer AAP methods 20
92	CKRTLVDRGWNGCGLFGKG	1	-0.41	53.5	-8.99	Stable	mer AAP methods 20
379	DPPFGDSYIVIGVGDKKITH	1	-0.2	87.5	21.41	Stable	mer AAP methods 20
61	YEASISDMASDSRCPTQGEA	0.991	-0.745	34.5	33.25	Stable	mer AAP methods 20
220	DIPLPWHAGADTGTPHWNNK	0.986	-1.06	49	20.16	Stable	mer AAP methods 20
37	DKPTVDIELVTTTVSNMAEV	0.946	0.11	102	17.86	Stable	mer AAP methods 20
167	VEVTPNSPRAEA	1	-0.642	65	32.42	Stable	mer FBC Pred 12
332	YAGTDGPCKVPA	0.999	-0.258	40.83	40.57	Unstable	mer FBC Pred 12
376	LELDPPFGDSYI	0.986	-0.108	97.5	57.32	Unstable	mer FBC Pred 12

25	LEHGGCVTVMAQ	0.984	0.558	89.17	1.45	Stable	FBC Pred 12 mer
228	GADTGTPHWNNK	0.981	-1.708	8.33	-4.04	Stable	FBC Pred 12 mer
79	EAYLDKQSDTQY	0.979	-1.658	40.83	33.35	Stable	FBC Pred 12 mer
186	LGLDCEPRTGLD	0.954	-0.35	97.5	11.86	Stable	FBC Pred 12 mer
98	DRGWGNGCGLFG	0.944	-0.442	32.5	-22.15	Stable	FBC Pred 12 mer
152	IVNDTGHETDEN	0.924	-1.442	56.67	-17.51	Stable	FBC Pred 12 mer
165	AKVEVTPNSPRAEATL	1	-0.419	79.38	21.51	Stable	FBC Pred 16 mer
331	QYAGTDGPCKVPAQMA	1	-0.4	36.88	35.93	Stable	FBC Pred 16 mer
24	VLEHGGCVTVMAQDKP	0.997	0.119	85	5.61	Stable	FBC Pred 16 mer
224	PWHAGADTGTPHWNNK	0.996	-1.525	12.5	12.47	Stable	FBC Pred 16 mer
374	MMLELDPPFGDSYIVI	0.993	0.7	115.62	38.38	Stable	FBC Pred 16 mer
74	CPTQGEAYLDKQSDTQ	0.988	-1.394	30.63	34.84	Stable	FBC Pred 16 mer
182	GFGSLGLDCEPRTGLD	0.949	-0.188	73.12	11.39	Stable	FBC Pred 16 mer
144	HGSQHSGMIVNDTGHE	0.937	-1.031	42.5	-5.07	Stable	FBC Pred 16 mer
159	ETDENRAKVEVTPNSPRAEA	1	-1.45	44	19.21	Stable	FBC Pred 20 mer
327	TVEVQYAGTDGPCKVPAQMA	0.999	-0.11	58.5	30.74	Stable	FBC Pred 20 mer
222	PLPWHAGADTGTPHWNNKEA	0.999	-1.195	34.5	21.6	Stable	FBC Pred 20 mer
24	VLEHGGCVTVMAQDKPTVDI	0.996	0.32	102	-1.03	Stable	FBC Pred 20 mer
66	SDMASDSRCPTQGEAYLDKQ	0.993	-1.2	29.5	44.61	Unstable	FBC Pred 20 mer
181	GGFSLGLDCEPRTGLDFSD	0.991	-0.245	58.5	13.52	Stable	FBC Pred 20 mer
370	ENSKMMLELDPPFGDSYIVI	0.991	-0.025	92.5	49.01	Unstable	FBC Pred 20 mer

TABLE 2: PREDICTIONS OF GRAVY, ALIPHATIC INDEX AND INSTABILITY INDEX VALUE FOR DENGUE VIRUS

Start position	Sequence	Score	Gravy	Aliphatic index	instability index	Result	Name of tool
317	HGTIVIRVQYEGDGSP	0.94	-0.400	85.00	10.32	Stable	ABC Pred 16 mer
178	YGTVTMECSPTGLDF	0.94	-0.181	42.50	33.65	Stable	ABC Pred 16 mer
58	KYCIEAKLNTTTASR	0.9	-0.613	61.25	28.87	Stable	ABC Pred 16 mer
219	PWLPGADTQGSNWIQK	0.9	-0.944	55.00	-3.88	Stable	ABC Pred 16 mer
98	DRGWGNGCGLFG	0.999	-0.442	32.50	-22.15	Stable	BC Pred 12 mer
386	QLKLSWFKKGS	0.999	-0.708	65.00	-8.53	Stable	BC Pred 12 mer
64	KLTNTTTASRCP	0.988	-0.750	40.83	14.59	Stable	BC Pred 12 mer
367	IEAEPFPGDSYI	0.986	-0.217	73.33	109.98	Unstable	BC Pred 12 mer
354	VNPIVTEKDSPV	0.903	-0.167	105.00	77.38	Unstable	BC Pred 12 mer
64	KLTNTTTASRCPTQGE	1	-1.069	30.63	4.02	Stable	BC Pred 16 mer
216	LPLPWLPGADTQGSNW	0.998	-0.388	79.38	20.20	Stable	BC Pred 16 mer
363	SPVNIEAEPFPGDSYI	0.995	-0.269	73.12	151.95	Unstable	BC Pred 16 mer
321	VIRVQYEGDGSPCKIP	0.995	-0.319	85.00	15.59	Stable	BC Pred 16 mer
97	VDRGWGNGCGLFGKGG	0.993	-0.362	42.50	-26.41	Stable	BC Pred 16 mer
386	QLKLSWFKKGSIGQM	0.991	-0.375	73.12	-3.90	Stable	BC Pred 16 mer
160	KEVKVTPQSSITEAEL	0.971	-0.494	91.25	60.54	Unstable	BC Pred 16 mer
177	GYGTVTMECSPTGLD	0.958	-0.381	42.50	33.06	Stable	BC Pred 16 mer
141	ITPHSGEENAVGNDTG	0.94	-0.950	48.75	-0.28	Stable	BC Pred 16 mer
25	LEHGSVCVTTMAKNKPT	0.93	-0.544	48.75	26.34	Stable	BC Pred 16 mer
382	VEPGQLKLSWFKKGSIGQM	0.999	-0.365	73.00	8.51	Stable	BC Pred 20 mer
64	KLTNTTTASRCPTQGEPSLN	0.999	-0.960	44.00	24.48	Stable	BC Pred 20 mer
217	PLPWLPGADTQGSNWIQKET	0.994	-0.855	63.50	8.53	Stable	BC Pred 20 mer
353	TVNPIVTEKDSVNIEAAPP	0.987	-0.505	87.50	113.63	Unstable	BC Pred 20 mer
317	HGTIVIRVQYEGDGSPCKIP	0.978	-0.245	87.50	0.80	Stable	BC Pred 20 mer
96	MVDRGWGNGCGLFGKGGIVT	0.914	0.205	68.00	-31.86	Stable	BC Pred 20 mer
478	SLVLVGVVTLYL	1	2.400	226.67	-4.98	Stable	AAP Methods 12 mer
106	GLFGKGGIVTCA	1	1.117	97.50	-15.92	Stable	AAP Methods 12 mer
79	EPSLNEEQDKRF	1	-2.100	32.50	120.63	Unstable	AAP Methods 12 mer
389	LSWFKKGSIGQ	1	-0.358	65.00	5.62	Stable	AAP Methods 12 mer
150	AVGNDTGKKGKE	1	-1.450	32.50	-47.38	Stable	AAP Methods 12 mer

121	CKKNMEGKIVQP	1	-0.925	56.67	3.99	Stable	AAP Methods 12 mer
32	TTMAKNKPTLDF	1	-0.683	40.83	26.62	Stable	AAP Methods 12 mer
165	TPQSSITEAELT	1	-0.475	73.33	93.93	Unstable	AAP Methods 12 mer
65	LTNTTTASRCPT	1	-0.483	40.83	21.67	Stable	AAP Methods 12 mer
288	RMDKLQLKGMSY	1	-0.86	65.00	44.65	Unstable	AAP Methods 12 mer
324	VQYEGDGSPCKI	1	-0.642	56.67	19.86	Stable	AAP Methods 12 mer
369	AEPFGDSYIII	1	0.450	105.83	73.37	Unstable	AAP Methods 12 mer
355	NPIVTEKDSPVN	1	-0.808	80.83	77.38	Unstable	AAP Methods 12 mer
93	KHSMVDRGWGNG	1	-1.283	24.17	-34.68	Stable	AAP Methods 12 mer
13	EGVSGGSWVDIV	1	0.533	105.00	-7.23	Stable	AAP Methods 12 mer
249	DVVVLGSQEGAM	1	0.667	113.33	34.19	Stable	AAP Methods 12 mer
221	LPGADTQGSNWI	1	-0.433	73.33	-16.38	Stable	AAP Methods 12 mer
47	KTEAKQPATLRK	1	-1.567	49.17	93.10	Unstable	AAP Methods 12 mer
100	GWGNGCGLFGKGGIVT	1	0.375	66.88	-22.91	Stable	AAP methods 16 mer
384	PGQLKLSWFKKGSSIG	1	-0.400	73.12	-3.90	Stable	AAP methods 16 mer
67	NTTTASRCPTQGEPSL	1	-0.931	30.63	42.79	Unstable	AAP methods 16 mer
357	IVTEKDSPVNIEAAPP	1	-0.531	91.25	141.34	Unstable	AAP methods 16 mer
321	VIRVQYEGDGSPCKIP	1	-0.319	85.00	15.59	Stable	AAP methods 16 mer
245	AKKQDVVVLGSQEGAM	1	-0.094	91.25	54.98	Unstable	AAP methods 16 mer
47	KTEAKQPATLRKYCIE	1	-1.038	61.25	99.79	Unstable	AAP methods 16 mer
19	SWVDIVLEHGSCVTTM	1	0.675	103.12	-11.47	Stable	AAP methods 16 mer
216	LPLPWLPGADTQGSNW	1	-0.388	79.38	20.20	Stable	AAP methods 16 mer
144	HSGEENAVGNDTGKHG	1	-1.556	24.38	-12.06	Stable	AAP methods 16 mer
177	GYGTVTMECSPRTGLD	1	-0.381	42.50	33.06	Stable	AAP methods 16 mer
119	FTCKKNMEGKIVQPEN	1	-1.000	42.50	16.36	Stable	AAP methods 16 mer
437	HQVFGAIYGAAAFSGVS	0.999	0.819	79.38	-11.26	Stable	AAP methods 16 mer
385	GQLKLSWFKKGSSIGQMFET	1	-0.390	58.50	-1.12	Stable	AAP methods 20 mer
314	ETQHGTIVIRVQYEGDGSPC	1	-0.580	68.00	2.71	Stable	AAP methods 20 mer
349	GRLITVNPIVTEKDSPVNIE	1	-0.090	121.50	94.37	Unstable	AAP methods 20 mer
37	NKPTLDFELIKTEAKQPATL	1	-0.610	88.00	50.29	Unstable	AAP methods 20 mer

255	SQEGAMHTALTGATEIQMSS	1	-0.250	54.00	91.75	Unstable	AAP methods 20 mer
61	IEAKLTNTTTASRCPTQGEP	1	-0.795	49.00	36.82	Stable	AAP methods 20 mer
139	IVITPHSGEENAVGNDTGKH	1	-0.680	73.00	-6.71	Stable	AAP methods 20 mer
96	MVDRGWGNGCGLFGKGGIVT	1	0.205	68.00	-31.86	Stable	AAP methods 20 mer
187	PRTGLDFNEMVLLQMENKAW	1	-0.450	78.00	25.86	Stable	AAP methods 20 mer
5	GISNRDFVEGVSGGSWVDIV	0.997	0.260	97.00	-8.35	Stable	AAP methods 20 mer
118	MFTCKKNMEGKIVQPENLEY	0.985	-0.755	53.50	15.09	Stable	AAP methods 20 mer
478	SLVLVGVVTLYL	1	2.400	226.67	-4.98	Stable	FBC Pred 12 mer
106	GLFGKGGIVTCA	1	1.117	97.50	-15.92	Stable	FBC Pred 12 mer
79	EPSLNEEQDKRF	1	-2.100	32.50	120.63	Unstable	FBC Pred 12 mer
63	AKLTNTTTASRC	1	-0.467	49.17	-1.46	Stable	FBC Pred 12 mer
388	KLSWFKKGSIG	1	-0.392	65.00	-1.46	Stable	FBC Pred 12 mer
142	TPHSGEENAVGN	0.999	-1.258	32.50	15.90	Stable	FBC Pred 12 mer
78	GEPNLNEEQDKR	0.999	-2.367	32.50	114.34	Unstable	FBC Pred 12 mer
217	PLPWLPGADTQG	0.998	-0.400	73.33	16.19	Stable	FBC Pred 12 mer
367	IEAEPFGDSYI	0.993	-0.217	73.33	109.98	Unstable	FBC Pred 12 mer
307	KVVKEIAETQHG	0.993	-0.658	89.17	0.82	Stable	FBC Pred 12 mer
47	KTEAKQPATLRK	0.988	-1.567	49.17	93.10	Unstable	FBC Pred 12 mer
265	TGATEIQMSSGN	0.982	-0.508	40.83	72.71	Unstable	FBC Pred 12 mer
25	LEHGSCVTTMAK	0.981	0.083	65.00	18.33	Stable	FBC Pred 12 mer
181	VTMECSPTGLD	0.98	-0.275	56.67	61.97	Unstable	FBC Pred 12 mer
353	TVNPIVTEKDSP	0.965	-0.575	80.83	61.33	Unstable	FBC Pred 12 mer
160	KEVKVTPQSSIT	0.959	-0.542	80.83	61.33	Unstable	FBC Pred 12 mer
241	KNPHAKKQDVVV	0.951	-1.050	80.83	42.55	Unstable	FBC Pred 12 mer
321	VIRVQYEGDGSP	0.948	-0.550	80.83	33.22	Stable	FBC Pred 12 mer
98	DRGWGNGCGLFG	0.944	-0.442	32.50	-22.15	Stable	FBC Pred 12 mer
463	ITWIGMNSRSTS	0.855	-0.183	65.00	49.31	Unstable	FBC Pred 12 mer
63	AKLTNTTTASRCPTQG	1	-0.738	36.88	8.73	Stable	FBC Pred 16 mer
142	TPHSGEENAVGNDTGK	1	-1.475	24.38	-5.58	Stable	FBC Pred 16 mer
386	QLKLSWFKKGSIGQM	1	-0.375	73.12	-3.90	Stable	FBC Pred 16 mer
307	KVVKEIAETQHGTIVI	0.999	0.287	133.75	-7.50	Stable	FBC Pred 16 mer
216	LPLPWLPGADTQGSNW	0.999	-0.388	79.38	20.20	Stable	FBC Pred 16 mer
365	VNIEAEPFGDSYIII	0.996	0.444	121.88	112.45	Unstable	FBC Pred 16 mer
25	LEHGSCVTTMAKNKPT	0.995	-0.544	48.75	26.34	Stable	FBC Pred 16 mer
261	HTALTGATEIQMSSGN	0.984	-0.275	61.25	52.32	Unstable	FBC Pred 16 mer
237	LVTFKNPHAKKQDVVV	0.963	-0.156	103.12	27.43	Stable	FBC Pred 16 mer
80	PSLNEEQDKRFVCKHS	0.957	-1.431	42.50	80.93	Unstable	FBC Pred 16 mer
177	GYGTVTMECSPTGLD	0.95	-0.381	42.50	33.06	Stable	FBC Pred 16 mer
63	AKLTNTTTASRCPTQGEPSL	1	-0.695	49.00	24.48	Stable	FBC Pred 20 mer
139	IVITPHSGEENAVGNDTGKH	1	-0.680	73.00	-6.71	Stable	FBC Pred 20 mer

307	KVVKEIAETQHGTVIRVQY	1	-0.025	121.50	-7.77	Stable	FBC Pred 20 mer
380	IGVEPGQLKLSWFKKGSSIG	1	-0.080	92.50	8.51	Stable	FBC Pred 20 mer
214	LDLPLPWLPGADTQGSNWIQ	0.999	-0.245	102.50	18.16	Stable	FBC Pred 20 mer
353	TVNPIVTEKDSPVNIEAEP	0.996	-0.505	87.50	113.63	Unstable	FBC Pred 20 mer
23	IVLEHGSCVTTMAKNKPTLD	0.994	0.015	92.50	18.82	Stable	FBC Pred 20 mer
256	QEGAMHTALTGATEIQMSSG	0.982	-0.230	54.00	82.12	Unstable	FBC Pred 20 mer

Consensus Epitope Prediction: BC pred predicted 1, 4, and 3 consensus epitopes (12mer, 16mer, 20mer) respectively. 1, 3 (16mer, 20mer) consensus epitopes were predicted by AAP method. FBC pred predicted Most of the 2, 5, 4 consensus epitopes (12mer, 16mer, 20mer) respectively. In an analysis of DENV protein, 2 (16mer) ABC predicted consensus epitopes pred

whereas BC pred predicted 4, 4 consensus epitopes (16mer, 20mer) respectively. 4, 5 (16mer, 20mer) consensus epitopes were predicted by AAP method. FBC pred predicted Most of the 1, 3 and 4 consensus epitopes (12mer, 16mer, 20mer). 24 (47%) consensus epitopes have a confirmed length of 16mer. There were 23 (45%) 20 mer and 4 (8%) 12 mer, respectively.

TABLE 3: PREDICTIONS OF CONSENSUS EPITOPES FOR ZIKA AND DENGUE

Sequence	Zika			Sequence	Dengue		
	Score	Epitope length	Tool		Score	Epitope length	Tool
AKVEVTPNSPRAEATL	0.94	16 mer	ABC pred	HGTIVIRVQYEG DGSP	0.94	16 mer	ABC pred
RAKVEVTPNSPR	0.944	12 mer	BC pred	PWLPGADTQGS NWIQK	0.9	16 mer	ABC pred
NRAKVEVTPNSPRAEA	0.998	16 mer	BC pred	KLNTTTASRCP TQGE	1	16 mer	BC pred
VEVQYAGTDGPCKVPA	0.996	16 mer	BC pred	VIRVQYEGDGSP CKIP	0.995	16 mer	BC pred
LVDRGWGNGCGLFGK G	0.991	16 mer	BC pred	VDRGWGNGCGL FGKGG	0.993	16 mer	BC pred
AGADTGTPHWNNKEA L	0.99	16 mer	BC pred	QLKLSWFKKGSS IGQM	0.991	16 mer	BC pred
DIPLPWHAGADTGT PHWNNK	0.988	20 mer	BC pred	VEPGQLKLSWFK KGSSIGQM	0.999	20 mer	BC pred
TDENRAKVEVTPNSPR AEAT	0.985	20 mer	BC pred	KLNTTTASRCP TQGEPSLN	0.999	20 mer	BC pred
VEVQYAGTDGPCKVPA QMAV	0.962	20 mer	BC pred	PLPWLPGADTQ SNWIQKET	0.994	20 mer	BC pred
VCKRTLVD RGWNGC G	1	16 mer	AAP meth	HGTIVIRVQYEG DGSPCKIP	0.987	20 mer	BC pred
ENRAKVEVTPNSPRAE ATLG	1	20 mer	AAP meth	PGQLKLSWFKK GSSIG	1	16 mer	AAP meth
LSVHGSQHS GMIVNDT GHET	1	20 mer	AAP meth	LPLPWLPGADTQ GSNW	1	16 mer	AAP meth
CKRTLVD RGWNGC G	1	20 mer	AAP meth	GYGTVTMECS PR TGLD	1	16 mer	AAP meth
VEVTPNSPRAEA	1	12 mer	FBC Pred	FTCKKNMEGKIV QPEN	1	16 mer	AAP meth
DRGWGNGCGLFG	0.944	12 mer	FBC Pred	GQLKLSWFKK GSSIGQMFET	1	20mer	AAP meth
QYAGTDGPCKVPA QMA	1	16 mer	FBC Pred	ETQHGTVIRVQ YEGDGSPC	1	20 mer	AAP meth

PWHAGADTGTPHWNN K	0.996	16 mer	FBC Pred	IEAKLTNTTTASR CPTQGEP	1	20 mer	AAP meth
CPTQGEAYLDKQSDTQ	0.988	16 mer	FBC Pred	IVITPHSGEENAV GNDTGKH	1	20 mer	AAP meth
GFGSLGLDCEPRTGLD	0.949	16 mer	FBC Pred	MFTCKKNMEGK IVQPENLEY	0.985	20 mer	AAP meth
HGSQHSGMIVNDTGHE	0.937	16 mer	FBC Pred	DRGWGNGCGLF G	0.944	12mer	FBC pred
ETDENRAKVEVTPNSP RAEA	1	20 mer	FBC Pred	AKLTNTTTASRC PTQG	1	16 mer	FBC pred
TVEVQYAGTDGPCKVP AQMA	0.999	20 mer	FBC Pred	TPHSGEENAVGN DTGK	1	16 mer	FBC pred
PLPWHAGADTGTPHW NNKEA	0.999	20 mer	FBC Pred	LEHGSCVTTMA KNKPT	0.995	16 mer	FBC pred
GGFGLSLGLDCEPRTGL DFSD	0.991	20 mer	FBC Pred	AKLTNTTTASRC PTQGEPSL	1	20 mer	FBC pred
				KVVKEIAETQHG TIVIRVQY	1	20 mer	FBC pred
				IGVEPGQLKLSW FKKGSSIG	1	20 mer	FBC pred
				LDLPLPWLPGAD TQGSNWIQ	0.999	20 mer	FBC pred

Predictions of Antigenicity and Allergenicity: Allergen FP predicts non-allergic effects of the final vaccination formulation. Antigenicity and allergenicity were evaluated on 24, 27 Zika and Dengue consensus epitopes. BC pred had the most antigenic and Non Allergenic 10 epitopes (35.71%), followed by FBC pred 9 epitopes (32.14%) and 7, 2 epitopes (25 %, 7.14%) from the AAP Methods and ABC pred.

TABLE 4: PREDICTIONS OF ANTIGENICITY AND ALLERGENICITY FOR ZIKA VIRUS

Epitope length	sequence	Vaxijen score	Antigenicity	Allergenicity	Result	Tool
16 mer	AKVEVTPNSPRAEATL	0.6917	Antigen	0.55	Non Allergen	ABC pred
12 mer	RAKVEVTPNSPR	0.6219	Antigen	0.59	Non Allergen	BC pred
16 mer	NRAKVEVTPNSPRAEA	0.5131	Antigen	0.55	Non Allergen	BC pred
16 mer	VEVQYAGTDGPCKVPA	0.2715	Non Antigen	0.52	Non Allergen	BC pred
16 mer	LVDRGWGNGCGLFGKG	0.4848	Antigen	0.58	allergen	BC pred
16 mer	AGADTGTPHWNNKEAL	0.8268	Antigen	0.57	Non Allergen	BC pred
20 mer	DIPLPWHAGADTGTPHWNNK	0.7757	Antigen	0.61	allergen	BC pred
20 mer	TDENRAKVEVTPNSPRAEAT	0.5860	Antigen	0.63	Non Allergen	BC pred
20 mer	VEVQYAGTDGPCKVPAQMAV	0.4222	Antigen	0.6	Non Allergen	BC pred
16 mer	VCKRTLVDGRWGNGCG	0.4492	Non Antigen	0.53	Non Allergen	AAP methods
20 mer	ENRAKVEVTPNSPRAEATLG	0.7850	Antigen	0.63	Non Allergen	AAP methods
20 mer	LSVHGSQHSGMIVNDTGHE	0.1743	Non Antigen	0.67	allergen	AAP methods
20 mer	CKRTLVDGRWGNGCGLFGKG	0.1147	Non Antigen	0.62	Non Allergen	AAP methods
12 mer	VEVTPNSPRAEA	0.5990	Antigen	0.61	Non Allergen	FBC Pred
12 mer	DRGWGNGCGLFG	0.1661	Non Antigen	0.65	Non Allergen	FBC Pred

16 mer	QYAGTDGPKVPAQMA	0.1466	Non Antigen	0.59	allergen	FBC Pred
16 mer	PWHAGADTGTPHWNNK	0.8358	Antigen	0.58	Non	FBC Pred
16 mer	CPTQGEAYLDKQSDTQ	0.3721	Non Antigen	0.61	Allergen	FBC Pred
16 mer	GFGSLGLDCEPRTGLD	1.3220	Antigen	0.58	Non	FBC Pred
16 mer	HGSQHSQMIVNDTGHE	0.0332	Non Antigen	0.65	Allergen	FBC Pred
20 mer	ETDENRAKVEVTPNSPRAEA	0.4149	Antigen	0.62	allergen	FBC Pred
20 mer	TVEVQYAGTDGPKVPAQMA	0.1270	Non Antigen	0.69	allergen	FBC Pred
20 mer	PLPWHAGADTGTPHWNNKEA	0.9325	Antigen	0.61	Non	FBC Pred
20 mer	GGFSLGLDCEPRTGLDFSD	1.5554	Antigen	0.64	Allergen	FBC Pred

TABLE 5: PREDICTIONS OF ANTIGENICITY AND ALLERGENICITY FOR DENGUE VIRUS

Epitope length	Sequence	Vaxi Jen score	Antigenicity	Allergenicity	Result	Tool
16 mer	HGTIVIRVQYEGDG SP	0.9363	Antigen	0.55	Non Allergen	ABC pred
16 mer	PWLPGADTQGSNWI QK	0.2599	Non Antigen	0.57	Non Allergen	ABC pred
16 mer	KLTNTTTASRCPTQ GE	0.8589	Antigen	0.59	Non Allergen	BC pred
16 mer	VIRVQYEGDGPCKI P	0.2868	Non Antigen	0.5	allergen	BC pred
16 mer	VDRGWGNGCGLFG KGG	0.6497	Antigen	0.6	Non Allergen	BC pred
16 mer	QLKLSWFKKGSSIG QM	1.0680	Antigen	0.63	Non Allergen	BC pred
20 mer	VEPGQLKLSWFKKG SSIGQM	1.0872	Antigen	0.67	Non Allergen	BC pred
20 mer	KLTNTTTASRCPTQ GEP LN	0.8825	Antigen	0.66	allergen	BC pred
20 mer	PLPWLPGADTQGSN WIQKET	0.5089	Antigen	0.65	Non Allergen	BC pred
20 mer	HGTIVIRVQYEGDG SPCKIP	0.5904	Antigen	0.62	allergen	BC pred
16 mer	PGQLKLSWFKKGSS IG	1.0629	Antigen	0.61	Non Allergen	AAP methods
16 mer	LPLPWLPGADTQGS NW	0.5417	Antigen	0.54	Non Allergen	AAP methods
16 mer	GYGTVTMECSPRTG LD	0.7913	Antigen	0.58	Non Allergen	AAP methods
16 mer	FTCKKNMEGKIVQP EN	0.3722	Non Antigen	0.61	Non Allergen	AAP methods
20mer	GQLKLSWFKKGSSI GQMFET	0.6671	Antigen	0.68	Non Allergen	AAP methods
20 mer	ETQHGTIVIRVQYE GDGSPC	0.9347	Antigen	0.61	Non Allergen	AAP methods
20 mer	IEAKLTNTTTASRCP TQGE P	1.0315	Antigen	0.65	Non Allergen	AAP methods
20 mer	IVITPWSGEENAVGN DTGKH	0.1396	Non Antigen	0.65	Non Allergen	AAP methods
20 mer	MFTCKKNMEGKIV QPENLEY	0.5536	Antigen	0.64	allergen	AAP methods
12mer	DRGWGNGCGLFG	0.1661	Non Antigen	0.65	Non Allergen	FBC pred

16 mer	AKLTNTTTASRCPT QG	0.8092	Antigen	0.6	Non Allergen	FBC pred
16 me	TPHSGEENAVGNDT GK	-0.1015	Non Antigen	0.59	Non Allergen	FBC pred
16 me	LEHGSCVTTMAKN KPT	0.4300	Antigen	0.56	Non Allergen	FBC pred
20 mer	AKLTNTTTASRCPT QGEPSL	0.9032	Antigen	0.65	Non Allergen	FBC pred
20 mer	KVVKEIAETQHGTI VIRVQY	0.7464	Antigen	0.67	allergen	FBC pred
20 mer	IGVEPGQLKLSWFK KGSSIG	1.3301	Antigen	0.65	Non Allergen	FBC pred
20 mer	LDLPLPWLPGADTQ GSNWIQ	0.3370	Non Antigen	0.65	allergen	FBC pred

Predictions of Toxicity, Hydrophobicity, Hydrophobicity, Charge and Mol. Weight of Zika: All epitopes for ZV and DV in my study were non-toxic, hydrophobic and hydrophobic with Charge and Molecular Weight.

TABLE 6: PREDICTIONS OF TOXICITY, HYDROPHOBICITY, HYDROPATHICITY, CHARGE, MOL. WT. OF ZIKA

Peptide Sequence	SVM Score	Prediction	Charge	Mol. Wt.	Hydrophobicity	Hydrophobicity
AKVEVTPNSPRAEATL	-1.05	Non-Toxin	0.00	1683.11	-0.20	-0.42
RAKVEVTPNSPR	-0.88	Non-Toxin	2.00	1353.70	-0.43	-1.20
NRAKVEVTPNSPRAEA	-0.86	Non-Toxin	1.00	1739.13	-0.37	-1.11
AGADTGTPHWNNKEAL	-0.59	Non-Toxin	-0.50	1682.01	-0.16	-1.04
TDENRAKVEVTPNSPRAEAT	-0.84	Non-Toxin	-1.00	2185.60	-0.38	-1.31
VEVQYAGTDGPKVPAQM AV	-0.91	Non-Toxin	-1.00	2063.66	-0.03	-0.14
ENRAKVEVTPNSPRAEATLG	-1.05	Non-Toxin	0.00	2139.63	-0.30	-0.93
VEVTPNSPRAEA	-1.12	Non-Toxin	-1.00	1269.53	-0.22	-0.64
PWHAGADTGTPHWNNK	-0.58	Non-Toxin	1.00	1789.13	-0.18	-1.52
GFGSLGLDCEPRTGLD	-1.02	Non-Toxin	-2.00	1637.03	-0.09	-0.19
PLPWHAGADTGTPHWNNKE A	-0.80	Non-Toxin	0.00	2199.66	-0.14	-1.20
GGFGLSLDCEPRTGLDFSD	-1.16	Non-Toxin	-3.00	2043.48	-0.08	-0.25

TABLE 7: PREDICTIONS OF TOXICITY, HYDROPHOBICITY, HYDROPATHICITY, CHARGE, MOL.WT. OF DENGUE

Peptide Sequence	SVM Score	Prediction	Charge	Mol.Wt.	Hydrophobicity	Hydrophobicity
HGTIVIRVQYEGDGSP	-0.60	Non-Toxin	-0.50	1728.14	-0.10	-0.40
KLTNTTTASRCPTQGE	-0.98	Non-Toxin	1.00	1708.10	-0.32	-1.07
VDRGWGNGCGLFGK GG	-1.04	Non-Toxin	1.00	1580.00	-0.06	-0.36
QLKLSWFKKGSSIGQ M	-1.14	Non-Toxin	3.00	1838.45	-0.13	-0.38
VEPGQLKLSWFKKGS SIGQM	-1.26	Non-Toxin	2.00	2220.93	-0.10	-0.37
PLPWLPGADTQGSNW IQKET	-0.87	Non-Toxin	-1.00	2238.79	-0.11	-0.86
PGQLKLSWFKKGSSIG	-1.32	Non-Toxin	3.00	1733.29	-0.10	-0.40
LPLPWLPGADTQGSN W	-1.03	Non-Toxin	-1.00	1752.20	0.01	-0.39
GYGTVTMECSPRTGL D	-0.17	Non-Toxin	-1.00	1687.10	-0.13	-0.38
GQLKLSWFKKGSSIG QMFET	-1.27	Non-Toxin	2.00	2272.96	-0.11	-0.39
ETQHGTIVIRVQYEGD GSPC	-0.80	Non-Toxin	-1.50	2189.69	-0.16	-0.58
IEAKLTNTTTASRCPT QGEP	-0.94	Non-Toxin	0.00	2118.63	-0.24	-0.80

AKLTNTTTASRCPTQ G	-0.79	Non-Toxin	2.00	1650.06	-0.26	-0.74
LEHGSCVTTMAKNKP T	-0.34	Non-Toxin	1.50	1717.22	-0.18	-0.54
AKLTNTTTASRCPTQ GEP SL	-0.95	Non-Toxin	1.00	2076.59	-0.23	-0.70
IGVEPGQLKLSWFKK GSSIG	-1.50	Non-Toxin	2.00	2131.82	-0.04	-0.08

Predictions Epitope Cluster: All epitopes were organized into clusters. Alignment TDENRAKVEVTPNSPRAEATLG has the most Consensus epitopes 5, followed by Alignment PLPWHAGADTGTTPHWNNKEAL with 3 Consensus epitopes and Alignment GGFGSLGLDCEPRTGLDFSD with 2 Consensus epitopes for ZIKV. DENV works in a similar way.

Alignment IGVEPGQLKLSWFKKGSSIGQM, VEPGQLKLSWFKKGSSIGQMFET and IEAKLTNTTTASRCPTQGEPSL, had the most consensus epitopes 4, while Alignment LPLPWLPGADTQGSNWIQKET and ETQHGTIVIRVQYEGDGSPC had 2 consensus epitopes.

TABLE 8: PREDICTIONS EPITOPE CLUSTER OF ZIKA

Clique No.	Peptide No.	Alignment	Position	Description	Peptide
1	Consensus	TDENRAKVEVTPNSPRAEATLG	-	-	-
1	1	TDENRAKVEVTPNSPRAEAT--	1	seq9	TDENRAKVEVTPNSPRAEAT
1	2	--ENRAKVEVTPNSPRAEATLG	3	seq13	ENRAKVEVTPNSPRAEATLG
1	3	---NRAKVEVTPNSPRAEA---	4	seq5	NRAKVEVTPNSPRAEA
1	4	----RAKVEVTPNSPR-----	5	seq3	RAKVEVTPNSPR
1	5	-----AKVEVTPNSPRAEATL-	6	seq1	AKVEVTPNSPRAEATL
3	Consensus	PLPWHAGADTGTTPHWNNKEA L	-	-	-
3	1	PLPWHAGADTGTTPHWNNKEA-	1	seq21	PLPWHAGADTGTTPHWNNKEA
3	2	--PWHAGADTGTTPHWNNK---	3	seq17	PWHAGADTGTTPHWNNK
3	3	----AGADTGTTPHWNNKEAL	6	seq7	AGADTGTTPHWNNKEAL
4	Consensus	GGFGSLGLDCEPRTGLDFSD	-	-	-
4	1	GGFGSLGLDCEPRTGLDFSD	1	seq23	GGFGSLGLDCEPRTGLDFSD
4	2	-GFGSLGLDCEPRTGLD---	2	seq19	GFGSLGLDCEPRTGLD
5	Singleton	VEVQYAGTDGPCKVPAQMAV	-	seq11	VEVQYAGTDGPCKVPAQMAV

TABLE 9: PREDICTIONS EPITOPE CLUSTER OF DENGUE

Clique NO.	Peptide No.	Alignment	Position	Description	Peptide
1	Consensus	IGVEPGQLKLSWFKKGSSIGQM	-	-	-
1	1	IGVEPGQLKLSWFKKGSSIG--	1	seq33	IGVEPGQLKLSWFKKGSSIG
1	2	--VEPGQLKLSWFKKGSSIGQM	3	seq7	VEPGQLKLSWFKKGSSIGQM
1	3	----PGQLKLSWFKKGSSIG--	5	seq11	PGQLKLSWFKKGSSIG
1	4	-----QLKLSWFKKGSSIGQM	7	seq26	QLKLSWFKKGSSIGQM
2	Consensus	VEPGQLKLSWFKKGSSIGQMFET	-	-	-
2	1	VEPGQLKLSWFKKGSSIGQM---	1	seq7	VEPGQLKLSWFKKGSSIGQM
2	2	--PGQLKLSWFKKGSSIG----	3	seq11	PGQLKLSWFKKGSSIG
2	3	---GQLKLSWFKKGSSIGQMFET	4	seq17	GQLKLSWFKKGSSIGQMFET
2	4	----QLKLSWFKKGSSIGQM---	5	seq26	QLKLSWFKKGSSIGQM
3	Consensus	IEAKLTNTTTASRCPTQGEPSL	-	-	-
3	1	IEAKLTNTTTASRCPTQGE--	1	seq21	IEAKLTNTTTASRCPTQGE
3	2	--AKLTNTTTASRCPTQGEPSL	3	seq30	AKLTNTTTASRCPTQGEPSL
3	3	---AKLTNTTTASRCPTQG----	3	seq23	AKLTNTTTASRCPTQG
3	4	---KLTNTTTASRCPTQGE---	4	seq3	KLTNTTTASRCPTQGE
4	Consensus	LPLPWLPGADTQGSNWIQKET	-	-	-
4	1	LPLPWLPGADTQGSNW-----	1	seq13	LPLPWLPGADTQGSNW
4	2	-PLPWLPGADTQGSNWIQKET	2	seq9	PLPWLPGADTQGSNWIQKET
5	Consensus	ETQHGTIVIRVQYEGDGSPC	-	-	-

5	1	ETQHGTIVIRVQYEGDGSPC	1	seq19	ETQHGTIVIRVQYEGDGSPC
5	2	---HGTIVIRVQYEGDGSP-	4	seq1	HGTIVIRVQYEGDGSP
6	Singleton	GYGTVTMECSPRTGLD	-	seq15	GYGTVTMECSPRTGLD
7	Singleton	LEHGSCVTTMAKNKPT	-	seq28	LEHGSCVTTMAKNKPT
8	Singleton	VDRGWGNGCGLFGKGG	-	seq5	VDRGWGNGCGLFGKGG

Conservancy Analysis: IEDB tool with sequence identity criteria of 80% chooses homologous protein sets. In my research, we discovered that the epitopes PLPWHAGADTGTPHWNNKEA and GGFGSLGLDCEPRTGLDFSD length 20 mer had 100% Conservancy, but the epitope TDENRAKVEVTPNSPRAEAT length 20 mer has a low Conservancy of 40.74 percent and was eliminated from the vaccine selection procedure for

ZIKV. The DENV epitopes LPLPWLPGADTQGSNW (16mer) and IGVEPGQLKLSWFKKGSIG, VEPGQLKLSWFK-KGSSIGQM and IEAKLTNTTTASRCPTQGE length 20 mer all have 100% conservation. Conservancy 97.30 percent for the remaining epitope ETQHGTIVIRVQYEGDGSPC with a length of 20 mer had been discarded.

TABLE 10: PREDICTIONS OF CONSERVANCY ANALYSIS

Sl.	Epitope length	Name of vectors	Epitope sequence	% of protein sequence matches	Maximum identity	Minimum identity
1.	20	Zika	TDENRAKVEVTPNSPRAEAT	40.74% (22/54)	95.00%	100.00%
2.	20	Zika	PLPWHAGADTGTPHWNNKEA	100.00% (54/54)	100.00%	100.00%
3.	20	Zika	GGFGSLGLDCEPRTGLDFSD	100.00% (54/54)	100.00%	100.00%
4.	20	Dengue	IGVEPGQLKLSWFKKGSIG	100.00% (74/74)	100.00%	100.00%
5.	20	Dengue	VEPGQLKLSWFKKGSIGQM	100.00% (74/74)	100.00%	100.00%
6.	20	Dengue	IEAKLTNTTTASRCPTQGE	100.00% (74/74)	100.00%	100.00%
7.	16	Dengue	LPLPWLPGADTQGSNW	100.00% (74/74)	100.00%	100.00%
8.	20	Dengue	ETQHGTIVIRVQYEGDGSPC	97.30% (72/74)	95.00%	100.00%

DISCUSSION: Defeat dengue viruses. The sudden outbreaks of Zika virus has brought additional difficulties to solve the dengue fever, the antibodies elicited by dengue vaccines also have the potential to augment ZIKV infection. However, like with any other scientific issue, the enormous worldwide outbreak of ZIKV has made it difficult to combat the infections.

Apart from forcing affected individuals to live with these neglected tropical illnesses, they also prevent children from attending school, jeopardise job security, raise the financial burden on nations owing to medical costs, and have a severe impact on developing countries' economies³⁷. When examining the whole ZIKV and DENV proteome, which contained B cell epitopes, we discovered consecutive amino acids³⁸. The adoption of the immunoinformatic technique epitope cluster will open the door for more study into the creation of a

precise ZIKV and DENV synthetic epitope vaccine. The epidemics of DV and ZV inspired much study into flavivirus virology, immunology and vaccinology. Epitope based vaccines provide specific immune responses without causing any adverse effects.

Research has very productive when B cells an emphasis on the E protein. Using different prediction method total 1529 B cell epitopes were predicted from all ZV and DV proteomes in this work. 27 and 24 consensus epitopes were separated for DV and ZV serotypes. The consensus epitopes identified in this work might be beneficial in developing a multipathogenic vaccine. Peptides with high antigenic and non-allergenic values were deemed to be strong B-cell epitopes using the VaxiJen server and the Allergen FP tool. 5 Alignment of DV and 3 Alignment of ZV had more Consensus epitopes.

Epitopes PLPWHAGADTGTPHWNNKEA and GGFGSLGL-DCEPRTGLDFSD had 100 percent conservation for ZIKV. Other DENV epitopes, including the 16-mer LPLPWLPADTQGSNW and the 20-mer IGVEPGQLKLSWFKKGSSIG VEPGQLKLSWFKKGSSIGQM and IEAKLTNTTTASRCPTQGEF, all have 100% conservation.

CONCLUSION: Based on this knowledge, we provided possible ZIKV and DENV epitopes. In our research, the anticipated immunogenic epitopes PLPWHAGADTGTPHWNNKEA, GGFGSLGLDCEPRTGLDFSD of ZIKV and IGVEPGQLKLSWFKKGSSIG, VEPGQLKLSWFKKGSSIGQM and IEAKLTNTTTASRCPTQGEF, LPLPWLPADTQGSNW of DENV were the most Two-pronged methods combining human preventative vaccinations with vector blockade to stop transmission cycles are being used to combat mosquito-borne viruses.

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