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CYTOCHROME B ANALYSIS FOR HYDROPHOBICITY, SURFACE ACCESSIBILITY AND ANTIGENICITY

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ABSTRACT

In this study Cytochrome b (mitochondrion) protein has been used to investigate its role in antigenicity. The cytochrome b protein sequences (367 aa protein) is analyzed through different types B- cell epitope prediction methods.We found that the region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because the terminal regions of antigen protein is solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein. It was seen that an antigen protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility. The predicted antigenic protein segments of Cytochrome b (mitochondrion) can take active part in the host immune reactions. In future study the predicted antigenic protein Cytochrome b (mitochondrion) fragments can be used in the investigation of MHC molecules binding and it can be the first bottlenecks in vaccine design.

Keywords: *Dracunculusmedinensis, Dracunculiasis, Epitope, Antigen, Protein, Cytochrome b (mitochondrion).*

I. INTRODUCTION

D.medinesis (a little dragon from Medina) infects humans and causes "Guinea worm disease (GWD)" ,This is the only species from all the *Dracunculus 12* species(Muller ,R., 1971;Muller,R., 1979; Jones,h.I., and Mulder, E., 2007; Moravee,F,and Santos C.P,2009)which infects human. The other *Dracunculus* species generally resides in the internal tissues and body cavities of non-human mammals and reptiles (snake and turtles)(Bimi,L., et al., 2005).This little dragon undergo a very unusual life cycle of six developmental stages with incubation period last for 1 to one an half years approximately. This is one of the most neglected tropical parasites which bears clinical importance and needs to be eradicated after small pox(Greenaway C. 2004). After reaching to the maturation stage, these worms copulate and an adult female produces millions of eggs in its uterus whereas mail dies. Later on, the female worm release the larvae which induces a painful blister (1 to 6cm diameter) on the skin of lower limbs (predominantly localized in the lower extremities(80-90%) in most of the reported cases).The infected person develops slight fever , local skin redness , swelling and severe pruritus around the blister . Other symptoms include: diarrhea, nausea, vomiting and dizziness. The blister burst within three days and female worms one or more slowly comes out from the wounds which causes an excoriating burning sensation and pain (Miillner A, Helfer A ,KotlyarD.Oswald J, EfferthT, 2011).Immersing or pouring water over the blister provides pain reliever. But this the moment that adult female is exposed to the external environment (Ruiz-Tiben E, Hopkins DR., 2006). Duringemergence of the limbs in open water sources it recognizes the

temperature difference and releases the milky white liquid in the water which contains millions of immature larvae, when larvae released in water are ingested by copepods where they mount twice and become infective larvae within two weeks(IriemenamNC,Oyibo WA, Fagbenro-BeyiokuAF, 2008).The *D.medinensis* antigen peptides can be most desirable segment for the subunit vaccine development because with the single epitope, the immune response can be generated in large population. This approach is usually based on the phenomenon of cross-protection, whereby infected with the mild strain and is protected against a more severe strain of the same. The phenotype of the resistant transgenic hosts includes fewer centers of initial infection, a delay in symptom development and low accumulation. In this study Cytochrome b (mitochondrion) has been used to investigate its role in antigenicity and hydrophobicity.Cytochrome b protein present in the mitochondria of eukaryotic cells. It acts as an important part of the electron transport chain and is the main subunit of transmembrane cytochrome bc1 and b6f complexes. Cytochrome b/b6 isan integral membrane protein consists of the approximately 400 amino acid residue which probably has 8 transmembrane segments. Cytochrome b/b6 non-covalently binds to b562 and b566 heme groups. The four conserved histidine residue in the ligands of the iron atoms of these two heme groups has been postulated (Howell N .,1989; Esposti MD, Crimi M, Ghelli A, Patarnello T, Meyer A, De Vries S., 1993). The cytochrome b is the central redox catalytic subunit of the quinol: cytochrome c/plastocyanin oxidoreductases .The cytochrome b functionally involves in the binding of the quinine substrate and actively responsible for the transmembrane electron transfer through which the protonmotive force is created/ generated from redox energy.Cytochrome b also carries the binding domain of the various inhibitors and quinine antagonists bindind sites, which subsequently inhibits the oxidoreductase. Esposti MD et al., able to identify the amino acid residues in cytochrome b which has the probability to be involved in the binding of the inhibitors and, by extrapolation, quinone/quinol(Esposti MD, Crimi M, Ghelli A, Patarnello T, Meyer A, De Vries S., 1993).Cytochrome b is also known as the bc1 complex or ubiquinol-cytochrome c reductase , which is the part of the respiratory chain complex III. The cytochrome b6/ b6f complex is the analogous protein present in the plant chloroplasts and cyanobacteria, which is a part of the plastoquinone-plastocyanin reductase,. These complexes are involved in pumping of protons in order to create a PMF,electron transport. The proton gradient is finally promote the ATP generation. These complexes play a vital role in cells(Blankenship, Robert., 2009).The intolerance in human patients is due to the result of the mutation in cytochrome; though very few severe multi-system pathologies have also been reported (Blakely EL, Mitchell AL, Fisher N, Meunier B, Nijtmans LG, Schaefer AM, Jackson MJ, Turnbull DM, Taylor RW., 2005). In *Plasmodium falciparum* and *P. berghei* a single- point mutations in cytochrome b is associated with resistance to the anti anti-malarial drug atovaquone(Siregar JE, Syafruddin D, Matsuoka H, Kita K, Marzuki S., 2008). It has also reported that mutations in the MT-CYB(mitochondrial cytochrome b) geneis responsible for the deficiency of mitochondrial complex III. This mutation in the gene is the cause of the condition which characterized by muscle weakness (myopathy) and pain especially during exercise (i.e., exercise intolerance). The severity due to this mutation in affected individual can suffer from the problems like liver, kidneys, heart, and brain.These tissues required a huge amount of the energy for its functionality, researcher believes that impaired oxidative phosphorylation can lead to cell death which inturns causes the deficiency of the various features of the mitochondrial complex. The accurate position of the MT-CYB gene in mitochondrial DNA (mtDNA) can reveal and unfold the underlying

the reason that only few people suffer from more severe features of the condition than others. The percentage of mutated mitochondrial DNA is highest in the skeletal muscles in most of people with MT-CYB-related mitochondrial complex III deficiency, which further interprets the finding of myopathy in these individuals. The mutation is most prevalent in muscle tissue is still imprecise.Antigenicity prediction of the protein from *D. medinensis*can play an important role in prototype synthetic vaccine development and as well as for target validation.

II. METHODOLOGY

B-cell epitopes are the sites of molecules that are recognized by antibodies of the immune system. Knowledge of B-cell epitopes may be used in the design of vaccines and diagnostics tests. It is therefore of interest to develop improved methods for predicting B-cell epitopes(Larsen JE, Lund O, Nielsen M., 2006).Inthis research work, antigenic epitopes of antigen protein Cytochrome b (mitochondrion) from *D.medinensis* is determined using the Gomase in 2007, Bepipred Linear Epitope Prediction, Emini Surface Accessibility Prediction, Karplus& Schulz Flexibility Prediction,Kolaskar&Tongaonkar Antigenicity, Parker Hydrophilicity Prediction(Gomase VS, Chitlange NR .,2012;Gomase VS and KaleKV., 2008; Gomase VS and Kale KV .,2008; Gomase VS, Chitlange NR., 2012; Gomase VS, Kale KV, Chikhale NJ, ChangbhaleS.S., 2007;Mishra Sonu and Virendra S. Gomase.,2015).

III. RESULT AND INTERPRETATIONS

Cytochrome b protein consists of the 367 amino acid sequence. It is analysed through different types B- cell epitope prediction methods.In the Bepipred Linear Epitope Prediction- the highest peak with highest score is observed between 205-210 position(Fig. 1),Emini Surface Accessibility Prediction-the score of the amino acid residue is between position220-225 (Fig. 2),Karplus& Schulz Flexibility Prediction: between 15-20 (Fig. 3),whereas in Kolaskar&Tongaonkar Antigenicity the highest score is between 320-325 position (Fig. 4) and in Parker Hydrophilicity Prediction between 15-20 (Fig. 5). Considering all the output of the result we can predict that the residue higher peak with higher score is between the positions 15-20[Tables 1& 2]. This in turns indicates that there might be probability of residue to be a part of the epitope. We found that the region of maximal Hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because the terminal regions of antigen protein is solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein [Figs-6-11] . It was seen that an antigen protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility [Figs.12-13]. The predicted antigenic protein segments of Cytochrome b (mitochondrion) can take active part in the host immune reactions. In future study the predicted antigenic protein Cytochrome b (mitochondrion) fragments can be used in the investigation of MHC molecules binding and it can be the first bottlenecks in vaccine or drug development.

IV. FIGURES AND TABLES

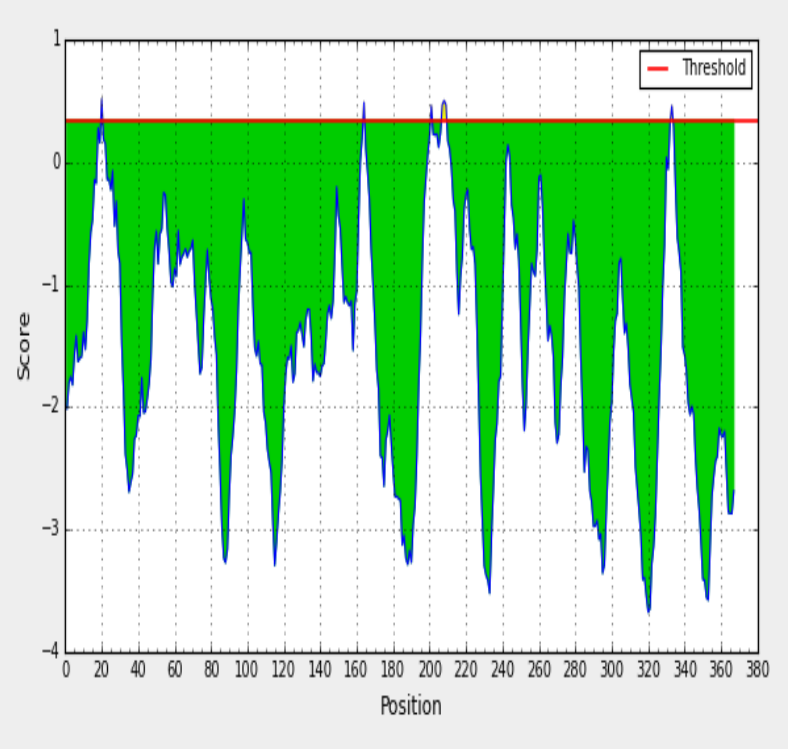

Fig. 1.Bepipred Linear Epitope Prediction Graph

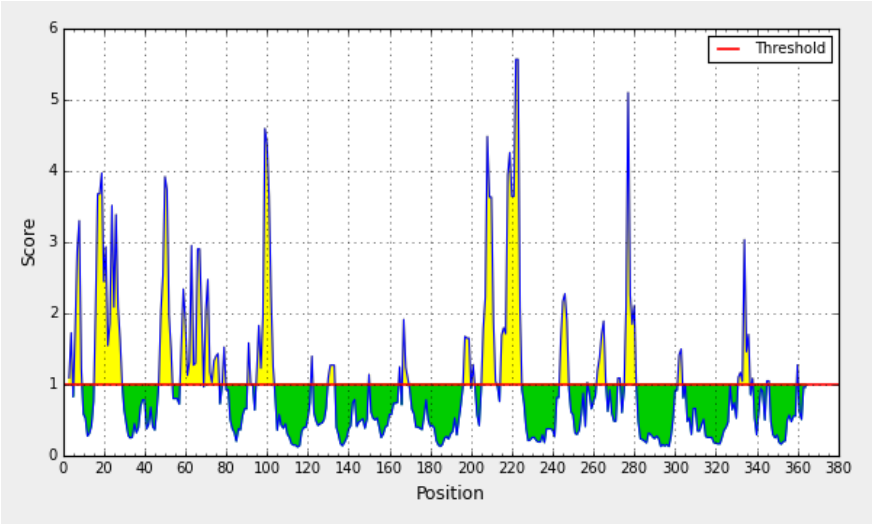

Fig.2. Emini Surface Accessibility Prediction Graph

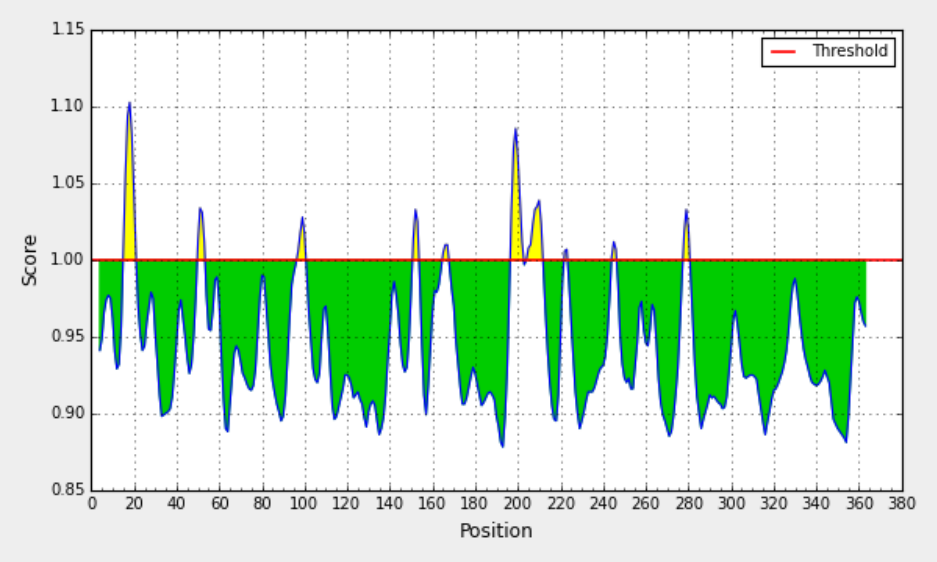

Fig.3. Karplus& Schulz Flexibility Prediction Graph

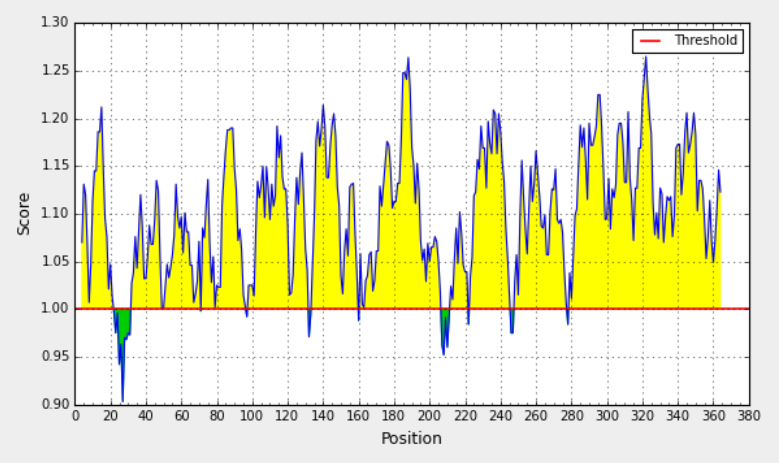

Fig.4. Kolaskar&Tongaonkar Antigenicity Graph

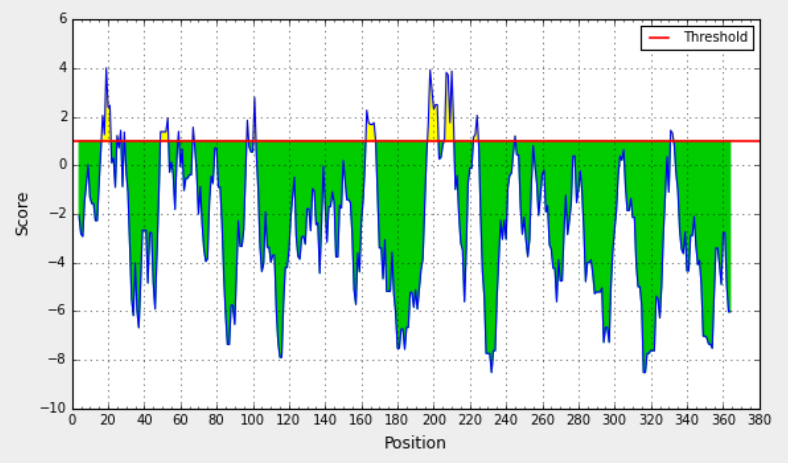

Fig.5. Parker Hydrophilicity Prediction Graph

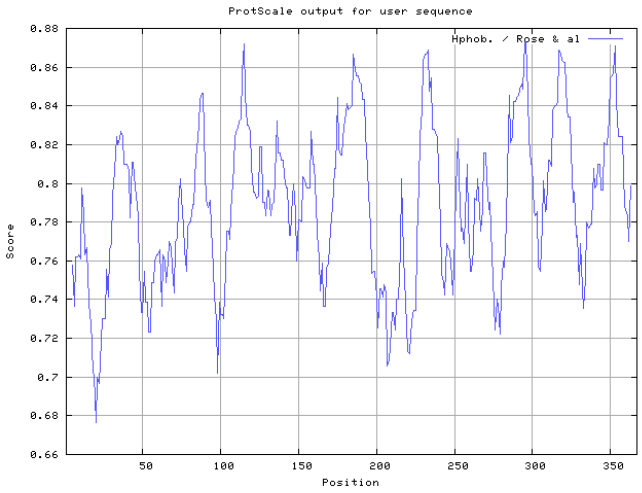

Fig.6. Hydrophobicity plot of antigen by Hphob/Rose & al., scale

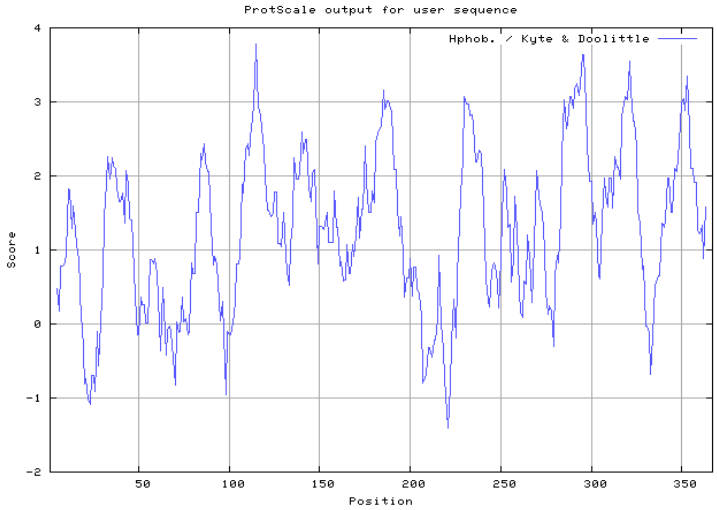

Fig.7. Hydrophobicity plot of antigen by Hphob/Kyte& Doolittle scale

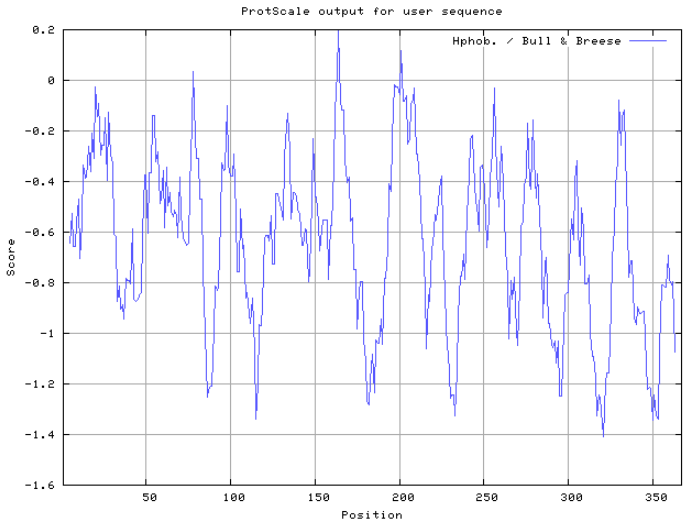

Fig.8. Hydrophobicity plot of antigen by Hphob/Bull & Breese scale

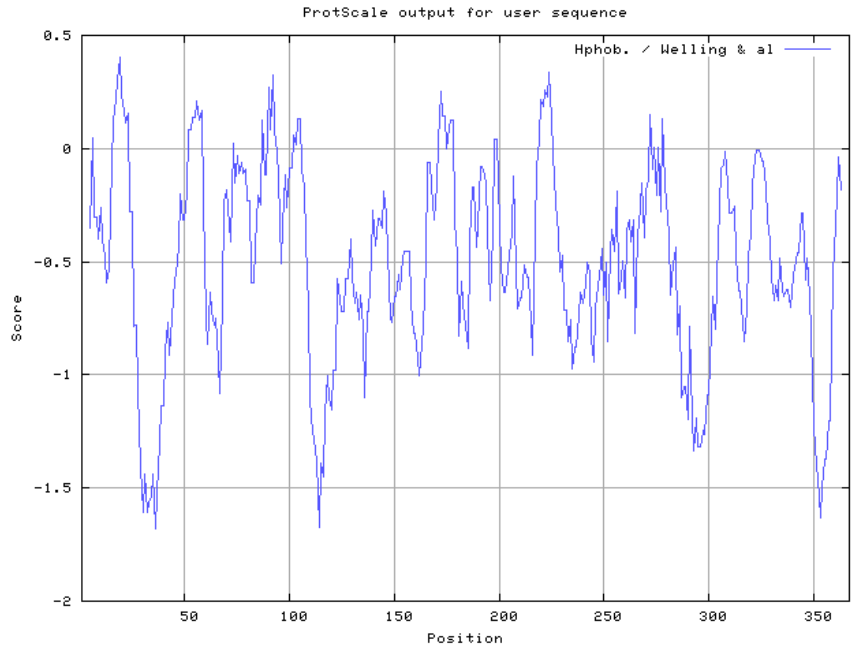

Fig.9. Hydrophobicity plot of antigen by Hphob/Welling & al., scale

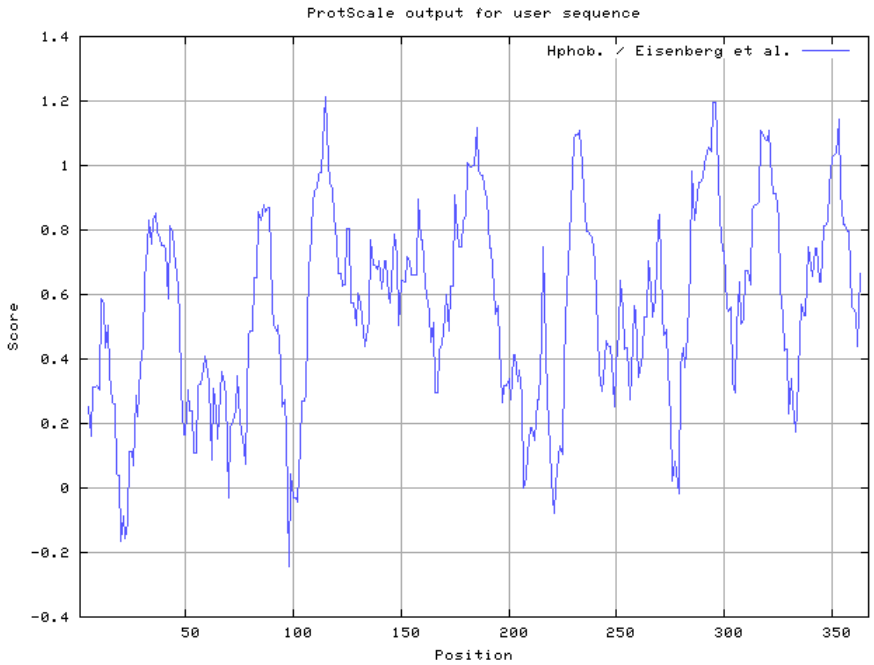

Fig.10. Hydrophobicity plot of antigen by Hphob/Eisenberg et al., scale

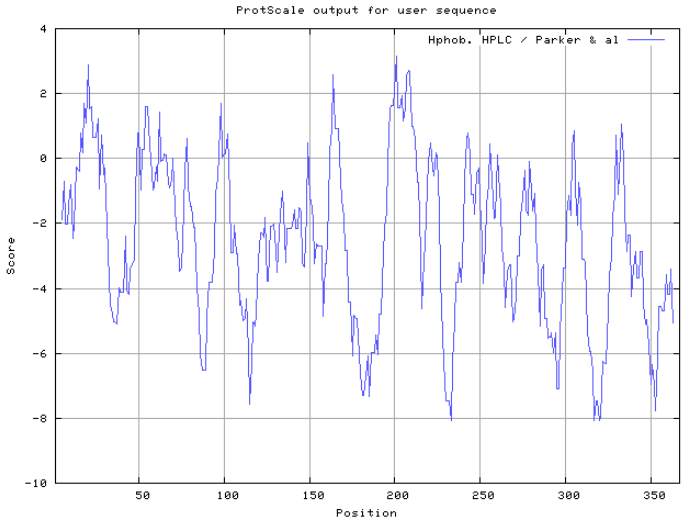

Fig.11. Hydrophobicity plot of antigen by Hphob. HPLC/Parker & et al., scale

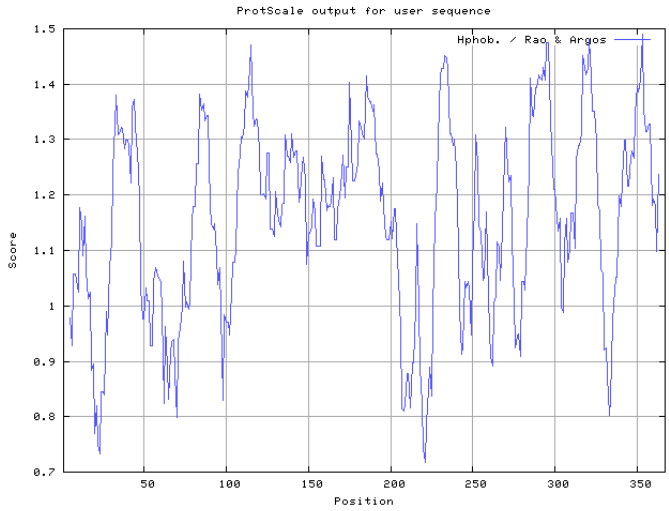

Fig.12. Antigenicity plot of antigen protein by Hphob. / Rao & Argos, scale

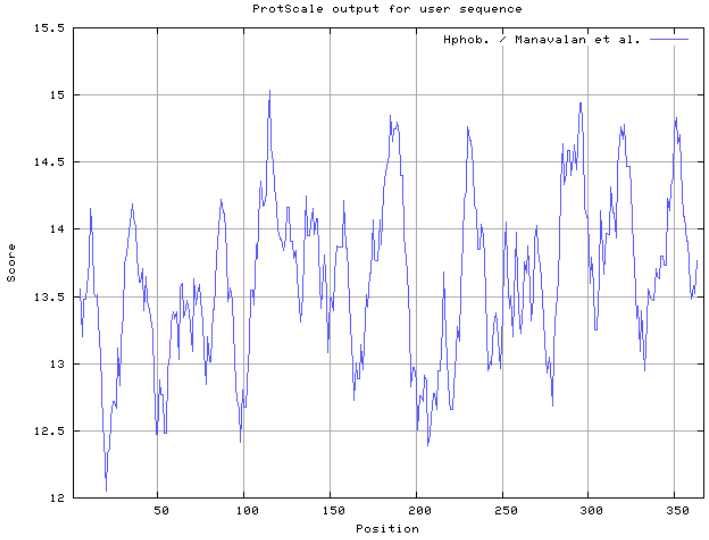

Fig.13. Antigenicity plot of antigen protein by Hphob. / Manavalan et al., scale

Table-1:Karplus& Schulz Flexibility Prediction-Predicted Residue Scores

	Position	Residue	Start	End	Peptide	Score
Karplus& Schulz Flexibility Prediction - Predicted residue scores	15	L	12	18	VVVLPSS	1.005
	16	P	13	19	VVLPSSK	1.056
	17	S	14	20	VLPSSKS	1.094
	18	S	15	21	LPSSKSL	1.103
	19	K	16	22	PSSKSLD	1.082
	20	S	17	23	SSKSLDL	1.044

Table-2:Parker Hydrophilicity Prediction-Predicted Residue Scores

	Position	Residue	Start	End	Peptide	Score
Parker Hydrophilicity Prediction - Predicted residue scores	15	L	12	18	VVVLPSS	-0.743
	16	P	13	19	VVLPSSK	0.6
	17	S	14	20	VLPSSKS	2.057
	18	S	15	21	LPSSKSL	1.271
	19	K	16	22	PSSKSLD	4.014
	20	S	17	23	SSKSLDL	2.4

V. CONCLUSION

The rational prediction of protein surface regions that are recognized by antibodies (antigenic epitopes) preferentially can guide in vaccine components design and immuno-diagnostic reagents. An antigenic protein Cytochrome b (mitochondrion) from *D. Medinensis*can plays an important role in vaccine development. The peptide fragments of antigen protein can be used to select nonamer for use in rational vaccine design and can develop the understanding of roles in the immune system in infectious disease.

VI. CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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