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FISHGUARD: a fast, cost-effective, user-friendly and in-field screening test Aitana Neves & Carlos Peña

Viral hemorrhagic septicemia and infectious hematopoietic necrosis are highly contagious fish diseases that cause important losses in fish farms. Ensuring early detection of the responsible viruses is therefore important to improve biosecurity of the aquaculture industry. The aim of this project is to develop a rapid in-field immuno-assay-based screening test. This requires generating highly sensitive and specific antibodies against viral antigens.

Bioinformatic tools were used to predict epitopes for antibody generation:

ANTIGEN Selection

Identify most abundant viral proteins in early and late responses to infection

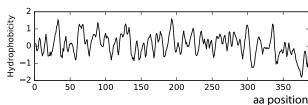
L and P proteins G (glycoprotein) (RNA polymerase) Lipid bilayer M (matrix protein) (-) strand RNA genome N (nucleocapsid protein) [VHSV cartoon]

PEPTIDE Pre-processing Slide a 20 amino-acids (aa) window to define all potential peptides. Discard peptides if:

- they contain repeats or post-translational modifications (Uniprot.org)
- they are hydrophobic on average (Cowan & Whittaker scale)

Post-process if cysteins in the middle, since used for carrier protein conjugation

protein sequence



SEQUENCE conservation Keep remaining peptides if:

20aa

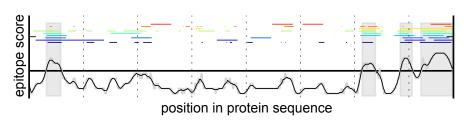
- they contain a strech of at least 12 consecutive amino acids perfectly conserved across all virus isolates
- the conserved stretch is on average hydrophilic
- there are no homologous sequences in related viruses or species of interest

RANKING Pareto dominance

3 CRITERIA to rank remaining peptides:

- Average hydrophilicity of the conserved strech
- Predicted average solvent accessibility (JPred)
- Epitope prediction (combination of 9 existing classifiers

based on hierarchical clustering of their predictions)



Epitopia [5] BCPred[4] COBEPro [ABCPred **AAP** [3] **BepiPred** 0.84 **ABCPred** 0.80 AAP **BCPred** 0.76 **Epitopia** 0.72 **COBEPro** 0.68 **TEPRF** 0.64 **CBTope** 0.60 **SVMTriP**

Based on our predictions, 18 peptides were selected and used to conduct mouse immunization experiments. Generated antibodies are currently being characterized by our partners.



REF. [VHSV cartoon] www.virology.ws [1] PMID: 16635264 [2]PMID: 16894596 [3] PMID:17252308 [4] PMID: 18496882 [5]PMID: 19751513 [6] PMID: 19074155 [7] PMID: 24721579 [8] PMID: 20961417 [9] PMID: 22984622