

**INACTIVATED VACCINE AGAINST *FLAVOBACTERIUM COLUMNARE* FOR FISH**

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**Technical details:**

*Flavobacterium columnare* inactivated vaccine is a formalin inactivated vaccine for protecting fresh water fish against *F. columnare* infection mainly affects gill and external surface of fresh water fish. Laboratory and field trials of developed inactivated vaccine of *F. columnare* in *Labeo rohita* have been successfully tested. The efficacy and performance of vaccine is estimated in terms of relative percent survival along with antibody titer and some vital cellular immune responses. In this process, a new pathogenic strain of *F. columnare* was isolated from *L. rohita* (sample collected from aqua farm of Pen, Raigard, Maharashtra), and characterized by biochemical (griffin test) and molecular techniques (species specific PCR (ColF & ColR)). The CRP strain was further deposited in the NCBI nucleotide sequence databases under accession numbers MK351268 - MK351269.

Technology of vaccine preparation primarily depend on selection and preparation of immunogenic antigen and for this isolation of extracellular protein, whole cell protein, outer membrane protein and inactivated whole cell were tested for different properties. Further, based on positive agglutination test the whole cell was selected for vaccine preparation.

- Preparation of whole cell inactivated bacteria for use in immunization: inactivation of bacteria was performed using formalin, sonication, heat and alkali treatment. After comparing economic and efficiency aspect, protocol for formalin inactivation of bacteria was selected for development of bacterial vaccine due to its economical and practical viability and efficient immunogenicity.
- Preparation of inactivated bacterin (inactivation of bacteria using 0.3% formaldehyde for 4°C for 48 h), further sterility was tested for testing the effective inactivation procedure and its safe administration to host fish. The inactivated vaccine, bacterial pools, and media are all screened for the absence of adventitious agents and endotoxins.

**Application:** Vaccine would be suitable for field application on farms/hatcheries/brooders

**Brief Description of Technology Including Salient Features**

- A formalin-killed vaccine of *Flavobacterium columnare* is developed for protecting fish against *columnaris* disease
- The vaccine is available in inactivated lyophilized form in vials facilitate the ease to prepare and administer to farm fishes

- The recommended dose of vaccine is  $10^5$ cfu/mL administered through immersion mode
- Primary and booster vaccination dose (after 21 days of primary vaccination) is  $10^5$ cfu/mL for 1 h and 2 h respectively through immersion mode only
- The recommended age for primary vaccination is 15 to 45 days old fingerlings and a booster on 21 days post first vaccination
- Ease in application: Single Dose Vial- first withdraws the entire volume (5 ml) of diluent into the syringe to be used for reconstitution. Inject all the diluent in the syringe into the vial of pelleted vaccine, and agitate to mix thoroughly. Transfer the diluted vaccine in a 150 L water stir it properly and immediately transfer 500 fish in the same tank. Expose the fish to immersion vaccine for 1 h after that transfer them into 150 L of fresh water and finally to the rearing tank.
- A safety aspect of *Flavobacterium columnare* killed vaccine was evaluated for administration in fish.
- Stability testing was performed for *F. columnare* killed vaccine
- Storage: Vaccine can be store even at room temperature however it is stable in lyophilized form at -20 and 4 °C. Protective duration has been recorded up to 4 months post vaccination.
- Vaccine would be suitable for field application on farms/hatcheries/brooders

Field demonstration of vaccine was done at selected fish farms near by Mumbai and found RPS 81%.

#### **Benefits and Utility:**

- Alternative to antibiotics for controlling columnaris, a bacterial disease of fresh water fishes.
- Vaccine would be suitable for preventing columnaris disease in fish under different aquaculture system like farms and hatcheries.
- Effective and environmental friendly approach for controlling columnaris disease.
- Inactivated vaccine has benefit of safety aspects of host and practitioners
- This vaccine can be used for both fingerlings and adult stage of fish.

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