

**STANDARD OPERATING PROCEDURE (SOP) FOR AQUATIC QUARANTINE OF  
SPECIFIC PATHOGEN FREE (SPF) *LITOPENAEUS VANNAMEI* BROODSTOCK**

## SCOPE OF THIS DOCUMENT

The importation of Specific Pathogen Free *Litopenaeus vannamei* has been permitted by the Government of India and specific guidelines for pre-quarantine, quarantine and post-quarantine have been notified under the Livestock Importation Act. Unless otherwise stated specifically, this Standard Operating Procedure for the Aquatic Quarantine for *L. vannamei* is based on the Guidelines. This protocol aims to confirm the SPF status of the imported shrimp before permitting the transfer of the consignment to the importing hatcheries.

## DEFINITIONS

The following words are used in the context this SOP:

**Shrimp:** Specific Pathogen Free *L.vannamei* imported from overseas.

**Consignment:** Shrimp in oxygenated polythene bags with seawater and packed in thermocol boxes with coolant packets.

**Vehicle:** Four wheel covered truck

**MPEDA:** The Marine Products Export Development Authority

**CAA:** Coastal Aquaculture Authority

**CIBA:** Central Institute of Brackishwater Aquaculture

**NFDB:** National Fisheries Development Board

**RGCA:** Rajiv Gandhi Centre for Aquaculture

**AQF:** Aquatic Quarantine Facility

## REFERENCES

Derived from the expertise of MPEDA-RGCA/ CIBA/ NFDB/ CAA

### 1. PROCEDURE FOR USING AQUATIC QUARANTINE FACILITY

The following are the procedures for using the Aquatic Quarantine Facility at Neelankarai, Chennai for the import of SPF broodstock of *L. vannamei*.

#### 1.1 PERMITS REQUIRED FOR IMPORTATION

- Hatcheries which have been permitted by CAA to import broodstock and to produce seed of *L. vannamei* can only use the AQF.
- The importer should have obtained a specific letter of permission from CAA for the import of SPF *L. vannamei* indicating the quantity of import.

- They should also have obtained a valid Sanitary Import Permit from the Ministry of Agriculture, Government of India.
- With these valid documents, the prospective importer can inform AQF for reserving the quarantine space, 30 days prior to the date of arrival of the consignment. The space at AQF will be allotted based on first come first serve basis.
- The reservations should be confirmed 10 days prior to the arrival of the consignment in the proper format (Annexure I). Along with the application necessary fee and an undertaking to the effect that the importer agrees to the terms and conditions of the AQF should be submitted (Annexure I).
- Delay/failure from the part of the importer to import the consignment on the allotted period shall invite forfeiture of the fee as the holding space is reserved for the importer.

## 1.2. CERTIFICATES TO ACCOMPANY THE CONSIGNMENT

- As per the quarantine guidelines, the following certificates in original, issued by the competent authority of the exporting country should accompany the consignment of SPF broodstock and copies of the certificates should be made available to the Aquatic Quarantine Officer at least two days before arrival of the consignment.
  - A certificate indicating the SPF status of the broodstock in relation to the pathogens excluded in the shrimps.
  - A certificate indicating the history of disease occurrence in the broodstock rearing facility of the exporter for the last two years.
  - A certificate indicating that the batch of exported broodstock was held in pre-quarantine for a period of 12 days and the results of the tests for the following pathogens should be included.
    1. Taura syndrome virus (TSV) (OIE listed)
    2. Yellowhead virus (YHV) (OIE listed)
    3. Infectious myonecrosis virus (IMNV) (OIE listed)
    4. Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV) (OIE listed)
    5. *Baculovirus penaei* (BP) (OIE listed)
    6. White spot syndrome virus (WSSV) (OIE listed)
    7. Necrotising hepatopancreatitis bacterium  $\alpha$ -Proteobacterium (NHPB)- (presently under study for inclusion in OIE list)
  - The testing should have been done not earlier than 10 days before the actual shipment
  - The necessary customs clearance will be issued by the Animal Quarantine Officer as per the technical recommendations provided by the Aquatic Quarantine Officer.

## 1.3 CLEARANCE AT THE AIRPORT

- Consignment shall be cleared from airport by the importer in the presence of staff of AQF designated for the purpose. All the expenditures in connection with clearance, loading and unloading at the airport will be borne by the importer.

- After the clearance, the designated staff of AQF will take charge of the consignment. Specific dress code with badges shall be provided to the AQF staff responsible for collection of broodstock from airport. Gross observation on the condition of the cartons, Number of cartons, damage if any, will be recorded by the AQF representative in the presence of the importer or his representative.
- The consignment should be accompanied by a package slip with the following detailed information in English
  - (a) Identification: Name of the species (Zoological name and the common name), age and total number, males and females
  - (b) Country of origin: Name and address of the Supplier of SPF
  - (c) Destination: Importing country, name and address of the importer
  - (d) No. of animals per carton – Males and females

#### 1.4 TRANSPORTATION TO AQF

- A sanitised truck with covered space shall be used to transport the imported broodstock from airport to the Aquatic Quarantine Facility. The vehicle will be driven by the route designated for the purpose by the Aquatic Quarantine Officer only. Vehicle shall not stop en route to the AQF at any other place.

#### 1.5 DISSEMINATION OF INFORMATION

The information on shipping details, health certificates, and other relevant particulars listed below regarding each batch of shrimps that passes through the AQF will be hosted in CAA website.

- a. Name and address of the importer
- b. Country of origin
- c. Name and address of the Supplier of SPF
- d. Consignment date
- e. Consignment number
- f. Date of arrival of consignment at the facility
- g. Details of accompanying certificate
- h. CAA letter of permission
- i. Total number of *L.vannamei* broodstock imported (male and female separately)
- j. Transit mortality, if any
- k. Date of release from the quarantine facility
- l. Number of broodstock cleared from the facility (male and female separately)

## 2. STANDARD OPERATING PROCEDURE FOR AQF

### 2.1 RECEIVING AT AQF

- Vehicle shall pass through a tyre bath and reach the receiving point. The following procedures will be adopted for unloading and further action.
  - a) Unload the entire consignment at the receiving area. (All the staff involved in unloading must disinfect their hands, apron, coat and shoes).
  - b) Only the polythene bags containing shrimps shall be taken inside the Primary Quarantine Unit for acclimatization.
  - c) One representative of the importer/consignee shall be permitted to observe the shrimps in the receiving area and general health condition of the shrimps shall be assessed jointly by AQF technical staff and the importer. Accordingly, the importer will acknowledge the status of the consignment at the arrival point (receiving area) as per the format (Annexure-II).
  - d) Polythene bags with shrimps shall be shifted to Primary Quarantine for acclimatization. The empty cardboard/Styrofoam boxes and other items used during the transport shall be burnt under the supervision of the quarantine staff.
  - e) In addition to the regular sampling, if there are dead/ moribund shrimp, samples from all these shrimp will be collected and the dead/moribund shrimp shall be immediately removed from the receiving area for proper disposal by incineration. A record of all dead/ moribund shrimp shall be kept.
  - f) Shrimp bags which arrive with a temperature 15-20 degree Celsius shall be left in a large tank with filtered, chlorinated, dechlorinated, UV sterilized seawater with 28 to 30 degree C. Once the bag temperature equals the container temperature, shrimp shall be collected from the bag using an aquarium scoop net and after dipping in 200 ppm formalin for 2-3 minutes followed by a rinse in fresh seawater from AQF, shrimps shall be transferred to the quarantine holding tanks in the respective rooms.
  - g) Check quality of seawater in the bags for salinity, pH, ammonia and temperature. It is necessary to make sure that water from the imported shrimp consignment shall not get into the AQF and hence should be discarded in the primary quarantine unit itself which ultimately gets sanitized in ETS with ozone.

### 2.2 SAMPLING FOR PATHOGEN TESTING

- The total number of samples will follow the OIE protocol. Exopodite of the 3<sup>rd</sup>/4<sup>th</sup> pleopod shall be extracted using a sterilized scissor. Exopodites sourced from one CONSIGNMENT shall be pooled into four samples. Faecal matter from the different bags will also be collected and pooled as above. For DNA viruses, extracts of DNA will be made as per Standard Operating Procedure for PCR as suggested by the concerned OIE

recognized primer supplier. One half of the extracted DNA will be stored for further confirmation tests, if required. For RNA viruses, one half of the tissue sample will be stored in RNA later (Buffer) and the other half used for the extraction of RNA and further diagnostics. For BP, DNA from the faecal matter will be extracted as per the standard OIE primer supplier.

- Care shall be taken to avoid any physical injury to the shrimp during the process. Samples collected shall be transferred to the PCR lab for further analysis.

### 2.3 PCR TESTING AND ISSUE OF QUARANTINE CERTIFICATE

- The nested PCR test will be carried out as per the Standard Operating Procedures as suggested by the OIE approved primer supplier.
- In case any of the 7 OIE listed viral pathogens are tested positive, the extracted DNA/ stored tissues for RNA along with fresh exopodite pleopod and faecal samples, as per the requirement, shall be sent to Central Institute of Brackishwater Aquaculture(CIBA), Chennai, for confirmation immediately.
- In the event of confirmation of the presence of virus by CIBA, it will be reported to the technical committee by CIBA and the final decision on destroying the entire consignment will be taken by the committee. The decision of the committee will be intimated to the importer and the consignment destroyed within 4 days of receiving confirmatory report from CIBA. The presence of disease in the consignment shall be notified to the source company by the technical committee. If two consignments are tested positive for any OIE listed diseases, the supplier shall be recommended to CAA for blacklisting.
- Effort should be made to complete the entire process within the scheduled quarantine period of 10 days.
- In case the samples are negative for all the OIE listed diseases, the quarantine officer shall accordingly issue a Quarantine Certificate (QC)

### 2.4 MAINTENANCE OF QUARANTINE FACILITY

- Shrimps from the primary quarantine unit shall be transferred into the Quarantine rooms. There are four rooms in AQF with 158 tanks of 650 litre capacity each. Shrimp shall be stocked @ 6-7 per tank. Tanks in each room shall be serially connected to a re-circulation system.
- Re-circulation system consists of drain system with suitable PVC pipes/valves, a sump, a heavy-duty pump with non-reactive impellers, physical filtration system and seawater delivery plumbing. Suspended sediments (unfed feed and faeces shall be removed from the re-circulation sump through a drain pipe).
- Each room shall be air conditioned to maintain an ambient room temperature not more than 25 degree C.
- Shrimps shall be fed @ 4-8% of their biomass with pelletised biosecure certified feed preferably produced in a country where shrimp diseases are not reported. Actual feed ration shall be based on demand feeding.
- Pelletised feed ration for the day shall be split into 6 meals and fed to the shrimps besides one meal a day with SPF frozen polychaete worms.

- Feed consumption shall be checked in each tank two hours after each feeding and recorded. Pelletised feed ration shall be appropriately increased/decreased based on the level of consumption.
- Unfed feed and faeces shall be siphoned out twice daily from the tanks using ¾” siphon pipes
- Pelletised feed and frozen SPF polychaetes shall be stored in a deep freezer at sub zero temperature in separate containers.
- Water quality parameters like pH, ammonia and nitrite shall be analysed twice daily for each tank. Since continuous aeration is provided in the tanks, oxygen analysis is not necessary. Water exchange @ approximately 100% everyday shall be carried out to maintain the water quality parameters in the desired range.
- Rooms should be kept dark to reduce stress to the animals. Entry to the rooms should be strictly restricted and only when absolutely necessary during feeding, water exchange and water quality monitoring.

## **2.5 OPERATIONAL MANUAL OF AQF**

- Detailed manual on the operation of different sections of AQF will be developed by the AQF technical team and will be validated by the Technical Committee. The manual will contain sections on Water treatment, Water quality management, Feeding, Staff personal hygiene, Biosecurity protocol, Diagnostic tests etc.,

## **2.6 DESPATCH OF CERTIFIED SHRIMP BROODSTOCK**

- In case the animals are found to be negative for the OIE listed pathogens, shrimps shall be packed in polythene bags with oxygen at the packing area and shall be handed over to the importer.
- The importer shall make necessary arrangements for transporting the shrimps to the respective hatchery.
- The importer shall be provided with Delivery Challan (Annexure-III) along with Quarantine Certificate.

## **2.7 RECORD KEEPING**

- Daily data sheets on the broodstock management in the quarantine facility shall be maintained by the AQF technical team during the period of holding the broodstock in the AQF. This will include the details of diagnostics, water quality characteristics, mortality etc.