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المملكة العربية السعودية
KINGDOM OF SAUDI ARABIA

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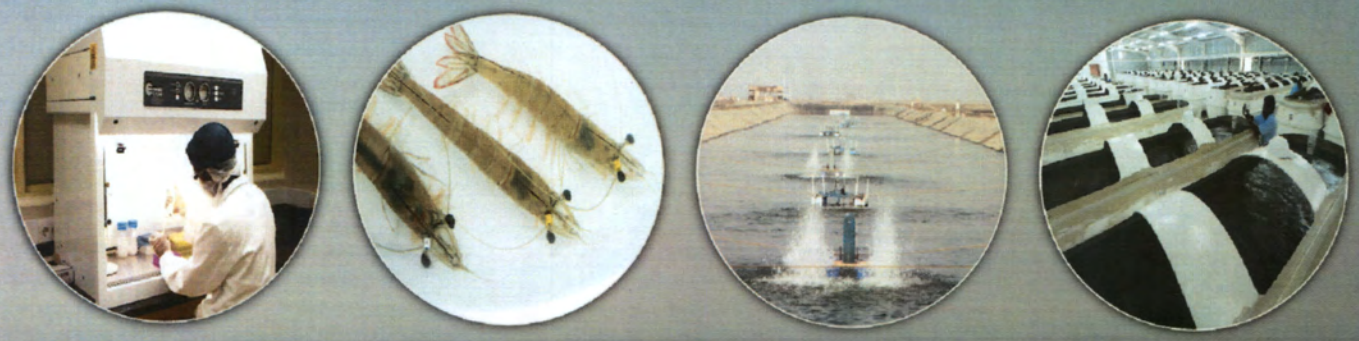
Ministry of Environment Water & Agriculture
Kingdom of Saudi Arabia المملكة العربية السعودية

دليل الأمن الحيوي للروبيان المستزرع في المملكة العربية السعودية

National Biosecurity Manual for Shrimp culture in Kingdom of Saudi Arabia



Issue No. 2, July 2018

The General Directorate of Fisheries
Ministry of Environment, Water and Agriculture
Kingdom of Saudi Arabia

The General Directorate of Fisheries hereby declares that:

- This National Biosecurity Manual stipulates the official regulation and procedures concerned with Biosecurity and Aquatic animal health management in aquaculture production in Saudi Arabia.

- The General Directorate of Fisheries of Ministry of Environment, Water and Agriculture (GDF-MEWA) shall be the Competent Authority in Kingdom of Saudi Arabia in dealing with all matters related Biosecurity, Aquatic animal health, emergency preparedness, and to issue Aquaculture Licensing, inspection of Production, approval for imported live aquatic species and issuance of HC for Export.

The General Directorate of Fisheries hereby approves the stated contents of 'National Biosecurity Manual for Shrimp Culture" to be followed in all matters related to the Biosecurity and Aquatic Animal Health in the Kingdom.

Any interim decision(s) taken by concerned government agencies shall be incorporated in the manual during subsequent revisions.

Director General – General Directorate of Fisheries
Ministry of Environment, Water and Agriculture

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1. Introduction

Biosecurity of the shrimp industry in the Kingdom of Saudi Arabia (KSA) is a priority for the Government in order to develop a sustainable industry that generates food, employment and wealth for the kingdom. Diseases are part of animal rearing and therefore, a proactive approach for prevention is required to minimize their presence.

The objective of the National Biosecurity Plan is to describe criteria and procedures in order to establish Standard Operating Procedures (SOP) to be adapted to each of the shrimp farming facilities in the Kingdom.

KSA has suffered severe epidemics due to White Spot Syndrome virus (WSSV) with devastating effect. This triggered the reaction from both, Government and Private sector, to establish a Private Public Partnership (PPP) to develop and implement a joint Biosecurity Strategy and Plan for the control of aquatic animal diseases.

The main shrimp pathogens reported in the shrimp industry in KSA have been WSSV, Taura Syndrome virus (TSV), Infectious Hypodermal and Hematopoietic Necrosis virus (IHHNV), Monodon Baculovirus (MBV) and Baculovirus penaei (BP). These are only a reduced number of pathogens compared with other national industries; therefore, considering its advantage, KSA has set up an effort to control and eradicate, if possible, the existing diseases and prevent the appearance of new ones.

2. National Biosecurity Plan of KSA

The National Biosecurity Plan of KSA has 12 key points that are strategic for the prevention and control of diseases in the aquaculture industry.

1. Switch to Specific Pathogen Free (SPF) stocks
2. National Reference Diagnostic Laboratory
3. KSA listed pathogens
4. National surveillance program
5. Health certificate for animal movement
6. National Zoning and Compartmentalization
7. Aquaculture Production Unit zoning
8. Compulsory reporting of disease outbreaks
9. Emergency response and contingency plan
10. Control of importation of live shrimp

- *Pre-approved supplier of any live imported aquatic animals (on site audit)*
- *Quarantine and testing on reception*

11. Ban on the use of wild broodstock
12. Restriction on aquatic products based on the SPS agreement of the WTO

In addition to these National Strategic points, each aquaculture company has its own biosecurity measures applied to company level.

2.1 General Biosecurity Management Procedures

To maintain the high Biosecurity standards achieved, and to minimize the risks of a disease outbreak throughout the year, the following general management procedures must be strictly adhered to:

- Farms must dry out their earthen ponds for a duration of at least 1 month during winter period.
- Farms are allowed to be stocked with WSSV tolerant SPF stocks ONLY (see section 2.2)
- Prior to any stocking in the grow-out ponds, a cold challenge and PCR testing is mandatory and the results submitted to JFRC for final approval of stocking.
- All earthen pond nurseries used have to be lined, or concrete nursery pond may be used.
- The water use, filtration and treatment must strictly follow the SOP 6 –'Water use in the shrimp culture' guidelines.
- In addition to the national Biosecurity surveillance program, implemented by SAS, farms which maintain shrimp stock in the ponds during winter period must implement an internal fortnightly (every 2 weeks) PCR surveillance program. The winter period internal surveillance sampling schedule will be submitted to JFRC for approval, and the results of the PCR test will be uploaded in the GDF-MEWA/SAS Biosecurity database. The cost of the fortnightly PCR analyses at JFRC will burden the project (farm).
- All farms must have a concise and clear biosecurity plan and the farm's staff must be familiar with it.
- All farms must have a concise and clear contingency plan in the event of WSSV, or any other disease outbreak, and the farm's staff must be familiar with it. The plan must mandatorily include at least the following:
  - ✓ Immediate notification of GDF-MEWA and SAS.
  - ✓ Sealing of outlet water gate to prevent the release of the pathogen into the environment
  - ✓ Harvesting of the infected pond(s) and subsequent disinfection of the pond(s) using trichlorfon IMMEDIATELY. Culling of the pond is suggested when the shrimp are of no commercial size/value (ie: <10gr)

2.2. Switch to Specific Pathogen Free (SPF) stocks

KSA had traditionally farmer *Penaeus indicus*, however, the WSSV epidemic resulted in the infection of the broodstock population and the evidence of very high sensitivity to the disease. After attempts to implement biosecurity still using *P. indicus*, a decision was taken to switch to SPF stocks that were Specific Pathogen Tolerant (SPT) for WSSV. The only stocks available with these characteristics were *Penaeus vannamei* from a specific breeding program in Ecuador. After an Import Risk Analysis (IRA), that covered the infectious, environmental and genetic risk assessment, these stocks were imported and are the only ones allowed into the country.

2.3. National Reference Diagnostic Laboratory

The Government of KSA has appointed Jeddah Fisheries Research Center (JFRC) as the National Reference Diagnostic Laboratory. This laboratory has full facilities and highly trained staff for diagnostic purposes through a range of techniques: polymerase chain reaction (PCR), histology and microbiology in addition to other techniques to support aquaculture production and aquaculture product quality. Continuous update and upgrade of facilities and staff are fundamental for maintain the excellence of this center.

Participation in international ring tests is routinely done every year in order to validate the quality of the work performed.

2.4. KSA listed pathogens

Part of the National Strategy has been to identify the shrimp pathogens KSA targets for their control and exclusion. These include all pathogens listed by the World Animal Health Organization (OIE) and additional ones that may be relevant to the economic success of the local industry. The list of pathogens is included below in table 1 indicating which one of them are OIE listed, has been present in the kingdom at one point and the severity of their impact.

This list of pathogens is dynamic and updated on regular basis based on the information generated within KSA and worldwide.

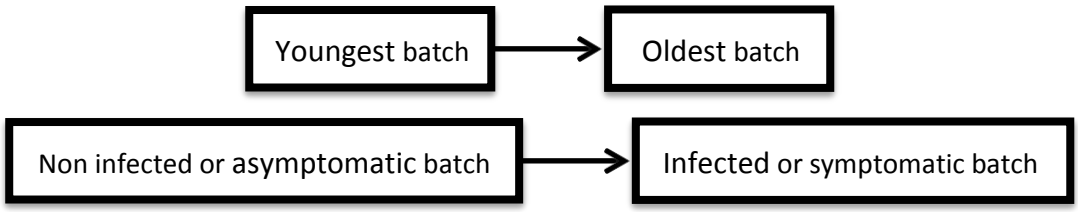
Table1. GDF-MEWA listed diseases important for the shrimp culture in the Kingdom of Saudi Arabia, their status regarding OIE listing and their detection in KSA

Pathogen	OIE listed	KSA listed	Ever detected in KSA
Acute Hepatopancreatic Necrosis Disease (AHPND)/Early Mortality Syndrome (EMS)	Yes	Yes	No
Enterotizooan penaei (EHP)	No	Yes	No
White Spot syndrome virus (WSSV)	Yes	Yes	Yes
Taura syndrome virus (TSV)	Yes	Yes	Yes
Yellow Head virus (YHV)/Gill Associated virus (GAV)	Yes	Yes	No
Infectious Hypodermal and hematopoietic Necrosis (IHHNV)	Yes	Yes	Yes
Infectious Myonecrosis virus (IMNV)	Yes	Yes	No
Necrotizing Hepatopancreatitis (NHP)	Yes	Yes	No
Monodon Baculovirus (MBV)	No	Yes	Yes
Baculovirus Penaei (BP)	No	Yes	Yes
Microsporidia	No	No	Yes

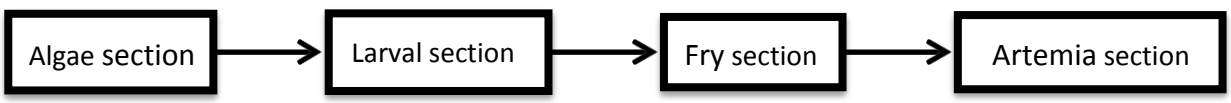
2.5. National surveillance program

The Government of Saudi Arabia has a National Surveillance Program that collects samples from every aquaculture company in the country in order to detect pathogens prior to disease outbreaks. These samples are collected monthly and tested by PCR for pathogens that have been endemic in the country or there is concern at international level. Currently, the pathogens tested are: WSSV, TSV, IHHNV, AHPND and NHP. In addition, samples are also collected for histology in case of new pathogens may arise.

Results of this surveillance program are shared with the stakeholders at the quarterly Biosecurity Workshop organized by the Saudi Aquaculture Society (SAS)



When samples are collected, these are done from populations of lower chances of infection to **higher chances:**
In the case of samples collection in hatcheries, the order is be as follow:



2.6. Health certificate for animal movement

All animals moved within the country need to be accompanied by a Health Certificate issued by the National Reference Diagnostic Laboratory at JFRC. Currently, the requirement is to be free WSSV by PCR technique after cold challenge. Depending on the national shrimp health situation, other pathogens may be included in the certification.

2.7. National Zoning and Compartmentalization

As one more component of the control of diseases, as recommended by the OIE, Saudi Arabia is divided in zones.

Fourteen zones / Compartments identified in the Kingdom as follows:

i. Al Bahah
ii. Al Hudud ash Shamaliyah
iii. Al Jawf
iv. Al Madinah
v. Al Qasim
vi. Ar Riyadh
vii. Ash Sharqiyah
viii. Asir
ix. Hail
x. Jizan
xi. Makkah (include Al Lith)
xii. Najran
xiii. Tabuk

Considering that all shrimp production facilities are placed in the Red Sea coast without any geographical barriers and cannot be classified as sanitary zones as per OIE description as they don't have geographical barriers, some companies are considered a compartment based on their own Biosecurity Plan and results from their surveillance program.

2.8. Aquaculture Production Unit Zoning

Aquaculture production units have different activities with a different risk associated to them.

These can be ranked by their level of business risk impact. Considering that the earlier animals can get infected, the higher the risk of spread of the disease and the impact, the classification is as follows:

- **High risk activities:**

-Nuclear Breeding Center

-Broodstock Production
-Broodstock Maturation and nauplia production
-Larval rearing
-Live and Fresh feeds

- **Moderate risk activities:**

-Nurseries
-Grow out ponds
-Harvest

- **Low risk activities:**

-Transit areas
-Accommodation and office area

Such classification of risk has led to the definition of sanitary zones within companies based on the areas where these activities take place. Areas where High Risk activities take place are considered Zone 1 and identified with Red Color. Areas where Moderate Risk activities take place are considered Zone 2 and identified with Yellow Color and areas where Low Risk activities take place are considered Zone 3 and identified with Green Color.

ZONE 1

ZONE 2

ZONE 2

1. **High Risk –** Quarantine, Nuclear Breeding Center, Broodstock production unit, Broodstock maturation and nauplia production, larval rearing, and Live and fresh feeds.
2. **Moderate Risk –** Nurseries and Farms.
3. **: Low Risk –** Processing Plant*, transit and offices -accommodations

*Processing plants are classified as low risk and allocated in zone 3 because they have efficient effluent treatment procedures in place and proper disposal of solids. The release of infectious pathogens during processing poses a significant risk to the surrounding industry. If imported product is process the risk of introducing exotic pathogen is high, the impact may affect the whole regional shrimp industry and therefore, it is an unacceptable risk

The level of risks determines the type of biosecurity measures involved.

High risk activities:
All activities within the high---risk category must be indoors and operated under strict biosecurity measures including incoming water treatment (SOP 6) and waste incineration.

2.9. Broodstock Breeding Center and Broodstock production units

The Broodstock Breeding Center and the broodstock production unit are the backbone of shrimp production. The scope is to produce Specific Pathogen Free (SPF) broodstock for maturation and to improve shrimp culture performances through its Family Selection Broodstock Breeding Program.

Broodstock health is the primary focus of the disease surveillance program (see SOP 18 surveillance).

Production is performed indoors; all incoming water is disinfected; all solid wastes are incinerated. Introduced animals will be tested by PCR on multiple tissues to ensure optimum detection levels (See SOP 14 PCR sampling).

Broodstock maturation and Nauplii production facilities

Nauplii production is performed indoors; all incoming water is disinfected and water temperatures must be kept stable. All wastes are incinerated. Only broodstock originated from the Broodstock Breeding Center can enter the maturation.

Fresh feeds are a significant biosecurity risk. Fresh feeds of crustacean origin are banned except for Artemia biomass. Such feeds must be supplied only from prequalified suppliers and provide evidence of freedom from WSSV, IHHNV and AHPND. (See SOP 13. Fresh feed and Artemia cysts)

Dry feeds are non-infectious, therefore PCR screening is not necessary, however, they must be free of crustacean by - products.

Eggs and Nauplii must be disinfected before being transferred.

2.9.1 Nuclear Breeding Center (NBC)

Nucleus Breeding Centre (NBC) means a facility where Specific Pathogen Free (SPF) shrimp broodstock are raised over a number of generations in a highly bio-secure environment, excluding a number of pathogens of concern from the facility. A strict surveillance protocol is followed to ensure that the pathogens are excluded. A highly bio-secure NBC is used for producing multiple generations of the SPF stock.

2.9.1.1 Broodstock Multiplication Centre

Shrimp Broodstock Multiplication Centre (BMC) means a facility which receives the Specific Pathogen Free (SPF) post larvae (PL) from a Nucleus Breeding Centre (NBC) and rears post larvae up to adult broodstock for supply to hatcheries. BMC is a facility for developing broodstock from the post larvae to adult, under strict biosecurity and close disease surveillance.

2.9.1.2 Registration and approval process

a) Site Selection

The breading center shall be located in an area where any Shrimp Hatchery, Aquaculture Activities or Fish Landing Centers (FLCs) do not already exist within a radius of 1000 meters (1.0 km) and the promoters put in place adequate biosecurity measures and follow the standards as prescribed in this National Biosecurity Manual.

b) General Requirement

The Nuclear Breading center shall establish and maintain infrastructural requirements according to the capacity of the facility:

i. The breading center shall have proper designs and drawings of the breading center buildings ensuring prescribed bio-security protocols and standards, an illustrative list of which is give as hereunder:
   a. Totally bio-secure area with fencing, shower room(s), disinfection for working staff, contractors, visitors and materials.
   b. Water treatment protocol with required reservoirs, filters and mandatory Ozonization.
   c. An Effluent Treatment System (ETS) to meet environmental management requirements
   d. Full- fledged facility for incineration of dead/ diseased animals.
   e. A fully equipped disease diagnostic laboratory with stock of all required primers as well as qualified and trained technicians.
   f. The facility shall implement and meet requirement of the National Biosecurity Manual
   g. The nuclear breading center teams shall coordinate with MEWA officials during periodic audits and surveillance sampling program
   h. The breading center subject to annual approval of “Production of SPF P. vannamei Broodstock” certificate.

Larval Rearing:
Production is performed indoors; all incoming water is disinfected prior to its use. All solid wastes are incinerated. Only Nauplii originated from the Nauplii Production Unit (maturation) can be stocked in the Larval Production Unit (hatchery). Water temperature should be kept stable.

Fresh feeds and live feeds production:
All live feeds (algae and Artemia cysts, Nauplii and biomass) should be kept free of green TCBS colonies. Enrichment products must be free of shrimp pathogens. Fresh feed must be

from prequalified suppliers and provide evidence of freedom of shrimp pathogens as per KSA listed diseases.

2.10. Moderate Risk Activities

Nurseries and grow---out ponds are operated outdoor and therefore total exclusion of pathogens is not achievable. Such production systems require equal emphasis on animal health monitoring, water parameters monitoring and close track keeping of animal performance. All incoming water must be filtrated preferably at 250 um (See SOP 6 Water use in shrimp culture). Potentially, animals will be exposed to endemic pathogens entering the production system essentially through water or carriers. Mechanical filtration plays a very significant role but its efficacy is limited due to the large volumes of water needed. This antagonism must be well balanced and every effort must be made to anticipate problems by keeping filtration systems in proper conditions as well as water parameters adequate and stable.

In the case of nurseries and intensive ponds, the perimeter needs to be fenced and the bottom-lined. It is recommended to have these structures covered.

Low Risk

Even if a high-risk activity such as processing or packaging seafood or live aquatic animal transportation takes place, it does not represent a very significant risk as long as the outcomes from such activities are contained.

Regarding the Processing Plant, high biosecurity standard must be maintained. Vehicles delivering seafood raw material must be disinfected before and after delivery, before loading ice in the case of harvest vehicles. The same applies to the harvest equipment. Effluents must be contained, disinfected and disposed without any contact with production areas including farm drainage canals. Solid wastes must be incinerated except for wastes that are further processed through rendering or chitin production. Imported product cannot be processed unless; the effluent treatment plant is in place and proven efficiency.

Regarding Laboratory Services, movement of laboratory staff, tools and equipment towards production areas should be avoided. It is recommended to deliver the fixed samples to the laboratory. Wastes from laboratory services are categorized as high risk and treated as such.

Regarding site accommodations and offices, movement of staff, catering goods and other housing related items are permitted within site accommodations. However, depending on the biosecurity status of the unit, the site access can become restricted.

The movement of staff and their vehicles between zones should be therefore regulated and restricted when they move out of their area of operation.

2.11. Compulsory reporting of disease outbreaks

Any abnormal mortality at a production unit, needs to be immediately reported (within 24h) to SAS so that the emergency response is triggered. In addition, production units with diagnostic capacity need to report the detection of KSA listed pathogens within the same timeframe.

SAS will inform of the positive case to the Government and to the rest of the shrimp industry within the same day, confirm the emergency response and contingency measures taken.

2.12. Emergency response and contingency plan

As soon as mortality or KSA listed pathogen is detected, the emergency response is triggered. This implies the immediate sending of a technical team to collect suitable samples and assess the production conditions that may have triggered the mortality. Measures to minimize the risk of spread of pathogens into the sea will be implemented. This may mean the blocking of the effluent canal.

In case a primary pathogen is confirmed, the contingency protocol must be applied within the shortest possible time frame to minimize the risk of disease spreading (see SOP 8 contingency plan). Culling or emergency harvest will be decided based on the value of the affected stocks, season and other factors.

2.12.1 Control of importation of live shrimp

Importation of live shrimp for aquaculture production is restricted in KSA. The government has adopted a policy of SPF stocks only in combination with Specific Pathogen Tolerance for WSSV. Importations may be considered for improvements of shrimp breeding programs. There are two requirements to comply with importations:

2.12.1.1 Pre-approval of supplier after assessment of SPF status and health condition for the previous 2 years and on-site audit

SPF animals will be submitted to quarantine until their health status is validated as per quarantine protocol. (See SOP 2. proceedings for the importation of live shrimp species into the kingdom of Saudi Arabia)

2.12.1.2 The approved stocks will be subjected to quarantine where they will be tested for all KSA listed pathogens and only be released if no pathogen is detected. PCR and histology techniques will be used for this purpose. Sample size should target a 2% prevalence with 95% confidence. Quarantine is performed indoors; all incoming water is disinfected; all effluents are to be treated and solid wastes incinerated. (See SOP 1 Quarantine procedures)

2.13. No wild broodstock allowed

The use of wild animals as broodstock is not allowed in KSA due to the high risk they represent. Latent infections are common in wild animals, both with known and unknown pathogens. Only broodstock coming from an SPF program with a sanitary history of at least 2 years are allowed for aquaculture purposes.

2.14. Restriction on aquatic products based on the SPS agreement of the WTO

The importation of aquatic products presents a risk for the wild fisheries, environmental diversity and national shrimp industry. Therefore, restrictions in the importation from countries with poorer health status than KSA should apply. The Sanitary and Phytosanitary Agreement (SPS) from the World Trade Organization provides the criteria to implement such protection and will be applied case by case as considered necessary

SOP 1. Proceedings for the importation of live shrimp species into the kingdom of Saudi Arabia

The Aquaculture Division of the Ministry of Agriculture (GDF-MEWA) of Saudi Arabia has prepared the Standard Operating Procedures for Importation of live shrimp species into the Kingdom and several Proceedings related to the different activities involved in the process. The aim is to maintain the high sanitary status in the Kingdom, to avoid the introduction and propagation of exotic diseases that could affect the different shrimp species and to serve as a guide to the different shrimp farmers. Each company, interested in importing live shrimp species into the Kingdom must follow this manual and the different Proceedings accompanied.

General Proceedings for the Importation of live shrimp species:

1. Approval of suppliers

Importation of life animals requires approval of supplier, which will imply the visit to the supplier facility, analysis of diagnostic records of at least the last 2 years and records of introductions to the facility during this period.

2. Importation request and documents

The company that is interested in importing live shrimp species into the Kingdom must notify the Aquaculture Division of the Ministry of Agriculture (GDF-MEWA) their intention at least 30 days in advance prior to the expected arrival date. The information to be provided through the Request of Importation is shown in Table 1.

Table 1: Request of Importation Document necessary for the Importation of live shrimp species

1	Name of the importing company, address, identification # number, telephone and email ~~of~~ contact
2	Name of the exporting company and country of origin
3	Name of the live shrimp species to be imported (scientific and common names)
4	Number of animals per species to be imported
5	Stage of the live shrimp species to be imported (nauplii, post larva, juveniles, broodstock)
6	Origin of the water where the live shrimp species to be imported and their parents are / were kept (open system, recirculation system etc.)
7	Expected dates of arrival
8	Port of arrival in the Kingdom of Saudi Arabia (name of sea-port and or air-port)
9	Name of the farm and location where the live shrimp species will be Maintained

It is important to note here that the live shrimp species to be imported can ONLY be sourced from sites approved by GDF-MEWA. If the live shrimp species is exotic in the Kingdom of Saudi Arabia, and it is the first time that it is imported, an Import Risk Analysis (IRA) must be performed including environmental/ecological, genetics and pathogens risk analysis. While methodology for genetic and ecologic/environmental risk assessments are not codified, a standardized framework for pathogen risk analysis (import risk analysis, IRA) for live aquatic animals and their products is laid out in the World Organization for Animal Health's (OIE) Aquatic Animal Health Code (OIE, 2012) and FAO documents.

3. Health Certificates

Along with the presentation of the Request of Importation document, the interested importing company must present the corresponding Health Certificates signed by the Official Authority of the exporting country. Table 2 shows the Information required for the Health Certificate.

Table 2: Health Certificates information required for the Importation of live shrimp species

a)	Date and place where the Certificate was Issued
b)	Country of origin
c)	Official Authority of the Country of Origin
d)	Identification of the Exporter
e)	Identification of the live shrimp species to be exported
f)	Zone or compartment of origin
g)	Identification of the farm origin
h)	Stage of development at the time of exportation
i)	To mention if the live shrimp are coming from a farm or it is a wildlife animal
j)	Purpose of the live shrimp importation
k)	Mean of transportation (air, sea, terrestrial)
l)	Identification, signature and stamp of the Certification Company

The Health Certificate has to state that the consignment satisfies the following requirements:

1 Come from a country which is under veterinary supervision.

2 Come from a farm which is under veterinary supervision.

3 Show no clinical signs on the day of loading.

4 Come from a zone that is free of the GDF-MEWA listed diseases (Table 3).

5 The certificate is valid for 10 days from the date of issuing.

Table 3. List of the GDF-MEWA diseases for shrimp

Disease		Category
1.	White spot disease	C1
2.	Taura syndrome disease	C1
3.	Yellow head disease – Yellow head virus	C1
4.	Infectious hypodermal and hematopoietic necrosis	C1
5.	Infectious myonecrosis	C1, C2
6.	Necrotizing hepatopancreatitis	C2
7.	Monodon Baculovirus (MBV)	C2, C3
8.	Microsporidia	C2, C3
9.	Gill-associated virus	C1, C2
10.	Early Mortality Syndrome (EMS) or Acute hepatopancreatic necrosis disease (AHPND)	C1
11.	Hepatopancreatic microsporidia	C2

The verification of the sanitary status of the shrimp stock to be exported requires that the exporting facility send the samples for sanitary analysis in the reference laboratories for shrimp diseases (See annex 1).

Any Company interested in the importation of any live shrimp species into the Kingdom must have an Authorization provided by GDF-MEWA that allows them to proceed prior to importation. Only importation from GDF-MEWA approved suppliers will be considered. At least 72 hours before the expected arrival of the live shrimp species, the importer must show the documents present in table 3 along with the corresponding Health Certificates. These documents must be presented to the GDF-MEWA officer located both at the entrance place and also at the division where the species will be allocated.

Certificate of origin

The Certificate of Origin is a document that is used in International trade, where the concept of origin refers to the country where the goods were produced and not necessarily originated from. Each shrimp consignment must come with the Certificate of Origin.

Additional Importation documents required

Besides the request of Importation and the health certificates, the documents stated in the table 4 are required by GDF-MEWA to issue the authorization for importation:

Table 4: Additional Documents requested during the process of Importation of live shrimp species

1	Copy of the Invoice provided by the Exporter
2	GDF-MEWA approval certificate
3	Air bill tracking document – number
4	Import Risk Analysis (IRA) in the case of an importation of Exotic – non native species
5	Request of Quarantine (SOP 2)
6	GDF-MEWA Quarantine Certificate authorizing the unit where the consignment will be allocated (SOP 2)

--- All these documents will be submitted to GDF-MEWA. In a time frame of 1 month, GDF-MEWA will study the documents and will issue the authorization for importation.

--- In case that the species to be imported is considered as endangered species according to CITES (Convention on International Trade in Endangered species of Wild Fauna and Flora), GDF-MEWA needs to approve the sanitary status. In this case the office from the exporting country and the importing countries will issue the authorization for exporting and importing respectively.

--- Once the shrimp enter the KSA, no opening of the boxes is allowed until the final destination, where proper disinfection and elimination of the boxes, water and ice is done.

All shrimp in the consignment must be packaged in leak---proof bags, each bag containing only one species. The bag must be colorless and sufficiently transparent to enable proper inspection and identification of the shrimp and must not contain any extraneous matter, unapproved plant material or pests.

--- The consignment must be accompanied by documents that include the identification number of each box or carton, and the scientific name and number of the contained shrimp. It is recommended that the common names of the shrimp also be included on the papers.

--- All shipments of shrimp will be inspected by GDF-MEWA on arrival to ensure that they:

a. Are healthy

b. The veterinary certification and invoice is in order
c. Are an approved species
d. Do not contain prohibited material or material of quarantine concern.

--- Shrimp not meeting these criteria and prohibited material will be seized or exported or destroyed at the importer's expense

--- All shrimp will be ordered into quarantine at an GDF-MEWA approved location (See SOP 2 Quarantine procedures).

SOP 2. Quarantine procedures

Movement of shrimp from abroad

--- Only shrimp population with GDF-MEWA approval from the exporting country will be allowed to enter the primary quarantine facilities.

--- A primary quarantine is the one to receive the animals from abroad and will hold them until all the required testing has shown not to pose an infectious risk. A primary quarantine is used for the first introduction of a particular supplier. A secondary quarantine is a private sector quarantine that needs approval from GDF-MEWA before the reception of animals released from the primary quarantine. Based on GDF-MEWA approval, a primary quarantine may be a government or private sector facility. In case that the primary quarantine is a private sector facility, a secondary quarantine might not be required.

First level quarantine facilities for imported live shrimp from an GDF-MEWA certified supplier

1. A request for quarantine must be filled up and sent to GDF-MEWA for approval.

2. It is recommended to be adequately isolated from all of the rearing and production areas to avoid any possible cross contamination.

3. It must be in an enclosed and covered building facility.

4. There must be means provided for disinfection of feet (foot-dip deep containing hypochlorite solution at 50 ppm active ingredient) and hands wash facility (bottles containing 70% alcohol / or iodine solution at 100 ppm) to be used upon entering and exiting the unit.

5. Entrance to the quarantine area must be restricted to the personnel assigned to work exclusively in this area.

6. Quarantine unit staff must enter through a dressing room, where they remove their street clothes, take a shower and put on working clothes and boots specific for the quarantine. At the end of the working shift, the sequence is reversed.

7. Pumped water will go through a mechanical filtration including sand filter to obtain the water filtered to at least 1 um.

8. Water disinfection with either 15 ppm (72 h),30 ppm chlorine (24 h) or ozone at 0.5 mg/L (10 minutes contact time; 8 minutes for 1.5 ppm) any other disinfectant to ensure proper water disinfection would be used and

9. Must to be validated by bacteriology in a general media.

10. Water will pass through activated charcoal unit.

11. Water will pass through UV light at 10 ml/cm 2.

12. All the tanks must be washed with soap and water and disinfected with hypochlorite solution (100 ppm active ingredient) and rinsed with disinfected water.

13. All wastewater must be collected for chlorination (100 ppm for not less than 1 day) and de-chlorination before released to the environment.

14. Used plastic containers and hoses must be washed and disinfected with hypochlorite solution (100 ppm) or other disinfectants at an equivalent concentration, before reuse.

15. All the materials used in the quarantine unit must be clearly marked and should remain in the quarantine area. Facilities for disinfection of all equipment at the end of each day should be available.

16. On entering the quarantine area, the shrimp should be gradually acclimatized to the same temperature and salinity of the tanks.

17. Once the shrimp are placed in the holding tanks, the packing plastic bags, boxes, and any other disposable material related to the shrimp packing must be incinerated. Styrofoam boxes could be disinfected at 200ppm chlorine and allow to dry for 5 days.

18. Dead shrimp will be sampled for PCR of GDF-MEWA listed pathogens. For a first importation all GDF-MEWA listed pathogens must be tested. Gills/pleopods organ and hepatopancreas from each recent dead shrimp will be individually preserved in 95% ethanol (See SOP 14 Sampling for PCR analysis).

19. Any symptomatic shrimp will be fixed in Davidson's fixative by injection, maintained for 48 h and then transferred to a 70% ethanol plastic container for histological analysis (see SOP 14 for fixation).

20. When the shipment involves low temperature (animals have been packed and shipped at temperatures <=22C), it is assume that no further cold challenge is needed. If this situation does not happen, a cold challenge is required: 10 random shrimp will be individually passed through a dip of iodine---PVP solution (20 ppm for 10'' minutes) or formalin (100 ppm) and placed in the cold challenge facilities (See SOP 12 Cold challenge).

21. Cohabitation studies are required for a first introduction of a new species or new supplier: After acclimation, 5-10 random shrimp are taken and placed them in a different tank along with 10 healthy P. indicus from the Fish Research Center (JFRC) and 5 SPF P. vannamei of about the same weight (with respective controls for each species).

The P. indicus used, must have a history of freedom of any of the OIE and GDF-MEWA listed pathogens and no unusual mortalities (this may pose a problem depending on the health status of the local P. indicus stocks).

22. Ingestion challenge is required for a first introduction of a new species or new supplier: After acclimation, 5-10 random shrimp are taken from the quarantine and homogenized them by grinding the whole tissue to feed 10 healthy P. indicus from the Fish Research Center (JFRC) and 10 SPF P. vannamei. Shrimp will be fed with the 3% of biomass/day in two rations (AM, PM) for three days. The P. indicus used, must have a history of freedom of any of the OIE and GDF-MEWA listed diseases and no unusual mortalities (this may pose a problem depending on the health status of the local P. indicus stocks).

23. If presence of shrimp displaying any clinical signs during the cold, cohabitation or the ingestion challenges, shrimp samples will be immediately collected for histological and PCR analysis.

24. After 5 days of cold challenge, every shrimp will be individually sampled. Samples of gills and hepatopancreas will be taken and preserved in 95% ethanol for PCR analysis. 5 shrimp will be fixed for histological analysis (See SOP 15 fixation of shrimp and crustacean samples in Davidson's fixative).

25. After 10 days of cohabitation challenge, every P. indicus and P. vannamei will be individually sampled. Samples of gills and hepatopancreas will be taken and preserved in 95% ethanol for PCR analysis. 5 shrimp from each species will be fixed for histological analysis (See SOP 15 fixation of shrimp and crustacean samples in Davidson's fixative).

26. After 10 days of the ingestion challenge, every P. indicus and P. vannamei will be individually sampled. Samples of gills and hepatopancreas will be taken and preserved in 95% ethanol for PCR analysis. 5 shrimp from each species will be fixed for histological analysis (See SOP 15 fixation of shrimp and crustacean samples in Davidson's fixative).

27. Samples of a first introduction must be sent to an international reference lab (according to OIE) for histological analysis and PCR analysis for WSSV, TSV, YHV, IMNV, MBV, IHHNV, AHPND and NHP---B (See annex 1

28. Reference labs). Subsequent shrimp introductions can be analyzed at the national reference lab or GDF-MEWA approved laboratories.

29. In the case that any sample gives a positive result for any of the GDF-MEWA listed diseases (See table 3) or display histopathological changes which are not recognized and might pose concern, the whole population will be discarded.

30. Only shrimp stock free of any of the GDF-MEWA listed diseases by PCR and histology would be taken to the secondary quarantine.

31. Second level quarantine

32. This quarantine has the same requirements in terms of infrastructure and water treatments as a primary quarantine. The second level quarantine could be a private sector quarantine approved by GDF-MEWA that could maintain shrimp if the following requirements are met:

33. Shrimp SPF stock from a primary quarantine that met all the health sanitary status required by GDF-MEWA.

34. Shrimp SPF stock from an approved secondary quarantine.

35. In secondary quarantine, samples for PCR and histology analysis will be taken and only if the animals are free of the GDF-MEWA listed disease, the stock would go to the production area. The check list for the quarantine is described below:

36. Quarantine unit staff must enter through a dressing room, where they remove their street clothes, take a shower and put on working clothes and boots specific for the quarantine. At the end of the working shift, the sequence is reversed.

37. Pumped water will go through a mechanical filtration including sand filter to obtain the water filtered to at least 1 um.

38. Water disinfection with either 15 ppm (72 h),30 ppm chlorine (24 h) or ozone at 0.5 mg/L (10 minutes contact time; 8 minutes for 1.5 ppm) any other disinfectant to ensure proper water disinfection would be used and

Kingdom of Saudi Arabia Ministry of Agriculture

Department of Aquaculture, GDF-MEWA Biosecurity Division

Fish Facility:

Date: Location:

Check list for Quarantine Unit approval

Characteristic	Result	Notes
1- Identification of the name of the company and the specific farm, plus the location and contact information.		
2- Present a general design together with the description of the specific technical characteristics of each part of the unit, including drawing and maps.		
3- Must be enclosed and covered with no direct access to/from outside.		
4- has an exclusive entrance, restricted only to authorized people who are working – also exclusively- in the unit.		
5- Must be separated and isolated from any other farming operation.		
6- Has record systems of all activities and management.		
7- Has an independent entrance, circulation – flow and exit of the water.		
8- Has system of water recirculation.		
9- Has the water treatment systems that include filtration of the discharged water.		
10- Has a water treatment system that include disinfection of the discharged water.		
11- Has specific – properly identified areas for disinfection of people and materials at both the entrance and exit of the unit.		
12- Implements cleaning, aeration and disinfection system in all of the areas.		
13- Has special clothes and boots that are used exclusively inside the unit.		
14- Has an independent - physically separated room, for the inspection and sample preparation for the pathology test. This room is located inside the Quarantine Unit, but separated from where the animals are kept.		
15--- Has proper proceedings and infrastructures for the destruction of the used materials and mortalities.		
16--- Standard Operating Procedures are present and applied in the unit facilities.		

GDF-MEWA Name and Signature

Name of Importer			
Name and Location of Quarantine Unit			
Initial date		# animals	
Finish date		# Invoice	
Address		Phone	

Item	Common name and sex	Scientific name	Quantity	Inspection	
				1	2
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
Total					

Observations Date of Inspection

1

2

Date of Quarantine start

Name and signature of Inspector Official Stamp Quarantine extension Details

Date of Quarantine release

Name and signature of Inspector Official Stamp

Quarantine Records

Mortality Records

Approval #_____

Days	**Item**					**Observations**
	1	2	3	4	5	
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
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22						
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24						
25						
26						
27						
28						
29						
30						
31						

Name and signature of Quarantine Unit responsible

SOP 3. Certification of shrimp movement within the country

In order for GDF-MEWA to issue a Movement certificate, the necessary information must be provided:

1. Written Request of shrimp movement 5days before the movement.

2. Include the following information:

a) Description of to/from the shrimp will be moved
b) Date of the movement
c) Species and stage
d) Origin of the stock

PCR results for endemic pathogens from an GDF-MEWA approved lab. Only stocks with negative results for endemic pathogens would be allowed to be moved

SOP 4. Shrimp transfer / Movements

1. Transfer of brood stock

- Non-SPF stocks raised in the same facility have to be directed to Quarantine (SOP 2 Quarantine procedures).
- SPF stocks transferred within the same operation, while recommended, do not require further testing.
- SPF stocks transferred to another operation will require PCR testing for endemic pathogens and histology analysis.

2. Transfer of Nauplii

- NAUPLII originated from SPF stocks need no certification for movement within the same facility.
- NAUPLII that are transferred to another operation will only be allowed from brood stock with proven sanitary records.

3. Transfer of PL's

- PLs will require cold challenge (SOP 12 Cold challenge) and PCR certification for endemic pathogens before transferring to nurseries or grow-out ponds.
- Only stocks with negative results for endemic pathogens would be allowed to be moved.

4. Transfer of juveniles

- Juveniles in nurseries that have maintained at the same biosecurity level as in larval rearing will not require PCR testing when transferred within the same operation. However, during winter stocking PCR screening after the cold challenge is mandatory
- If juveniles have already been exposed to a lower biosecurity level and therefore, PCR testing for endemic pathogens is required prior transfer to a pond even within the same facility.

Procedure for movement/transfer clearance

- Requesting shrimp farms place their order with SAS defining anticipated yearly requirement, based on the stocking density, spatial size and carrying capacity of the Farms.
- SAS forwards the request to JFRC for evaluating status and suitability of the shrimp grow out and/or Hatchery installations of the requesting farm
- JFRC conducts an audit on the suitability and status of the shrimp grow out and/or hatchery through an approved check list & forwards its recommendation to SAS
- Accordingly SAS advises supplier to allot the quantity based on the audit report issued by JFRC

- SAS will conduct health screening (PCR) through a cold challenge test at supplier's project site Supplier will distribute the approved quantities only upon performing PCR test. Only for negative results (no pathogen present) will be cleared for transfer. The counting process will be carried out under the supervision of an authorized representative of JFRC.
- JFRC & ADMEW will reserve the right of issuing approval
- SAS will be the center point for any dispute that may arise.

Responsibilities of parties involved in the process

Requesting Shrimp Company

1. Shrimp Company nominates a member of its staff with the competence and authority to deal with the issue as its focal point. His contact details (email and mobile) need to be communicated to all parties involved.

2. Shrimp Company submits its requirements in the beginning of the year to the SAS by the appointed representative; in his absence, the other contact person details need to be submitted to all the persons involved in this operation.

3. The company representative will get all the relevant information on this distribution arrangement only from SAS-approved representative

4. The company will receive the shrimp after the certification "disease free" is issued from SAS biosecurity unit.

5. The company will receive from SAS the PCR analysis report of Jeddah Fish Health & Safety Laboratory as well as any analyses made by SAS as 'third party audit'

6. Company representative will communicate with SAS only on any possible issues or disputes in receiving the consignment.

7. In winter period, from November to March, an additional sampling for PCR test is recommended by the bio security committee and this will be carried out by the shrimp company. Qualified sampling staff of Shrimp Company will carry out this process and get tested with the Jeddah Fish Health & Safety Laboratory.

Saudi Aquaculture Society (SAS)

1. SAS will collect the requirements order from the requesting company.

2. Based on the quantity requirements, SAS will request JFRC to audit the site of the requesting farm and check the status for receiving the requested quantities ate the requested dates.

3. Based on the JFRC's audit report, SAS will advise the supplier to allot the requested quantity.

4. SAS will conduct the "third party audit" on the quality of the shrimp product through the PCR for the listed pathogen after the cold challenge test

5. SAS will collect the PCR analysis report from Jeddah Fish Health & Safety Laboratory and forward to the Shrimp company.

6. SAS reserves the rights for cancelling or postponing the shrimp distribution upon reasons that relate to the biosecurity of the farm and the industry.

7. SAS will function as a center point and coordinate with all the parties in this distribution process

Shrimp-Supplier

1. Supplier in principle agrees to supply animals from specific pathogen-free breeders.

2. Supplier representatives will coordinate with SAS/JFRC/Shrimp company representatives to organize the distribution from the hatchery.

3. Supplier will interact through SAS on arranging the JFRC-nominated representatives to supervise the counting related works and also provide the accommodation with food facility for the JFRC staff.

4. Supplier will coordinate with SAS to screen the listed pathogen on the products of shrimp larvae through cold challenge prior to the shipment. In this test, SAS will act as an external third-party auditor; SAS will collect a sample from the batch ready for shipment for the disease screening against the listed pathogens.

Jeddah Fisheries Research Center (JFRC)

1. Based on the request by SAS, the JFRC technical persons will conduct the site audit to evaluate the status and suitability of the shrimp grow out and/or Hatchery installations of the requesting farm
2. JFRC will send the status report to SAS with recommendation concerning the requested release of animals (PL's or nauplii) to the shrimp company.

3. JFRC will involve in the counting process of animals. All the records and documents related to the counting process will be maintained by the JFRC for any dispute that may arise between parties.

Jeddah Fish Health and Safety Laboratory (JFHSL)

1. JFHSL will process the health screening test (PCR) on the specimens brought by the SAS sampling team from suppliers after the cold challenge test.

2. JFHSL will not accept the samples for health screening on Thursday after 10 am, Friday and Saturday.

3. JFHSL will send the soft & hard copy of the analysis report to the SAS bio security manager or to the authorized person of SAS.

Critical Issues

1. SAS will take the whole responsibility of compiling the yearly requirements of PL's and nauplii of the shrimp company.

2. Supplier will take the responsibility of supply of disease-free animals from Specific pathogen free brood stock.

3. JFRC will take the responsibility of approving the shrimp farms for stocking and the quantity and the products.

SOP 5. Use of wild shrimp in aquaculture facilities

1. It is prohibited to use wild shrimp for direct aquaculture production.

2. Only hatchery produced animals can be used to stock aquaculture ponds.

3. Wild animals may be used for specific purposes (i.e. increase genetic diversity in breeding programs). In that case, animals must be subjected to Quarantine procedures (SOP 2 Quarantine procedures).

4. Prior to collection, GDF-MEWA must approve the collection of wild animals and must approve the quarantine facility to be used.

5. Wild animals to be used in a prawn hatchery must be subject to the following quarantine procedures:

    a. All animals brought from the wild must be maintained in the quarantine facilities in individual quarantine tanks.
    b. All animals brought from the wild must be tested individually by PCR for all GDF-MEWA listed shrimp pathogens.
    c. Target tissues to be analyzed are pleopods/gills and feces depending on the target pathogen.
    d. Any animal that tests positive, to any GDF-MEWA listed diseases must be destroyed.
    e. Animals that are negative to all GDF-MEWA listed diseases may be pooled together.
    f. Within the quarantine facility, the wild broodstock (negative to GDF-MEWA listed diseases may be used for production of offspring.
    g. Females will be tested a second time after ablation. After the spawning, both male and female will be sacrificed and analyzed by histology. If positive for any GDF-MEWA pathogen or unknown pathogen, offspring must be discarded. The remaining tissues/organs must go through histology and they need to be cleared by a veterinarian or pathologist.
    h. These offspring (First Generation) must be reared in a separate wing within quarantine facility and tested at 10 grams for all GDF-MEWA listed prawn diseases.
    i. Any batch that test positive, to any GDF-MEWA listed diseases, must be destroyed.
    j. Based on the sanitary data, GDF-MEWA would approve the introduction of the shrimp population into the Aquaculture facilities.

SOP 6. Water use in the shrimp culture

1. Breeding center, indoors broodstock, Nauplii production units and Larval rearing units

1.1 Pumped water will go through a mechanical filtration including sand filter to obtain the water filtered to at least 1 um.

1.2 Water disinfection with either 15 ppm (72 h),30 ppm chlorine (24 h) or ozone at 0.5 mg/L (10 minutes contact time; 8 minutes for 1.5 ppm) any other disinfectant to ensure proper water disinfection would be used and must to be validated by bacteriology in a general media.

1.3 Water will pass through activated charcoal unit.

1.4 Water will pass through UV light at 10 ml/cm ^{2}.

1.5 It is recommended that discharged water be retained in effluent treatment ponds especially in cases of disease outbreak.

1.6 All solid wastes (dead animals, molts, food left over, etc.) recovered must be disposed off properly (incineration or burial).

1.7 There will be appropriate documentation of each step in the process using the formats provided. In particular there must be clear documentation of ozone and/or chlorination procedures (see SOP 17 Disinfectants) of incoming water. Where chlorine is used as a disinfectant, the activity of the product will be tested and that value used in the calculation of dosing rates. Additionally there must be clearly documented evidence of sustained free chlorine availability in water held during the 72-hour period. When ozone is used as the disinfectant of choice there will be documentation of Oxidation Reduction Potential (ORP) levels.

2. Nurseries and outdoor ponds

2.1 Filter screens including the wooden water level control boards (known as slabs) are fixed in the main water supply canal. Generally, double layer filters (1,000 and 500µ) are used in large water control structures, like main and secondary reservoir channels. Main water supply canal is filtered initially with 1,000µ and installed additionally with 250µm bag nets.

2.2 All pond water filling requirements need to be checked by responsible managers before proceeding to next activity. When desired water level is achieved, ensure that inlet and outlet gates are soil-sealed before applying GDF-MEWA approved crusticide (0.5ppm) and copper sulphate (0.5ppm). Copper sulphate is used to eliminate zooplankton which is

known to be carriers of WSSV and which may be transmitted to shrimp. Secondary water supply canal is also disinfected with same concentration of GDF-MEWA approved crusticide and copper sulphate. Chlorination is another option and is widely used to disinfect production or culture ponds in preparation for post larvae stocking. The common dose used for killing disease vectors and for treating pond water requires 30ppm active ingredient in the water.

2.3 After application, filter screen with 250µ mesh can be fixed to each pond inlet gate only when desired water depth in a pond is achieved and when final disinfection is completed and as such is only used for water exchange. It is worth remembering that treatment has to be done as effectively as possible as chemicals represent an import part of the cost of production in semi-intensive culture.

2.4 All screens and control boards should be inspected regularly for leaks and all leaks must be sealed to prevent unfiltered water from entering ponds using rubber gasket type material. No debris recovered from screens or bag nets or other material is to be put back into supply canal or pond. Put all this material in buckets and dispose of in designated pit and cover it with hydrated lime.

It is recommended to take plankton samples of the reservoir ponds/tanks for WSSV by PCR 3 days after the treatment.

SOP 7. Criteria for shrimp sampling in ponds/tanks for sanitary analysis

1. Start the surveillance in the healthier ponds/tanks in order to avoid cross contamination from a symptomatic pond to a healthy pond/tank. This could be based on the following variables. Start with ponds that have:

a) High survival rate
b) No history of mortalities
c) No birds presence
d) New ponds

2. The last pond/tank sampled must be the ones that have reported mortality.

3. If a pond/tank displaying mortality is sampled, do not take samples in another pond/tank unless some mortality is observed as well.

Recommended procedure to increase the chance to take a shrimp displaying clinical signs

Grow out pond:

1. Let the selected pond drain for at least 20 min.

2. Take the sick shrimp adhered to the outlet gate net.

3. If no sick shrimp observed in the outlet net, take the samples in that area of the pond by cast netting.

4. Place the shrimp in a clear bucket to observe unusual discoloration.

5. If shrimp looks moribund in the bucket, select them for sampling.

6. Select preferably shrimp displaying any clinical signs such as:

a) Moribund
b) Dark /red discoloration
c) Melanization or dark spots in the exoskeleton
d) Soft shell
e) Weak behavior
f) Black gills

7. Take samples for PCR and histology according SOP 14 and SOP 15.

Tanks:

Cut the air flow.

1. Select preferably shrimp displaying any clinical signs such as:

a) Moribund
b) Dark /red discoloration
c) Melanization or dark spots in the exoskeleton
d) Soft shell
e) Weak behavior
f) Black gills

2. Take samples for PCR and histology according SOP 14 and SOP 15.

SOP 8. Contingency plan

Upon presence of any of the GDF-MEWA listed diseases in the category C1 in Quarantine, breeding program, commercial broodstock, post larva and nursery or massive mortality in grow-out ponds, the affected stock will be harvested or eliminated depending on the economic value. See table below.

Pathogen	Category	Quarantine	Breeding program	Commercial broodstock	Post larvae & nursery	Grow out
High risk						
EMS	C1	Eliminate	Cleaning	Eliminate	Eliminate	Harvest/eliminate
WSSV	C1	Eliminate	Cleaning	Eliminate	Eliminate	AM Temperature ≥30ºC: Monitor* AM Temperature ≤30ºC:: Harvest /Eliminate
IMNV	C1	Eliminate	Cleaning	Eliminate	Eliminate	Harvest/eliminate
TSV	C1	Eliminate	Cleaning	Eliminate	Eliminate	Harvest/eliminate
YHV	C1	Eliminate	Cleaning	Eliminate	Eliminate	Harvest/eliminate
Moderate risk						
EHP	C2	Eliminate	Cleaning	Eliminate	Eliminate	Eliminate /harvest if severe
NHP	C2	Eliminate	Cleaning	Eliminate	Eliminate	Eliminate /harvest if severe
Low risk						
IHHNV	C3	Eliminate	Cleaning	Individual selection	--	--
BP	C3	Individual selection	Cleaning	Individual selection	Eliminate if severe	--
MBV	C3	Individual selection	Cleaning	Individual selection	Eliminate if severe	--

* Increase monitoring of Animal Health and mortality on affected ponds kept in production

* Improve management of ponds to minimize stress that may trigger an increase in mortalities

***Increase aeration**
*Increase water exchange to improve water quality

SOP 9. Emergency harvest or termination of a pond

Procedure:

1. Upon presence of massive mortality from any of the GDF-MEWA listed diseases in the category C1 in a shrimp pond, the affected stock will be harvested or eliminated depending on the economical value

2. Harvest or termination should be done within the shortest possible time frame.

3. Termination will be carried out by applying GDF-MEWA approved crusticide with close gates and a 10-12 days retention time. After that, water can be drained safely and dead shrimp needs to be collected and disposed off appropriately (incineration or burial)

4. Harvest of these ponds should be prioritized over any other harvest and it should start as soon as water levels are appropriate.

5. Place a net of a mesh in the discharge canal that can contain any shrimp escape from the harvesting process.

6. Shrimp harvested will be taken to the processing plant avoiding to spill water

7. Apply the SOP 9 and SOP 10

8. All the material will be disinfected with chlorine at 100 ppm or any other disinfectant (SOP 17) and sun dry.

9. Personnel involved in the harvesting process will change their clothes and boots in order before continuing with their tasks.

10. Vehicles involved in the emergency harvest will be washed and disinfected (SOP17)

SOP 10. Crustacean eradication during pond preparation

Procedure:

1. After a pond harvested or terminated, crustaceans in the pond bottom need to be eradicated before stocking new shrimp.

2. All staff involved in handling chemicals must be wearing adequate protection equipment.

3. Fill up the pond with water to reach about 30 cm of water column or enough water to ensure the entire pond surface is covered.

4. Gates must be previously sealed.

5. Doses depending on the product applied:

Chemical	Doses	Application
GDF-MEWA approved crusticide	It will depends on the active ingredient (see SOP 17).	Unique application, retain the water for at least 5 days (It will depends on the chemical).
Calcium hypochlorite	50 ppm	Apply during the afternoon and re-enforce daily for 5 days.

6. After 10-12 days, drain the pond and with a shovel make a hole of 20 cm in different areas of the pond.

7. Check for live crustaceans. If present, repeat the steps 3 through 5.

SOP 11. Termination of a shrimp tank

1. Upon presence of massive mortality or positive PCR from any of the OIE listed diseases in the category C1, the affected tank will be immediately terminated.

2. All staff involved in handling the pesticides/disinfectant must be wearing adequate protection equipment.

3. Stop any water exchange and close the drainage pipes and seal the outlets.

4. Discard the tank by chlorinating at 100 ppm for 24 h. or by applying an equivalent crusticide (dosage based on table on SOP 17 Disinfectants).

5. All the materials/equipment that has been in contact with the shrimp and water must be disinfected by chlorination at 100 ppm, rinsed with water and sun---dried properly before storage.

6. This remains restricted area, sealed for any visit for 1 day.

7. After 1 day, tank water can be drained out if no chlorine residues.

8. Use bag nets when the tank is drained out to retain the dead shrimp.

9. Ideally, dead shrimp must be incinerated. Burial is also an acceptable disposal method.

10. Bags with dead shrimp must be transported to an identified pit area.

11. The entire surface of the pit should be covered with a layer of soil of at least 10cm.

12. Disinfection by chlorination of the water pipes must be done with 100 ppm of Chlorine for 24 h.

SOP 12. Cold challenge

1. Cold challenge methodology allows the replication of certain viruses, which results in a higher chance of detection of low level of infections. This procedure is required for any shrimp that go through a primary quarantine. Also, it is required for non---SPF broodstock, and different stages of animals, originated from SPF stock that have been exposed to lower levels of biosecurity.

2. The cold challenge room must be adequately isolated from all of the rearing and production areas to avoid any possible cross contamination.

3. There should be means provided for disinfection of feet (foot dip deep containing hypochlorite solution at 50 ppm active ingredient) and hands wash (bottles containing 70% alcohol / or iodine solution at 100 ppm) to be used upon entering and exiting the unit.

4. Tanks will be filled up with seawater previously chlorinated at 30ppm. Then if residual chlorine is present, sodium thiosulfate will be added or strong aeration.

5. Adjust water temperature 22ºC +/--- 2ºC.

6. Recommended densities for the challenge:
   --- 25 Post larva /L
   --- 4 juvenile/L
   --- 0.5 broodstock /L
7. 150 Pls will be used for a challenge test (2% expected prevalence) for 48 h.

8. 150 juveniles and broodstock will be the cold challenged for 5 days.

9. The effluent water will be discarded previous chlorine disinfection at 100 ppm.

10. Any dead shrimp gathered during cold challenge will be sampled. Tissues will be taken for WSSV, IHHNV and TSV analysis by PCR (See SOP 14 Sampling for PCR analysis).

11. The 150 Pls without the eye---stalk will be pooled in one sample for PCR analysis.

12. Juveniles and broodstock tissue such as pleopods, gills, lymphoid organ or haemolymph, will be taken and 10 subsamples of 15 pieces each (total 150 shrimp) will be pooled and analyzed for WSSV, IHHNV and TSV by PCR. See SOP 14 (Sampling for PCR analysis).

13. In the case of broodstock, if clinical signs are displayed during the challenge 10 affected shrimp in Davidson for histological analysis (See SOP 15 Fixation of shrimp and crustacean samples in Davidson's fixative).

14. Equipment used for transferring or disposing the animals must be disinfected by dipping it in 100 ppm sodium hypochlorite and rinsing with clean sea water and drying.

15. All the materials used for the cold challenge must remain in that place.

SOP 13. Fresh feed and Artemia cysts

1. Fresh feeds are a significant biosecurity risk. In the shrimp industry polychaete, squid, clams and artemia biomass are the most used in maturation/ Nauplii Production Units. Supplies cannot come from geographical areas where there are endemic diseases of significant commercial importance (GDF-MEWA listed diseases).

2. Fresh feed and artemia cysts suppliers must provide a certificate of origin and disease free status from an OIE reference laboratory.

3. Crabs or shrimp or any decapod cannot be used as a fresh feed.

4. These two conditions must be a pre---requisite for obtaining the importation license.

5. Artemia biomass must be analyzed by PCR for WSSV and IHHNV.

SOP 14. Sampling for PCR analysis

In order to protect the nucleic acid (DNA, RNA) for PCR, RT-PCR analysis, samples need to be preserved under proper conditions such:

- **DNA**: Frozen, 95% ethanol.
- **RNA**: RNA Later, 95% ethanol, frozen.

Procedure:

1. Samples for WSSV, YHV, IHHNV and TSV by PCR and any other etiology that infect cuticular epithelium, subcuticular epithelium and connective tissue will be Lethal samples: pleopods, gills, Lymphoid organ, haemolymph and PLs without eye-stalk Non-lethal samples: pleopods and haemolymph

2. Samples for IMNV by PCR any other etiology that infect striated muscle will be lethal samples: pleopods, abdominal muscle, lymphoid organ, haemolymph and PLs without eye-stalk. Non-lethal samples: pleopods and haemolymph

3. For hepatopancreatic viruses (MBV, BP, BMNV and HPV), bacteria (NHP), and HP microsporidia a piece of the hepatopancreatic lobule is collected.

   Lethal samples: Hepatopancreas
   Non-lethal samples: Feces

4. Samples for the Acute Hepatopancreatitis Necrotizing disease AHPND (EMS) could be pre-grown in a 1.5%NaCl containing TSB (trypticase Soy Broth) at 30ºC for 4 h in shaking conditions. If use of AP4 primers for AHPND PCR, this step could be omitted.

   Lethal samples: hepatopancreas, stomach
   Non-lethal samples: Feces

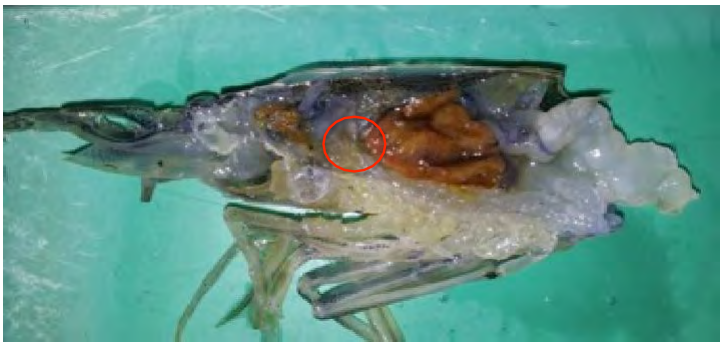
Lymphoid organ sampling (only broodstock):

Shrimp cephalothorax will be cut longitudinally in two halves (see picture).

1. Use sterile scissors and forceps to take the samples.

2. Pass the forceps/scissors by 70% alcohol and flame them before taking any other sample.

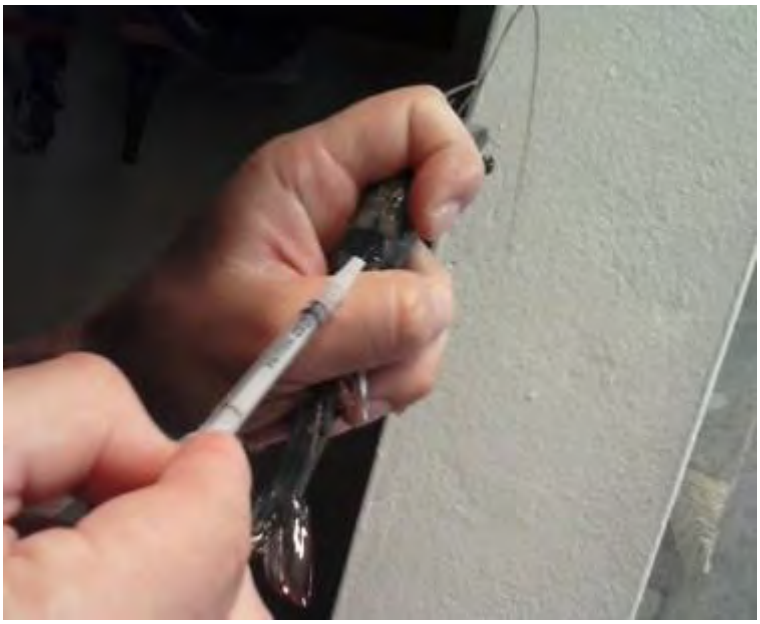
3. Area in front of the hepatopancreas (see red circle) will be taken with a forceps and placed in a 1 ml micro tube previously filled up with 95% ethanol.



Lymphoid organ location (red circle)

Haemolymph sampling:

Haemolymph will be withdrawn from the ventral area of the abdomen just close the junction to the cephalothorax (see picture). For this purpose, a 1 ml insulin syringe will be used.



1. Immediately the haemolymph is withdrawn, place it in a 1.5 ml micro tube containing 95% ethanol. The process needs to be done fast to avoid the haemolymph to clot.

2. The syringe and needle must be used only once.

Gills and pleopods sampling:

Pull out with a sterile forceps some gills lamella / pleopods and place them in a 1.5 ml micro tube containing 95% ethanol. Depend of the number of samples, a 15 ml tubes can be used.

1. Between samples, the forceps must be sterilized by dipping in ethanol and flamed.
2. Make sure that the whole tissue is immersed in 95% ethanol ration 1:10 (tissue: ethanol).
3. Label the micro tubes with pencil#2 with the details about the sample.
4. Micro tubes will be covered with parafilm plastic to avoid any leaking.

Back up samples:

Back up samples are samples taken from the **same** shrimp. e**.g.** If a pool of left pleopods / gill / lymphoid organ are used for PCR analysis. Tissue from the same organ must be taken as a back-up sample; i.e. if pleopods is chosen, back-up sample must be a piece of the same pleopods.

1. For back up sample of haemolymph, only half of the haemolymph withdrawn in the syringe will be placed in the sample tube and the other half will be placed in the back up sample tube.
2. In case that some positive results for any of the OIE listed diseases are found in a given sample, the backup sample will be ready to send to the diagnosis lab for confirmation purposes.
3. The backup sample will have the same codification that the sample tubes.

SOP 15. Fixation of shrimp and crustacean samples in Davidson's fixative

Davidson's AFA (alcohol, formalin, acetic acid) fixative. Davidson's AFA fixative is recommended for most histological applications. The fixative is rapid, reduces autolytic changes in tropical crustaceans (i.e. the penaeid shrimp), and its acidic content decalcifies the **cuticle.**

The formulation for Davidson's AFA is (for 1 liter):

1. 330 ml 95% ethyl alcohol
2. 220 ml 100% formalin (a saturated 37–39% aqueous solution of formaldehyde gas)
3. 115 ml glacial acetic acid
4. 335 ml tap water (for marine crustaceans, sea water may be substituted) Store the fixative in glass or plastic bottles with secure caps at room temperature.

For larvae and post larvae (PLs):

1. PLs that are too small to be easily injected with fixative using a tuberculin syringe, using a fine mesh screen or a Pasteur pipette, select and collect specimens.
2. Immerse shrimp selected for sampling directly in the fixative. Fix for 12–24 hours in fixative, and then transfer to 50–70% ethyl alcohol for storage.

For larger post larva, juveniles, and adults:

1. Inject Davidson fixative (use 1:1 volume (ml): weight (g) via needle and syringe
2. The hepatopancreas (HP) should be injected first and at two or more sites, with a volume sufficient to change the HP to a white to orange color; then inject fixative into adjacent regions of the cephalothorax, into the anterior abdominal region, and into the posterior abdominal region.
3. Immediately following the injection, cut the specimen after the first abdominal segment.
4. Cut the cuticle in the cephalothoraxic region, with dissecting scissors. The incision should be done just lateral to the dorsal midline.

5. Immerse the specimen in the fixative (use 10:1 fixative: tissue ratio).

6. Allow fixation to proceed at room temperature for 24–72 hours depending on the size of shrimp (or crustacean) being preserved. Longer fixation times in Davidson's AFA may be used to thoroughly decalcify the shell of crabs, lobsters, crayfish, etc.

7. Following fixation, the specimens should be rinsed with tap water and transferred to 70% ethanol, where they can be stored for an indefinite period.

8. Record a complete history of the specimens at the time of collection: gross observations on the condition of the shrimp (or other crustacean), species, age, weight, source (wild, or if culture pond or tank number, stock number, etc.), and any other pertinent information that may be needed at a later time.

9. The label should stay with the specimens in the same container during fixation, storage and transport to the laboratory. Always use no. 2 soft---lead pencil on water resistant paper (plastic paper is recommended; never use ink or marking pens as the ink is dissolved by alcohol).

Transport and shipment of preserved samples

Because large volumes of alcohol should not be posted or shipped, the following methods are recommended:

1 Remove the specimens from the 70% ethyl alcohol. For larvae, post larvae, or small juveniles, use leak---proof, screw---cap plastic vials if available; if glass vials must be used, pack to prevent breakage.

2 For larger specimens, wrap samples with white paper towels to completely cover (do not use raw cotton). Place towel---wrapped specimens in a sealable plastic bag and saturate with 70% ethyl alcohol. Insert the label and seal the bag.

3 Place the bag within a second sealable bag. Multiple small sealable bags can again be placed within a sturdy, crush---proof appropriately labeled container for shipment.

SOP 16. Plankton sampling for PCR analysis

1. Samples of plankton will be taken with a 60 µm net (see the picture below).

2. The net will be introduced in the water (above 30 cm below the water surface) and by boating or walking for 20 minutes by the border line, the sample will be collected in the collector bottles present in the net.

3. The samples will be extracted from the collector bottles with a sterile spatula and preserved in 95% ethanol in micro tubes.

4. Depend of the amount of plankton sample obtained from the sampling; a single sample will be conformed for no more than 1 g plankton sample.

SOP 17. Disinfectants

A key element of disinfection is a choice of a suitable disinfecting agent. Disinfecting agents are selected according to the nature of target pathogen.

For disinfection purposes virus falls into three basics groups

Category A: These viruses contain a lipid enveloped and are of intermediate to large size. These viruses are the easiest group to inactivate since the lipid envelope is sensitive to many lipophilic compounds.

Category B: These viruses are the most difficult to inactivate. They include small non lipid containing viruses and those protected within a protein matrix (occlusion).

Category C: These viruses are intermediate in their facility of inactivation by chemical agents. They do not contain lipids but are usually larger than viruses in category B.

Viruses	**Disinfection category**
1. White spot disease	A
2. Taura syndrome	B
3. Yellow head disease – Yellow head virus	B
4. Infectious hypodermal and hematopoietic necrosis	B
5. Infectious myonecrosis	
6. Monodon Baculovirus (MBV)	B
7. Gill-associated virus	A
8. Monodon slow growth syndrome	

The disinfection in bacteria can be divided in four groups depending on the cell wall nature.

Gram positive vegetative bacteria: Those tends to be most susceptible to disinfection.

Gram negative bacilli bacteria: Are most resistant to disinfectants agents than Gram negative cocci.

Mycobacteria: Tend to occupy an intermediate place between Gram-negative bacteria and bacterial spores.

Bacterial spores: Are most resistant to the action of disinfectants.

Reference table for water treatment and general disinfection

Chemical / disinfection method	Active ingredients	Dosage of active ingredient	Contact time	Scope	Application	Elimination of residues
Chlorine	Calcium hypochlorite	30ppm	1 hour	WSSV eradication in water	Water disinfection for hatcheries and grow-out	Sun light, aeration
		15ppm	1 hour plus 120 hours retention time	Zooplankton eradication	Water disinfection for hatcheries and grow-out	Sun light, aeration
		200ppm	1 hour	Surface disinfection	Disinfection of tanks and equipment	Dry out
		30ppm	1 min	Surface disinfection	Vehicles	Dry out, sun light
Formalin	Formaldehyde	100 ppm	30 sec	eggs, nauplii, PLs	Baths	Natural breakdown
Copper Sulfate	Copper	0.5ppm	48 hours	Zooplankton eradication (particularly rotifers)	Water disinfection for grow-out	Natural breakdown (10 days)
GDF-MEWA approved crusticide	Trichlorfon	0.5ppm	N/A	Crustacean eradication Zooplankton eradication except rotifers	Water disinfection for grow-out	Natural breakdown (10 days)
Quaternary Ammonium	Quaternary Ammonium	350ppm	<5 min	WSSV eradication and general disinfection	Foot bath, vehicle disinfection and of disinfection equipment	Natural breakdown
Potassium Permanganate	Potassium Permanganate	350ppm	<5 min	WSSV eradication and general disinfection	Foot bath, vehicle disinfection and of disinfection equipment	Natural breakdown
Povidine	Iodine	100 ppm	<5 min	WSSV eradication and	Hands dip and disinfection of delicate tools	Natural breakdown
		25 ppm	30 sec	eggs disinfection	Baths	
		50 ppm	30 sec	nauplii disinfection	Baths	
		200 ppm	1 min	foot dip	baths	
Ozone	Ozone	>0.5 mg/L for 1 minutes	8min for ORP values of 600-700	Eradication of any living organisms	Primary water treatment after mechanical filtration.	12 hours by oxidation (Bromine must be <0.05ppm)
UV	UV light	Radiation 200-300 nm	Irradiation must reach >10 mJ/cm2 in the incoming water flow	Eradication of micro-organisms bacteria and virus of category A	End of water treatment for hatcheries	N/A

SOP 18. Surveillance and prophylactic measures

Surveillance

1. Nucleus Breeding Center/ indoors broodstock tanks

1.1 Nucleus Breeding center

As per the GDF-MEWA operational surveillance protocol the following health monitoring must be done:

1.1.1 In case of Non-SPF broodstock, every batch of the spent females will be analyzed for WSSV and TSV by PCR. Samples of individual spent female will be pooled (ten samples for one pool) and analyzed for WSSV/TSV. In case of SPF broodstock, at least 10 samples per month will be analyzed for GDF-MEWA diseases list.

1.1.2 Daily mortality will be analyzed for WSSV and TSV by PCR. Pleopods from dead shrimp will be pooled (10 pleopods will be pooled in one sample).

1.1.3 If animals display any clinical signs they must be fixed for histological analysis.

1.1.4 PLs from SPF broodstock, while recommended, will not require further testing when moved within the same operation as long as the same level of biosecurity is maintained within the larval rearing process. PLs from SPF broodstock will require PCR certification for endemic pathogens when moved to a different operation from non-SPF broodstock will require going through cold challenge and PCR testing for WSSV and TSV.

1.1.5 150 Post larva will go through a cold challenge (See SOP 12. cold challenge). After the cold challenge, 3 pools of 50 PL each (total 150 PL per tank) will be analyzed for WSSV, IHHNV and TSV by PCR.

Each juvenile population will be sampled at least once per batch for WSSV, IHHNV and TSV by PCR. 3 subsamples of 50 pleopods each (total 150 shrimp) will be pooled in tube containing 95% ethanol

1.2 Nauplii Production unit and indoor broodstock tanks/ponds

1.2.1 For non SPF stock

a) Daily mortality present in the NPU will be analyzed for WSSV and TSV by PCR. Pleopods from dead shrimp will be pooled (10 pleopods will be pooled in one sample).

b) Every batch of the spent females will be analyzed for WSSV and TSV by PCR. Every 10 shrimp pleopods will be pooled in 1 sample (unless broodstock are used more than once).

c) Any shrimp displaying any clinical signs will be fixed for histological analysis in the NPU every month (See SOP 14: Fixation of shrimp and crustacean samples in Davidson's fixative.

d) Broodstock in the NPU units will not be fed with any fresh feed imported from areas where WSSV, TSV and AHPND are endemic (See SOP 13 Fresh feed).

1.2.2 For SPF stock

a) Daily mortality present in the NPU will be analyzed for WSSV, IHHNV and TSV by PCR. Pleopods/gills from dead shrimp will be pooled (10 pleopods will be pooled in one sample).

b) Any shrimp displaying any clinical signs will be fixed for histological analysis in the NPU every month (See SOP15: Fixation of shrimp and crustacean samples in Davidson's fixative).

c) Broodstock in the NPU units will not be fed with any fresh feed imported from areas where WSSV, TSV and AHPND are endemic (See SOP 13 Fresh feed).

d) Only eggs and nauplii produced by broodstock without history of any of the GDF-MEWA listed diseases with emphasis in WSSV and TSV will be allowed to continue in production.

e) Eggs will be dipped into at least 25 ppm iodine for 30 minutes and then rinsed with seawater.

f) Nauplii will be dipped into at least 50 ppm iodine for 30 minutes and then rinsed with sea water.

2. Larval Rearing / Post larval Rearing / Raceways

As per GDF-MEWA operational surveillance protocol the following health monitoring must be done:

2.1 Prior stocking the PLs, into the grow-out ponds / outdoors nursery ponds, each tank must be tested for WSSV and TSV by PCR. 150postlarvas will go through a cold challenge (See SOP 12: Cold challenge). After the cold challenge, 3 pools of 50 PL each (total 150 PL per tank) will be analyzed for WSSV and TSV by PCR. Only if negative results are obtained, the PLs could be stocked into the grow-ponds / outdoors nursery ponds (See SOP 14: Sampling for PCR analysis).

2.2 PLs from hatchery to indoors nursery will not require additional testing if the same biosecurity standard are met.

2.3 Prior stocking the juveniles into the grow-out ponds, each pond must be tested for WSSV and TSV by PCR. 150 juvenile will go through a cold challenge (See SOP 12: Cold challenge). After the cold challenge, 3 pools of 50 pleopods each (total 150 pleopods per tank) will be analyzed for WSSV and TSV by PCR. Only if negative results are obtained, the juveniles could be stocked into the grow-ponds. (See SOP14: Sampling for PCR analysis).

2.4 Any unusual mortality will be analyzed by PCR for WSSV and TSV. For this, 10 PLs will be pooled in one sample.

3. Outdoor Broodstock ponds

Only broodstock stocks raise isolated from the farm areas will be allowed to be stock in outdoor facilities. GDF-MEWA will approve the facilities before use them as a broodstock ponds.

3.1 All broodstock must be cold challenged and PCR tested for endemic pathogens before moving to maturation.

3.2 Each broodstock pond will be sampled at least once per month for WSSV and TSV by PCR (See SOP 14: Sampling for PCR analysis). 5 subsamples of 30 pleopods each (total 150 shrimp) will be pooled in tube containing 95% ethanol.

3.3 Five shrimp displaying any clinical sign will be fixed for histological analysis (See SOP 15 fixation of shrimp and crustacean samples in Davidson's fixative) in a monthly basis.

3.4 It is recommended to take plankton samples from the broodstock ponds for WSSV and TSV (See SOP 16: Plankton sampling).

3.5 Any mortality observed in broodstock ponds will be analyzed. Samples of recent dead shrimp must be analyzed for WSSV and TSV by PCR in pools of 5 (five recent dead shrimp pleopods will conform 1 sample).

4. Outdoor Nursery and grow out ponds

4.1 Each nursery ponds will be sampled at least once per month for WSSV and TSV by PCR). 5 subsamples of 30 pleopods each (total 150 shrimp) will be pooled in tube containing 95% ethanol.

4.2 If any mortality is observed in nursery ponds, samples of recent dead or moribund shrimp must be analyzed for WSSV and TSV by PCR or field rapid test (e.g. shrimple) / in

pools of 5 (five recent dead shrimp pleopods = 1 sample) or field test system (e.g. shrimple, pockit etc). Five samples of shrimp displaying clinical signs will be fixed for histopathology.

4.3 The sampling for grow-out ponds will be conducted based on the presence of clinical signs (See SOP 7: Sampling a shrimp pond for sanitary analysis). Each grow-out pond will be sampled at least once during the first month for WSSV by PCR. If history of TSV, samples for TSV will be taken as well. 3 subsamples of 50 pleopods each (total 150 shrimp) will be pooled in a tube containing 95% ethanol. The samples will be taken according the SOP 7 Criteria for shrimp sampling in ponds/tanks for sanitary analysis.

4.4 In case of any mortality or abnormal presence of birds observed in grow-out ponds, samples of moribund or recent dead shrimp must be analyzed for WSSV by PCR (5 recent dead shrimp pleopods = 1 pool sample) (See SOP 14: sampling for PCR analysis) or a field test system (e.g. shrimple, pockit etc).

4.5 In case of presence of moribund shrimp, samples of moribund shrimp (at least 5) must be fixed in Davidson fixative for histological analysis. See SOP 15 fixation of shrimp and crustacean samples in Davidson's fixative.

4.6 If there is a lower final survival in any grow-out pond (significantly lower than the final survival average), the cause of the mortality should be clearly explained and sanitary results must clearly state that analysis for WSSV and TSV was conducted.

5. Wild population

It is recommended to analyze crustacean samples taken from different areas of the farm including main canal, feeder canals and any mangrove area near the farm. Samples of gills (preferred), pleopods or haemolymph will be taken from the crustacean present in each area and pooled (Each 5 crustaceans samples will conform=1 pool) for WSSV analysis. At least 1 pools sample per area per month will be analyzed by PCR. Plankton samples must be analyzed by PCR for WSSV and TSV. Five samples of the main feeder canal per month must be preserved in 95% ethanol (See SOP 16 Plankton samples for PCR analysis).

SOP 19. Pre-requisites and procedures for approving experimental trials for new technologies and/or culture methods

1.This SOP is applicable to all trials and new culture methods (i.e. high density stocking, green water, biofloc etc), other than the ones already approved by GDF-MEWA.

2.For any such trial or new culture method to be approved and allowed to proceed, the interested party (Aquaculture Company) must submit a complete technical proposal to JFRC justifying the following:

a) The experimental trial / or new culture method with complete details about the trial / or new culture method proposed protocol
b) Description of the stocking cycle (process from PL to harvest) and expected densities at the various life production stages.
c) Available facilities, personnel and equipment in place ready to accommodate such stocking density (number and size of ponds, aerators, filtration, crab-fencing, water parameter equipment, cold-challenge facilities, PCR kits etc.).
d) Clear biosecurity protocol with contingency plan measures clearly defined.
e) Description of the surveillance and monitoring program to be applied.
f) The name of the assigned responsible person for the trial or the new culture method, along with a brief personal profile indicating the relevant skills and experience.
g) The organizational structure of the staff proposed to implement the trial or the new culture method

3.The proposal document (as described above) should be submitted at least two months earlier prior to expected start-up of the trial or new culture method

4.Based on the above, the process for approval of any such trial and/or new culture method is as following:

i. The technical proposal is submitted to JFRC.
ii. JFRC evaluates the proposal and the available infrastructure of the requesting farm, which is necessary to perform the trial or new culture method as per the technical proposal.
iii. The result of the evaluation (proposal and site evaluations) is forwarded to GDF-MEWA for approval or rejection.
iv. In the event of doubt and/or of inconclusive evidence that the requesting aquaculture project does not guarantee the biosecurity requirements, the matter can be referred to the next SABTG meeting for evaluation and consultation. Upon unanimous agreement of the members of SABTG the proposal can be forwarded to GDF-MEWA with the consultation for approval or rejection. GDF-MEWA evaluates the consultation of SABTG and proceeds accordingly to approval or rejection.
v. In the case the decision of the SABTG is not unanimous; the matter can be referred to the Higher Biosecurity Committee (HBC) for final decision, which in turn is forwarded to GDF-MEWA.

vi. During any stage of this process, any of the evaluating/ consulting bodies (JFRC, SABTG and HBC) has the right to request additional information concerning the proposed method, its protocols and the biosecurity and contingency plans concerned.
vii. At any point in the process SAS may be consulted.

ANEXURE 1 LIST OF THE REFERENCE LABS FOR SHRIMP DISEASES

Infectious hypodermal and haematopoietic necrosis	**Dr. Jie Huang** Maricultural Organism Disease Control and Molecular Pathology Laboratory, Yellow Sea Fisheries Research Institute (YSFRI), Chinese Academy of Fishery Sciences #106 Nanjing Road, Qingdao, Shandong Province 266071, CHINA (PEOPLE'S REPUBLIC OF)) Tel.: (86-532) 85.82.30.62 ext. 802, Fax: (86-532) 85.81.15.14; E-mail: huangjie@ysfri.ac.cn; aqudis@public.qd.sd.cn Web site: www.ysfri.ac.cn
Necrotisinghepatopan creatitis	**Dr. Grace Lo** National Cheng Kung University (NCKU) Center for Shrimp Disease Control and Genetic Improvement No. 500, Sec. 3 Anming Road Annan District Tainan City 709 CHINESE TAIPEI Tel: +886-6 384 24 48 Fax: +886-6 208.36.63 Email: gracelow@mail.ncku.edu.tw
Taura syndrome	**Dr. Arun Dhar** Aquaculture Pathology Laboratory School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ 85721 UNITED STATES OF AMERICA Tel: +1-520 621 87.27 Fax: +1-520 626.56.02 Email: adhar@email.arizona.edu

White spot disease	**Dr. Jie Huang** Maricultural Organism Disease Control and Molecular Pathology Laboratory, Yellow Sea Fisheries Research Institute (YSFRI), Chinese Academy of Fishery Sciences #106 Nanjing Road, Qingdao, Shandong Province 266071, CHINA (PEOPLE'S REPUBLIC OF)) Tel.: (86-532) 85.82.30.62 ext. 802, Fax: (86-532) 85.81.15.14; E-mail: huangjie@ysfri.ac.cn; aqudis@public.qd.sd.cn Web site: www.ysfri.ac.cn **Dr. G. Lo** Department of Life Science, Institute of Zoology, National Taiwan University, 1 Roosevelt Road, Section 4, Taipei, CHINESE TAIPEI Tel.: (886-2) 23.63.35.62, Fax: (886-2) 23.63.81.79 E-mail: gracelow@ntu.edu.tw
White tail disease	**Dr. A. SaitSahul Hameed** Aquaculture Biotechnology Division, Department of Zoology, C. Abdul Hakeem College, Melvisharam-632 509, Vellore Dt. Tamil Nadu, INDIA Tel.: (91-4172) 269.487, Fax: (91-4172) 269.487 E-mail: cah_sahul@hotmail.com
Yellow head disease	**Dr. Nick Moody** CSIRO Australian Animal Health Laboratory CSIRO Livestock Industries 5 Portarlington Road Private Bag 24 (Ryrie Street) Geelong 3220, Victoria AUSTRALIA Tel: +61-3 52 27 00 00 Fax: +61-3 52 27 55 55 Email: nick.moody@csiro.au Web: www.csiro.au

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