COASTAL AQUACULTURE AUTHORITY
Ministry of Agriculture & Family Welfare
Government of India
CHENNAI – 600 091.

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PUBLIC NOTICE

Invitation of Expression of Interest (EOI) for recognition of aquaculture disease diagnostic laboratories for shrimp seed testing

Recognition of Aquaculture Disease Diagnostic Laboratories is to be done for the purpose of providing tested disease free seed to shrimp farmers as PCR testing of shrimp seed is a mandatory requirement for shrimp hatcheries. In order to formulate a suitable mechanism to make the disease screening programme more reliable, it is decided that qualified PCR Disease Diagnostic Laboratories are to be given recognition jointly by Coastal Aquaculture Authority (CAA),the ICAR- Central Institute of Brackishwater Aquaculture (CIBA) and Rajiv Gandhi Centre for Aquaculture under MPEDA.

EOI is invited from existing PCR Disease Diagnostic Laboratories who fulfill the required conditions for enrolling as a recognised PCR laboratory for shrimp seed testing.

Shrimp farmers will be advised to test the seed only from such approved PCR laboratories in future. Registered shrimp hatcheries, whose in-house PCR laboratories, if not recognized under this process, would be required to get their seed tested from such recognized laboratories before sales.

The details of the process, application and terms and conditions are available in the official websites: www.caa.gov.in www.ciba.res.in www.rgca.org.in.

Application in the prescribed format shall be submitted in triplicate, within 30 days from the date of publication, to the Member Secretary, Coastal Aquaculture Authority, No.12-A, GDR Tower, Bharathi Street, Vanuvampet, Chennai – 600 091 (Tel. No.: 044-2260 3683/Fax No.: 2260 3780).

Member Secretary

Expression of Interest for recognition of Disease Diagnostic Laboratories through capacity building and harmonization in PCR diagnosis of shrimp pathogens and ring testing

(Terms and conditions)

Coastal aquaculture, especially shrimp farming, is an important economic activity carried out in the entire coastal belt of the country. Coastal Aquaculture Authority (CAA) is empowered to regulate all the activities connected with coastal aquaculture in coastal areas and one of the functions is to fix standards for all coastal aquaculture inputs viz. seed, feed, growth supplements as well as chemicals/medicines, etc. Production of healthy and disease free shrimp seed is the foremost requirement for sustainable shrimp farming and the Guidelines for *L. vannamei* farming stipulate that tested and certified seed should be procured from the hatcheries and shrimp farmers would be required to test the seed from approved PCR laboratories only.

Findings of the disease surveillance programme carried out by the Central Institute of Brackishwater Aquaculture (CIBA) indicate that the White Spot Syndrome Virus (WSSV) still cause major mortalities and production losses, the Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) is also widely prevalent. Recently, the microsporidian Enterocytozoan hepatopenaei (EHP) is reported to be occurring in a same pattern throughout the coast. Early pathogen detection is a crucial measure in preventing disease spread in shrimp aquaculture system and the DNA based polymerase chain reaction (PCR) technique is widely used as a major diagnosis tool for shrimp pathogens. However, there are various issues related to the application and the reliability of PCR diagnostic results due to the inconsistencies in the testing methods and the results mainly on account of lack of different levels of technical competency and technicians themselves are unable to prove beyond doubt the veracity of the diagnostic tests and the procedures adopted.

CAA, with the active technical support and cooperation of CIBA and RGCA, has taken the initiative to address the issue to train the technical personnel involved in PCR diagnostic laboratories involved in the field of aquatic animal health management as well as in the approved hatcheries through an intensive capacity building drive at three levels as per the following Terms of Reference:

Terms and conditions for the proposed capacity building, PCR harmonisation/ring testing

This section comprises the terms and conditions with regard to the following points on the proposed PCR harmonisation programme

1. PCR infrastructure - design and equipments; inspection, method of inspection and reporting
2. Capacity building: experience of technicians; brief note on the technical and practical aspects to be covered; evaluation of the technicians
3. Process of ring test: protocol for ring testing, method of sample distribution, submission of results, evaluation, etc.

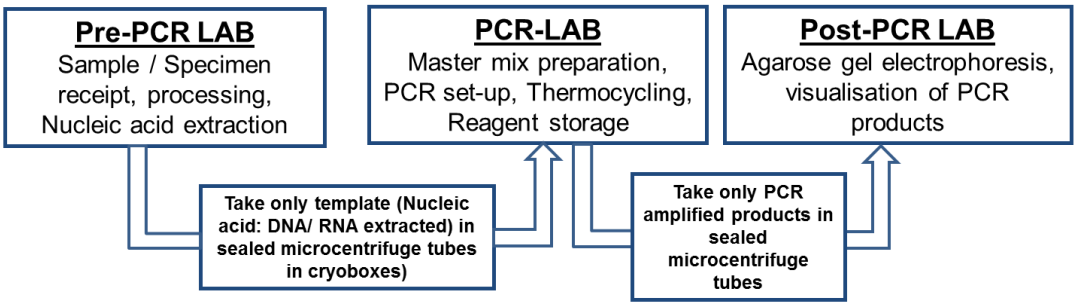
(1) PCR laboratory infrastructure and Inspection

The PCR laboratory and related facilities of the participating agency should be freely accessible to the inspection team to conduct inspection without hindrance. Inspection of laboratory will be carried out to ascertain the (i) availability of infrastructure in the participating laboratory (as given below in the succeeding sections), (ii) condition of equipment, maintenance and calibration, (iii) competence of technical staff, (iv) methodology of PCR Testing generally used in the laboratory, (v) protocols observed for disposal of laboratory wastes and (vi) laboratory record keeping. The laboratory should comply with the recommendations of inspection team. A model plan of the laboratory is provided below. The laboratory should carry out necessary corrective measures within the agreed time frame and send the compliance report to the inspection team as prescribed. The laboratory should depute one qualified technical staff to attend the training programme and later participate in the ring test.

Laboratory Requirements: Layout and equipment

The requirements for the polymerase chain reaction (PCR) diagnostic laboratory should include facilities such as specimen receiving, sample processing, nucleic acid extraction room, PCR procedure room, post-PCR room for electrophoresis, visualization and data recording. One of the main problems in PCR work is contamination that can lead to false positive results. Hence, the entire PCR work should be divided and performed in three different locations with physical barriers for each of the activities such as Pre-PCR, PCR and Post-PCR. All the three areas must be preferably in isolated rooms (size can be small, as required). A simple PCR laboratory layout can be as indicated in the figure given below and the flow of work should be in the order: Pre-PCR$\rightarrow$ PCR $\rightarrow$ Post-PCR. All the three areas can be organised in a total area of 15′ x 20′ room with partitions to provide required cabins.

Ideal PCR Laboratory plan



Pre-PCR area: Pre-PCR area will receive samples and the samples will be processed for diagnosis. This step involves dissection of animals, microscopy and extraction of DNA/RNA. The materials include scissors, forceps, microfuge racks, micropipettes of different capacities, etc. and consumables such as chemicals for buffer preparation and sample collection, gloves, tissue paper, tips for the micropipettes (10µl, 100 µl and 1000µl capacity), microcentrifuge tubes (1.5 ml and 2.0 ml capacity), tissue homogenisers, ice bucket/sample containers, etc.

Major equipment: Centrifuge (Refrigerated centrifuge preferable), refrigerator and an autoclave. Motorised tissue homogenisers will ease the work with large number of samples. A -20°C freezer is advisable for storage of samples/nucleic acid for reference.

PCR Area: The PCR part will involve setting up of the PCR reaction and amplifying the nucleic acids in a thermocycler. This will involve use of molecular biological reagents. Extreme care should be taken not to contaminate any of these components. Dedicated micropipettes (2.5 µl, 10 µl, 20 µl and 100 µl capacity) for PCR, PCR stand, Cryobox to hold temperature sensitive chemicals, tray for ice, PCR tubes/strips/plates, Filter tips, Tissue paper, Gloves, tube holding racks, etc. should be available in this area.

Major equipment: PCR work station or PCR hood or a simple inoculation hood used for microbiology with UV and fluorescent lights to set up the PCR test in a contaminant free environment.

Thermocycler: Quality brands could be used, e.g., Applied Biosystems, Eppendorf, Bio Rad, Perkin Elmer, etc. are available. A -20°C freezer is required for keeping all the PCR reagents.

Post-PCR area: Post PCR will involve electrophoresis of amplified PCR products and visualisation/detection of the PCR products. Minor equipment such as micropipettes (10µl, capacity), Electrophoresis apparatus (power pack, casting tray, comb) and a microwave oven for preparation of agarose gel for electrophoresis and consumables including the chemicals for buffer preparation, agarose, tips, gloves, tissue paper etc. will be required.

Major equipment: UV trans-illuminator or gel documentation system with a computer for imaging gels. A small refrigerator to keep PCR products for reference.

Method of inspection and reporting

Standard procedures will be followed for inspection and reporting as given below. The minimum facilities required for the PCR laboratory is indicated below:

Sl. No.	Facility/Equipment	Availability (Yes/No)
I.	**Pre-PCR Area (room /lab)**	
1.	-20°C Freezer (small, about 100 L capacity)	
2.	Centrifuge with rotor for 1.5 ml eppendorf tubes	
3.	Ice flaking machine (?) or cool box /ice box	
4.	Refrigerator	
5.	Autoclave /Pressure cooker (small)	
6.	Hot air oven (small)	
7.	Micropipettes (10µl, 100 µl and 1000µl capacity)	
II.	**PCR Area (room/lab)**	
1.	-20°C Freezer (small, about 100 L capacity)	
2.	PCR work station /PCR hood (Two numbers - one for preparation of PCR master mix and other for addition of nucleic acid template) Or A fabricated inoculation hood with UV and fluorescent lights.	
3.	Thermocycler	
4.	Micropipettes (2.5 µl, 10 µl and 100 µl capacity) for PCR	
5.	Cryoboxto hold temperature sensitive chemicals	
III.	**Post-PCR Area (room/lab)**	
1.	Microwave oven	
2.	Balance	
3.	Electrophoresis apparatus (Power pack, gel tank, casting tray, comb)	
4.	UV trans-illuminator or Gel documentation system with a computer	
	Consumables including chemicals for buffer preparation, agarose, tips, gloves, tissue paper etc.,	
IV.	Laboratory waste disposal area especially for Electrophoresis material and PCR products	

(2) Capacity building

Experience requirements of technicians: A minimum Bachelor's degree in Science with about two years working experience in an active PCR laboratory, preferably with exposure to diagnostics of aquaculture pathogens or Master degree with six months experience.

Brief note on technical and practical aspects to be covered:

The PCR training would cover theoretical classes on (i) Introduction to nucleic acids-DNA /RNA, (ii) Principles and practice of polymerase chain reaction (PCR) including reverse transcriptase-PCR, (iii) a detailed discussion on the various diagnostic tests used for white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), microsporidian parasite Enterocytozoan hepatopenaei (EHP) and acute hepatopancreatic necrosis disease (AHPND).

The second important part would comprise hands on training on (i) Extraction and quantification of nucleic acids (DNA and RNA) from shrimp samples, (ii) setting up PCR reaction for white spot syndrome virus (WSSV) using OIE protocols and (iii) setting up RT-PCR test for an RNA virus [Laem Singh virus (LSNV) or infectious myonecrosis virus (IMNV) (using positive control only, since IMNV is not reported and no positive tissue is available], (iv) agarose gel electrophoresis, visualisation, gel documentation, (v) documentation, reporting and record keeping and (vi) protocols for PCR laboratory waste disposal.

Examination of PCR technicians: After the training programme, a skill evaluation test will be conducted to the participants to evaluate their understanding of the concepts and proficiency.

(3) Process of ring test:

I. Method of sample distribution :

After the successful completion of training at CIBA Aquatic Animal Health Laboratory, blind tissue samples will be distributed to the participating laboratories by RGCA. The ring test will be conducted in three rounds as 1st, 2nd and 3rd. A set of four coded WSSV and IHHNV infected and SPF uninfected tissue samples adequate to extract nucleic acids and carry out the PCR test will be sent in microcentrifuge tubes by courier.

II. Protocol for ring test:

The laboratory should conduct the tests in their own laboratory itself and should not outsource/sub-contract testing. The PCR laboratories are free to use any diagnostic techniques of their choice. They should analyse the samples within their own laboratory by their own technical personnel and achieve required sensitivity and specificity and report the results of analysis to CIBA and RGCA. The participating laboratories are required to carry out PCR testing at least once in the presence of a PCR technical expert deputed from CIBA/RGCA.

III. Submission of results and evaluation ring test.

Participating laboratories are bound to report their results to CIBA/RGCA within a period of five working days from the receipt of the samples. The results given by the laboratories will be kept confidential. The results submitted by the laboratories will be examined vis-à-vis their codes jointly by CIBA and RGCA and the performance of each of the laboratories would be communicated individually. Corrective measures, if any, would be suggested to the laboratories for improving the performance and enhancing the skills of the PCR laboratory personnel.

An initial sensitisation workshop before starting this exercise and a concluding evaluation meeting will be held at CIBA, Chennai.

The laboratory should pass all the three levels successfully to be designated as "approved"/"recognized" laboratory. After the successful completion of the training and ring-test, an approval/recognition certificate will be issued to the participating laboratories by ICAR-Central Institute of Brackishwater Aquaculture (CIBA), Marine Products Development Authority (MPEDA)/Rajiv Gandhi Centre for Aquaculture (RGCA) and Coastal Aquaculture Authority (CAA). The validity of the approval/recognition is for a period of one year, and to renew the "approved"/ "recognized" status, each laboratory must undergo the ring test every year.

Capacity building and harmonization in PCR diagnosis of aquatic animal pathogens and ring test

APPLICATION FOR EXPRESSION OF INTEREST

1.	Name of the Applicant	:	
	Profession (Technician/Farmer/ Entrepreneur, etc.)		
2.	Address	:	
3.	Age	:	
4.	Educational Qualifications including technical trainings received, if any	:	
5.	Experience	:	
6.	Name of the PCR laboratory, when established, whether recognized by any national agency/NABL accredited	:	
7.	Details of PCR equipments installed in the laboratory	:	
8.	Number of technicians involved in PCR testing	:	
	Technicians' profile	:	

Name of the technician	Level of knowledge and practical experience	Specialised training acquired

……. Contd. 2 …….

9.	Whether qualified/appeared for ring test earlier (certificate to this effect to be furnished)	:	
10.	Technical/field-level problems experienced in relation to PCR diagnosis	:	

Date: (Signature)

Place: