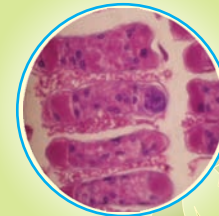


COLLECTION, PRESERVATION AND DISPATCH OF SHRIMP SAMPLES FOR DISEASE DIAGNOSIS



Where to send samples:

**National Surveillance Programme for Aquatic Animal and Diseases
Aquatic Animal Health and Environment Division**

Central Institute of Brackishwater Aquaculture

75, Santhome High Road, Raja Annamalaipuram, Chennai - 600 028

Telephone: 044-24618817; Fax: 044-24610311

E-mail: nspaad.ciba@gmail.com

URL: www.ciba.res.in



Aquatic Animal Health and Environment Division

CENTRAL INSTITUTE OF BRACKISHWATER AQUACULTURE

(Indian Council of Agricultural Research)

75, Santhome High Road, Raja Annamalaipuram,
Chennai - 600 028



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PROTOCOLS FOR SAMPLE COLLECTION AND SUBMISSION

Sample collection

Accurate sampling and fixation is one of the most crucial step for precise shrimp disease diagnosis.

- Before sampling, note any gross changes (behavioural abnormalities, change in appearance, colour, lesions etc.)
- Moribund (approaching death) shrimp is the most appropriate sample, since autolysis takes place immediately.

Sending live shrimp samples for diseases diagnosis

A representative live shrimps are most ideal for sampling and will help out disease diagnosis with the use of various techniques. Collect 3-6 diseased or moribund shrimp and an equal number of normal shrimps and pack separately in double polypack with oxygen. Pack these bags in a styrofoam or thermocol box to keep temperature cool. Place a couple of ice pack or gel pack while sending samples long distance. For smaller shrimp or post-larvae place adequate number ranging from 30 to 150 depending on size in the same manner. LABEL and dispatch through courier with completed history sheet (use separate photocopy for each batch of sample) within 24 hours.

Sending fixed shrimp samples for disease diagnosis

In case live sample delivery is not possible, the samples collected as above may be fixed appropriately as detailed below for PCR analysis and /or for histopathology.

Shrimp samples for Virological/PCR analysis (including screening of OIE listed diseases)

Smaller shrimp samples (up to PL) or dissected gills, pleopods etc. from larger shrimps may be collected in 95% Ethyl alcohol or frozen at -20°C or commercial transport medium (RNA Later) in tightly capped container and submit to the disease diagnostic lab at the earliest. Alternatively, these fixed samples may be kept in refrigerator (4°C) till the day of transportation to avoid evaporation of ethyl alcohol. Frozen shrimp packed in zip-lock bags should be shipped immediately on dry ice or ordinary crushed ice flakes.

Shrimp samples for Bacteriological, Mycological and Parasitological analysis

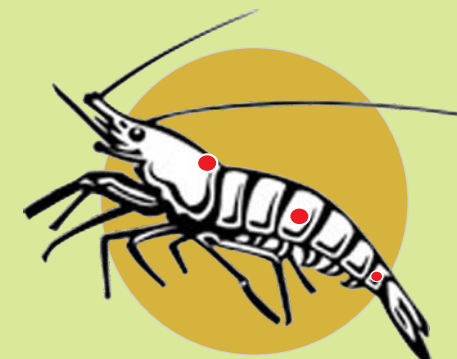
Preferably live moribund (weak, disease affected) specimens OR samples should be submitted on ice within the shortest possible time (24 hours of sampling). Water sample (100 ml) for bacteriological analysis should be collected in sterile container and transported in ice.

Shrimp samples for histopathological analysis

Fixation of sample: Shrimps are usually fixed in Davidson's OR Neutral Buffered Formalin (NBF) fixative (composition shown below) in a widemouth plastic bottle.

- Smaller shrimp (<1g) may be fixed directly by immersing in appropriate fixative for 24 hours and for larger shrimps it should be in the fixative for 48 hours (sample weight/volume should not exceed 1/10th of the volume of the fixative).

- For larger shrimp (>1g), inject fixative @ 10% of shrimp body weight (e.g. 1 ml for a 10 g shrimp) into live animals, first in hepatopancreas, then the region anterior to the hepatopancreas, and lastly two injections into the abdomen, one anterior and the other posterior.
- To ensure proper penetration of fixative, slit the cuticle open on both sides of the shrimp, from the 6th abdominal segment to the rostrum using scissors before placing in fixative. Seal the container, LABEL and dispatch with history sheet as soon as possible.



● Denotes area of fixative injection

Sending water samples for analysis

Pooled water samples collected from the middle of the water column at the centre and four corners of the culture pond (500 ml) are required.

Precautions for transportation of samples

- Do not send samples in glass containers
- Wrap sample containers in a non-fibrous material.
- Samples over ice may be packed in zip-lock bags.
- Labels should be written with pencil.
- Complete proforma with contact details.

Preparation of fixatives

1. Davidson's Fixative	1000 ml
1. Formalin (37% formaldehyde)	220 ml
2. 95% ethanol	330 ml
3. Distilled water (if not available, tap water)	335 ml
4. Glacial acetic acid	115 ml
2. Neutral Buffered Formalin (NBF)	1000 ml
1. Formalin (37% formaldehyde)	100 ml
2. Distilled water (if not available, tap water)	900 ml
3. Sodium phosphate, monobasic monohydrate	4.0 gm
4. Sodium phosphate, dibasic anhydrous	6.5 gm
3. 10% Formalin	1000 ml
1. Formalin	100 ml
2. Distilled water	900 ml
4. 95% Ethyl alcohol	1000 ml
1. Absolute alcohol	950 ml
2. Distilled water	50 ml

In case fixatives are needed please contact:

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