

METHOD OF SAMPLING FISH FOR DISEASE DIAGNOSIS

An abnormality in a fish could be recognised by observation of various clinical signs such as behavioural changes, morphological abnormalities, presence of external parasites, internal examination followed by histopathological, bacteriological, virological and mycological analysis of fish tissues. Diagnosis of the infection could be done from various observations and tests results in combination. While in some cases the infection can be easily diagnosed e.g. by the presence of parasites, in other cases, battery of tests may be required to find the aetiology of infection. The present experiment is level 1 diagnosis with observations followed by sampling of various tissues.

Fish, dissection kit, microscope, glass slides, sterile TSA plates, sterile containers, anaesthetic (MS222 or benzocaine), syringe with 24 g x 1” needle, fixative (NBF)

Neutral buffered formalin (NBF)

40% Formaldehyde	100ml
NaH ₂ PO ₄ H ₂ O	4 g
Na ₂ HPO ₄	6 g
Tap water	900 ml

Observation of behavioural changes

Wherever possible fish sampled for disease diagnosis should be examined for any behavioural abnormalities. This may include listlessness, swimming near surface sometimes putting the head out of water, gulping of air, loss of balance, corkscrew swimming, belly up rolling motion, flashing and scraping, lethargic movement, increased respiration, increased feed uptake followed by anorexia and reduced reflexes. Make a note of the above abnormalities, if any.

Gross examination

- Examination of fish can be done on sacrificed specimens.
- Kill the fish just prior to examination by decapitating - severance of spinal cord behind the head or anaesthetic over dose.
- Tricaine methanesulphonate (MS – 222) 1: 1000 or Benzocaine 0.1g/4l. (dissolve benzocaine in small quantity of acetone and then dissolve in water) will anaesthetise the fish within 5-10 minutes.

- Examine the external surface of the fish for any lesions or abnormalities. This may include damaged skin, ulcers, reddening and haemorrhages, changed colouration, abdominal swelling, abnormal eyes with exophthalmia, cloudiness and gas bubbles, skeletal deformities, eroded fins, abnormal growths and increased mucus production.
- The skin surfaces should also be examined for the presence of parasites.
- Gills are to be examined for its colour, lesions, presence of parasites and excessive mucus. Healthy gills should be red with a symmetrically outer periphery.

Collection of blood:

- Blood should be collected as soon as possible once the fish is received.
- Place the fish on its side and insert the needle behind the anal fin at an angle forward until it touches the spinal cord.
- Constantly apply a negative pressure and reposition or rotate the needle to withdraw blood.
- Once blood start flowing keep the negative pressure constant and do not shake or change the position of the needle.
- Blood will continuously flow into the syringe.
- Prepare blood smear for further analysis. Make a thin film of blood (dealt separately in another exercise).