

## Pathology

Submit fixed (preserved) tissues for the preparation of slides for histological (microscopic) examination.

Take tissues from freshly killed or moribund fish.

Samples taken from dead, decomposing or frozen animals are of little histological value.

The standard tissues required are skin, gill, liver, head and tail kidney, spleen, pyloric caecae, brain, eye, gastrointestinal tract and heart. Any lesions or other organs showing abnormalities should also be sampled. For very small fish (less than 5-6 cm in length) slit the belly and remove the operculum and place whole into fixative. When taking sections from organs for submission, target areas showing any abnormal signs, such as ulcers, lesions, irregular shape, colour or texture etc. Each tissue should be no more than 1 cm thick, and should be placed in a suitable (leak proof) specimen container, in a ratio of 10 to 1, fixative to tissues. The preferred routine fixative is 10% Neutral Buffered Formalin.

Exercise care when taking samples for histology. The use of a sharp scalpel and avoiding crushing of tissues will ensure good quality samples. Sample gills first and, if contaminated with blood or food, gently rinse in clean water before placement in fixative. Avoid cramming large gill arches into small containers, this leads to poor preservation, and may render the sample useless.

Seal the lids of any specimen containers with parafilm or tape to minimise leakage. Label the outside of the containers with the farm name, fish number, pen/cage/tank number, date sampled and species of animal.

Notes/observations from individual fish can be included on a separate piece of paper, submitted with your Aquatic Animal Submission Form. Contact us for a book of forms or download a copy from our website - <http://www.nre.tas.gov.au/AHLabsforms>

Following these simple submission guidelines will enable us to give you the most thorough report on the health status of your animals.

## Molecular Tests

Pathogens such as Tasmanian Aquareovirus, Tasmanian *Rickettsia*-like organism and Pilchard Orthomyxovirus can be detected by PCR.

The standard tissues required are liver, kidney, and spleen. Target areas showing any abnormal signs should be selected. Each tissue should be no more than 5mm thick and placed in a suitable (leak proof) specimen container. **Note:** The ratio of fixative to tissues should be at least 10 to 1. The preferred routine fixative is RNAlater solution. Containers with RNAlater are provided with this kit.

Specimen containers should be labelled as detailed for the Pathology section.

## Contact Information

*Postal address:*

PO Box 46, Kings Meadows, Tasmania 7249

*Street address:* (for couriers)

Specimen Reception

165 Westbury Rd, Prospect, Tasmania 7250

*Specimen Reception:*

Phone: (03) 6777 2111

Email: [specimenreception@nre.tas.gov.au](mailto:specimenreception@nre.tas.gov.au)

# Fish Disease Investigation Kit

## Instructions



Tasmanian  
Government

Natural Resources and Environment  
Tasmania  
Biosecurity Tasmania  
Animal Health Laboratory  
Prospect, Launceston

## Presentation

This kit contains material for bacteriological culture and molecular biology testing. On receipt, the box should be stored in a cool location such as a refrigerator. The culture plates have a **shelf life of 1 month** if stored correctly. Discard any expired plates and use a fresh kit containing in-date media. Spare loops, slides etc can be used for further sampling, or returned to the laboratory for recycling.

Once samples have been collected ensure that all plates are placed in the resealable plastic bags. Repack the box and address the box using the addressed adhesive label provided. The box may be sent either by post or a courier parcel delivery service. Return it as soon as possible to the Animal Health Laboratory.

There will be certain circumstances when the standard kit will not be entirely appropriate for your needs. In these cases please contact the Animal Health Laboratory so that a special pack can be provided.

## Selection of Fish

Choose fish with specific lesions (e.g. ulcers, pop-eye, fin rot etc.) for examination. In the absence of such characteristics, select moribund or languid fish. Dead fish should not be sampled as they may be too badly decayed for useful analysis.



Aquatic Animal Submission form



www.nre.tas.gov.au/AHLabs

## Bacteriology

The kit may contain a number of different culture media appropriate for the disease under investigation. Please note the expiry date on the plates provided and **do not use** if that date has been exceeded.

Freshwater Kit	Appearance	Purpose	Sampling Site
Blood Agar	Red	Non-fastidious pathogens	Kidney
Freshwater Ordal	Colourless	<i>Flavobacterium</i> spp.	Skin/gill lesions
Marine Kit	Appearance	Purpose	Sampling Site
Blood Agar/TCBS	Red/Green	Non-fastidious pathogens/Vibriosis	Kidney
Salt Blood Agar/SS	Red/Colourless	Marine Vibrios / <i>Tenacibaculum</i>	Skin/gill lesions

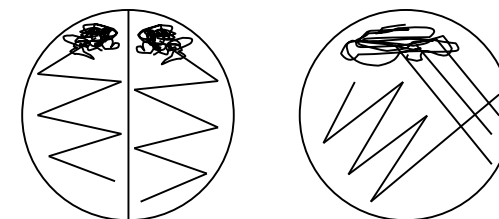
## Specimen Selection

**Kidney** Cut along the ventral line of the fish. Move the internal organs to expose the kidney lying under the swim bladder. Do not pierce through the swim bladder, gently move it to the side. Dip a sterile loop into the kidney and streak out onto an agar plate (half plate or whole plate) as illustrated. Repeat the procedure for the remaining agars using a fresh loop each time.

**Brain** At a point just behind the eyes, make a transverse cut through the head with a knife cleaned with an isopropanol wipe (e.g., Kimberly Clark "Isowipes"). With a sterile loop, collect as much of the fore brain as possible and transfer to a blood agar plate.

**Skin lesions** Using a sterile scalpel blade, scrape the edge of the lesion to collect a sample of skin and mucus. Inoculate a plate of Freshwater Ordal (FO) or SS medium with the sample.

**Smears** For every sample inoculated onto an agar plate, make a smear for microscopy. Place a small amount of the sample on a slide and spread it out very thinly using the handle end of a disposable loop. Let the slide air dry and return to the plastic slide box.



Streak pattern for half and whole plates

**Labelling** Using a waterproof pen or the labels provided in the kit. Label the culture plates with (i) fish number (ii) pen number (iii) sample type and (iv) date sampled. Label slides with a pencil and include fish number, pen number and sample type.

**Returning the culture plates** Forward the plates as soon as possible to the laboratory. Prior to dispatch, keep the plates cool and out of direct sunlight or heat.

**Accession Form** Place paperwork in document envelope and attach to the outside of the box.