



For sampling histopathology the following equipment is recommended:

- Submission form for histopathology
- Formalin pots* (10% formalin)
- Styrofoam/cardboard box and specimen transport bag/zip lock bag*
- Forceps and scalpel/scissors
- Clean surface and paper towels



* Formalin pots are sent to customers on request. The package with formalin pots from PHARMAQ Analytiq also contains submission form and barcode form for histopathology and transport bags to prevent leakage of formalin during transport.

It is very important to make sure that the formalin pots are properly closed before putting them in the transport bag/zip lock bag for shipping, as formalin is a harmful substance!

Sampling and shipping guide for histopathology samples



General recommendations

- **Anamnesis**
Fill in all relevant information about the medical history in the submission form. You can also upload pictures of necropsy findings in our customer portal when you register your electronic order. If you register the case in our customer portal (iWISE), please include the order number with the samples.
- **Parallel samples:**
 - It is always recommended to sample parallel samples for Realtime RT-PCR analysis. If this is not done it can be difficult to confirm or refute a suspicion of notifiable or serious disease.
 - If there is a suspicion of ulcers or bacterial disease it is recommended to take parallel samples for microbiological analysis.
- **Number of fish**
In general, we recommend sampling **5 fish** for disease investigation. In cases with more complex problems, it may be necessary to sample more fish. Contact us for advice on sampling.
- **Selection of individuals to sample**



In order to obtain good quality samples, it is best to sample moribund instead of dead fish. After death, the decomposition of cells and tissue starts, and it is difficult to

distinguish changes caused by decomposition from changes caused by disease. If there are no moribund fish to sample, it is important to sample as recently dead fish as possible. **N.B.** It is important to sample fish that is representative of the problem you want to investigate. Runts are normally not representative if there is an acute increase in mortality in an apparently healthy population.

- **Euthanasia**

It is important that the fish is euthanized by an overdose of sedative. Euthanizing the fish with a blow to the head can cause hemorrhage that makes the tissue unsuitable for histopathological evaluation.

- **Labelling of formalin pots**

To ensure traceability of the samples we recommend that all formalin pots are labeled with the name of the site/farm and date. If you register the samples electronically in our customer portal you will get a report number that you can label the pots with. Use one pot per fish and label the pot with fish number, for example F1 (fish1), F2, F3.

- **Single tissue samples**

If a single tissue (e.g. hearts or gills) is of interest and individual traceability is unnecessary, samples may be pooled with multiple gills/hearts from different individuals in the same formalin pot.

- **Size of tissue samples**

To secure optimal fixation of tissue by formalin, there should be a maximum of 1 part tissue per 10 parts formalin. The formalin penetrates and fixates at a rate of approximately 1 mm/hour, and it is therefore important that the tissue sample is not too thick. The optimal size of the sample is around 2-5 mm in thickness.

- **Cooling and storage of samples**

Samples can be stored in room temperature after sampling.

N.B. Avoid high temperatures and freezing of the sample, as this will destroy the tissue!

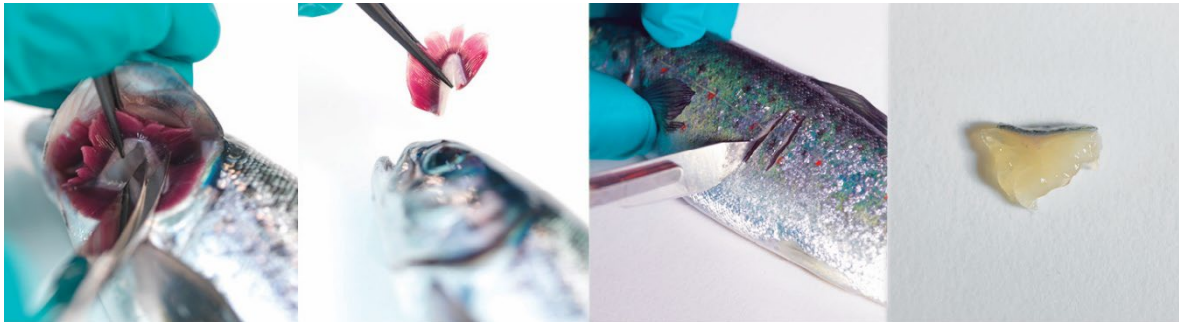




Sampling

1. Sampling of juveniles/larvae

Small juveniles can be fixated whole and several small juveniles/larvae can be put in the same formalin pot. If the fish is more than 4 mm thick an incision should be made in the abdomen of the fish, so that the formalin can enter the abdominal cavity for fixation of internal organs.



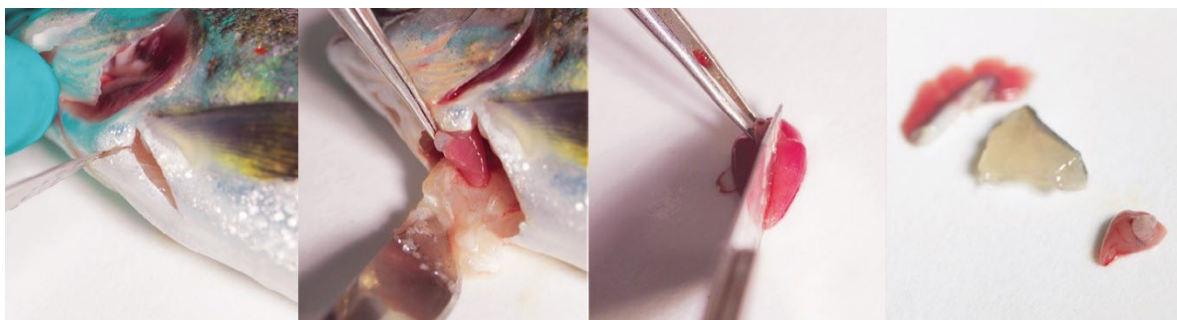
2. Organs

■ Gills

Sample the gill arch with most evident gross changes. Make sure to grasp the gill by the gill arch to avoid damage to the filaments. Always sample the gills first and put straight into formalin.

■ Heart

Make a section from the apex of the heart through the atrium so that the formalin can enter the lumen of the heart.



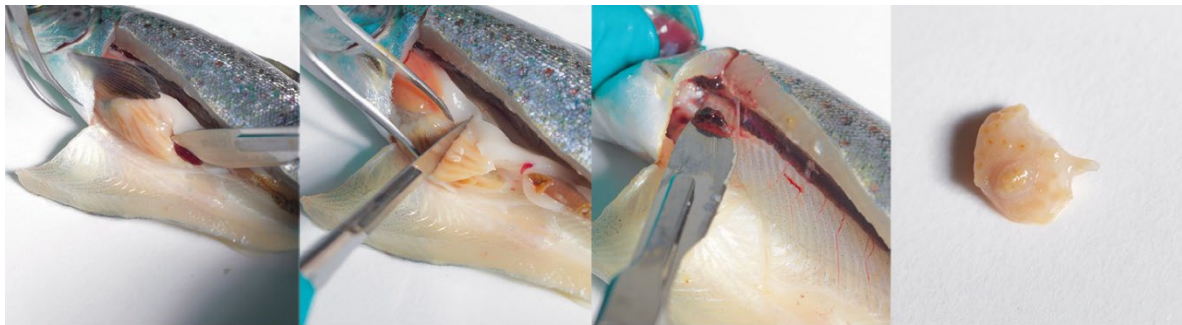


- **Liver, kidney, spleen**

We recommend sampling a disc-shaped piece of tissue with a maximum thickness of 4 mm if possible. The middle part of the kidney should be sampled to ensure the inclusion of both hematopoietic and tubular tissues in the sample. In small fish the kidney can be sampled and fixated on a tissue sample with muscle, and the spleen with the intestines.

- **Pancreas/intestines**

The pancreatic tissue is diffusely distributed in the visceral fat, between the pyloric caeca. Sample a cross section of the pyloric caeca to include pancreatic tissue and allow the formalin to enter the lumen of the intestines/pyloric caeca.

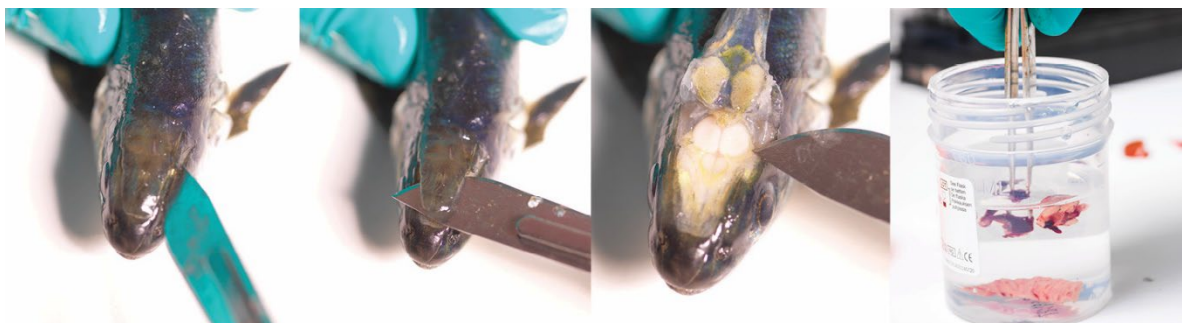


- **Muscle/skin**

Sample across the midline of the fish, beneath the dorsal fin. This will give a sample containing skin, red muscle and white muscle.

- **Brain and eye**

When there are symptoms indicating a central nervous system related cause of disease, such as spiral swimming or loss of equilibrium, the brain should be sampled. If there is suspicion of nodavirus in marine fish it is important to sample both brain and eye. If the





eye or lens of the eye is opaque, histology can be used to evaluate damage of the eye.

- **Other organs, ulcers, nodules, etc.**

In fish with ulcers it is important to sample the margin of the ulcer, so that tissue from the ulcer and normal, surrounding tissue are included in the sample. This also applies to tumors and nodules. We recommend taking pictures of ulcers/tumors/nodules before sampling and send to our histopathology team by e-mail, as this will make it easier to orientate the direction of sectioning when processing the tissue for analysis and optimizes the result.

When shipping

- Check that all pots/containers are properly closed so that the people handling the package will not be exposed to formalin leakages.
- Histopathology samples do not need to be chilled during transport and can be sent without cool packs.
- All samples should have three layers of packaging: formalin pots, closed secondary container (e.g. zip lock bag with absorbing material) and external container (insulated envelope, Styrofoam, cardboard box, etc.).
- Avoid spilling on the submission form if possible. The form can be put in a separate zip lock bag.
- The package is labelled «BIOLOGICAL SUBSTANCE, CATEGORY B», and check with your local courier regarding shipment of biological material.

- **Samples with urgent delivery time of results (24 h or 2 workdays)** should be sent with express delivery to:

PHARMAQ Analytiq AS
Hoffsveien 21-23
0275 Oslo
Norway

- **Samples with standard delivery of results** should be sent with express delivery to:

PHARMAQ Analytiq AS
Thormøhlens gate 53D
5006 Bergen
Norway

- **Samples from the Rørвик area can be delivered at the Rørвик laboratory:**
PHARMAQ Analytiq AS
Fjordgata 8
7900 Rørвик