

SAMPLING GUIDE: HISTOLOGY

Correct sampling procedures and treatment of the tissue samples are important to ensure optimal quality of the analyses. This user manual contains PatoGen's recommendations on how to ensure optimal quality of samples for histology.

In addition to histology, we recommend taking out tissue samples from heart, kidney and gill on RNAlater so that the diagnosis can be confirmed through two separate methods.

SUBMISSION OF ORGAN PACKAGES

- We recommend to sample organs from 5 fish in 10% phosphate buffered formalin per sampling or 5 fish per pen for increased sensitivity. The organ samples should be taken from moribund or fresh dead fish
- Organs from each individual fish are placed in their own formalin container
- Mark the container with an individual number/letter and write on the requisition form which organs that are included from each individual fish. In cases where PCR and histology samples are taken from the same fish, individual number/letter must match on the requisition form
- From each individual fish we recommend that tissue samples from the following organs are sent in: liver, pyloric caeca including exocrine pancreas, gills, heart, kidney, spleen and skin inclusive muscle. From marine fish, eye and brain should be included. Pseudobranch is included in the cases where applicable

SUBMISSION OF SINGLE ORGANS

- Single organs, meaning samples from the same type of organ tissue from different individuals, can be submitted together in the same formalin container

SAMPLING

Sampling of organs

Pyloric caeca including exocrine pancreas	Tissue samples (max 0.4 cm x 1 cm x 1 cm)
Gills	Second gill arch. In large fish: 2-3 cm is sampled around the apex of the gill arch. Gill tissue is very prone to post mortal changes (decay), and the tissue samples should be placed in 10% phosphate buffered formalin immediately after euthanizing the fish. To ensure fixation of the entire tissue sample, the formalin container should carefully be turned upside down 2-4 times immediately after the sample is placed in the container.
Brain	The fish should be euthanised with an anaesthetic overdose when sampling the brain. Furthermore, it is important to handle the brain tissue carefully to avoid damaging the tissue. In smaller fish: split the head vertically a little to the side of the centre. Carefully collect the largest half with the brain still in the skull and place it into the formalin container. In large fish: carefully dissect the whole brain and place it into the formalin container.
Heart	Collect the whole organ including the atrium, ventricle, and bulbus arteriosus. In medium sized fish: split the heart in the sagittal plane by place the heart with the tip toward you and the bulbus away from you. In large fish (>4kg) it will be necessary to collect separate tissue samples from the atrium (1 x 1 cm), a 0.4 cm thick slice of the ventricle wall from the lumen to the epicardium and a sample from the bulbus.
Skin/muscle	Collect a vertical slice (0.4 x 1 x 1 cm) of tissue which is symmetrical on the lateral line and below the dorsal fin. The tissue sample should include scales, skin and red and white skeletal musculature. Sample a piece that is three shell-lengths thick. Then the shells are more likely to be included in the sections, and there is no need to suspect scale loss.
Liver	Disc-shaped tissue sample (max 0.4 cm x 1 cm x 1 cm).
Spleen	Small fish: collect the whole spleen. Large fish: Collect a slice of tissue (max 0.4 cm x 1 cm x 1 cm)
Kidney	Collect the entire organ or a sample of a length of 1 cm from the mid kidney. In large fish: collect a sample symmetrical on the mid kidney so that the capsule is included.
Eye	Collect the entire organ
Fry	<5 mm thick: Fixate intact. >5 mm thick: Remove the gills from one side and make an incision into the abdominal cavity at the midline so that the formalin gains access to the abdomen.

Euthanising

- If mechanical euthanising causes damage to the relevant tissue or if the fish is small (< 4 cm), the fish should be euthanised with an anaesthetic overdose
- Small fish/fry should be euthanised with an anaesthetic overdose before fixation

General aspects regarding sampling for histopathology

- The volume of formalin should be at least 10 times the total tissue volume, to ensure good fixation of the samples
- Use a leak-proof formalin container that is large enough so that the tissue sample(s) can move freely. Formalin containers can be ordered from PatoGen AS
- Collect a disc shaped tissue sample (0,4 cm x 1 cm x 1 cm) with a smooth cut surface when possible and minimize squeezing/ crushing of the tissue with tweezers or other instruments. It is especially important that samples for urgent analysis are not too thick, as skin and muscle samples are difficult to fixate in a short time
- In cases where macroscopic lesions are present, an additional tissue sample should be collected at the transition between healthy and pathological tissue

Storage of formalin preserved tissue samples

- Formalin should be stored in a temperate room between 10-25°C and should not be stored for longer than 2 months. If the container is exposed to temperatures outside the recommended range, formaldehyde may be released

SHIPMENT

- Shipping should be done as soon as possible after sampling
- All samples should be packed in three layers of packaging: formalin containers, leak-proof secondary container (for example a zip-lock bag with absorbent material) and outer packaging that protects against impact during transport (padded envelope, cardboard box, polystyrene or similar packaging). Avoid freezing or contact with ice packs during shipping, as this can affect the results of the histopathological examination
- The package should be marked with "BIOLOGICAL SUBSTANCE CATEGORY B"
- <https://patogen.no/nb/sending-samples/>

Send the samples in Norway:

Fürst Medisinsk Laboratorium AS
Søren Bulls vei 25
1051 OSLO

Send the samples in UK:

PatoGen Ltd. (UK)
Suite 9, Malin House
European Marine Science Park
Dunbeg, Oban PA37 1SZ

SCOPE OF ANALYSIS ASSIGNMENTS AND LABORATORY RESPONSE TIME

Response time

Normal response time is 5 working days (in the start-up period 6 working days must be expected). No urgent delivery in UK. For urgent delivery in Norway, this can include one of these alternatives: 5 samples with full organ packages, 15 single organs (same tissue type) or 10 fry per submission.

Project

Submissions that include samples from more than 20 fish are considered a project, the response time needs to be agreed upon with PatoGen in advance. We will in every case do our best to give you a quick response. Analysis assignments where the submitter wants a systematic evaluation of tissue/organs beyond normal disease diagnostics are also considered projects and needs to be agreed upon with PatoGen in advance.

A filmed version of the sampling guide can be found here: <https://patogen.no/nb/how-to-carry-out-tissue-extraction-for-histology/>