

# 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 also recommend taking qPCR analyzes on tissue from the heart, kidney and gill on RNAlater.

## ORGAN PACKAGE

- 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

- Tissue samples from one organ from several individuals can be pooled in the same formalin container.

## SAMPLING

### Sampling of organs

<b>Pyloric caeca including exocrine pancreas</b>	Tissue samples (max 0.4 cm x 1 cm x 1 cm)
<b>Gills</b>	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.
<b>Brain</b>	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 and place it into the formalin container. In large fish: carefully dissect the whole brain and place it into the formalin container.
<b>Heart</b>	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.
<b>Skin/muscle</b>	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.
<b>Liver</b>	Disc-shaped tissue sample (max 0.4 cm x 1 cm x 1 cm).
<b>Spleen</b>	Small fish: collect the whole spleen. Large fish: Collect a slice of tissue (max 0.4 cm x 1 cm x 1 cm)
<b>Kidney</b>	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.
<b>Eye</b>	Collect the entire organ
<b>Fry</b>	<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.
- Use a leak-proof formalin container and 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 minimise 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

- The samples are kept cool (not above room temperature) until shipment. Avoid freezing the samples because this may compromise the histopathological examination.

### 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 place light absorbent material in a zip lock bag) and outer packaging (padded bag, cardboard, polystyrene or the like).
- If the formalin containers are sent together with qPCR samples, the formalin tubes must avoid direct contact with the ice packs.
- Avoid leakage of formalin, water, and biological material on the requisition form if possible. It could preferably be placed in a zip lock bag.
- The package should be marked with "BIOLOGICAL SUBSTANCE CATEGORY B".

#### Send the samples to:

PatoGen AS  
Rasmus Rønnebergsgate 21  
6002 Ålesund  
Norway

### 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). Haste deliveries can be performed but must be agreed upon in advance and will entail an addition in price. For haste deliveries the sampling material must arrive at PatoGen's laboratory by 07.45 and the order must be agreed with PatoGen by 12.00 the working day before the samples arrive.

#### Project

Submissions of samples from more than 20 fish are considered a project, and 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.