

SAMPLING GUIDE: TISSUE

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 Real-Time PCR analyses.

We recommend that the tissue sampling is done by experienced staff. PatoGen will be happy to answer any questions regarding sampling procedures. For statutory analyses samplings shall be performed by an authorized veterinarian, fish health biologist or a designated helper. For instructions on using a helper for parts of the sampling, refer to §6 in FOR 2017-08-29-1318: Regulations on measures to prevent, limit and fight pancreas disease (PD) in aquaculture animals.

PatoGen Sample Collection Kit: The kit has been developed as part of PatoGen's quality control system, and consists of pre-labelled tubes with RNAlater™, cooler brick/gel ice pack, and packaging for transport of the samples to our laboratory. The system is developed to prevent interchange of samples and ensure good quality of the sample tissue that is submitted to PatoGen's laboratory. The expiration date is printed on the side of the kit, and the kit should be stored at room temperature until use.

Recommended tissue: *Fry v-cut is considered to contain kidney, heart and gill and can be used in all cases where one of these tissues is among recommended tissues.*

Analysis	Recommended	Others that can be analysed	Analysis	Recommended	Others that can be analysed
AGD	Gill		Paranucleospora	Gill	Kidney, Heart
Atypical furunculosis (A.s type 5) ⁴	Kidney		Parvicapsula ⁸	Gill	Pseudobranch, Kidney, Heart
Atypical furunculosis (A.s type 6) ⁹	Kidney		Pasteurella skyensis O2	Kidney	Heart, Gill
Ballan wrasse birnavirus ^{1,2}	Fry, Kidney		Pasteurella atlantica	Kidney	Heart (only salmon)
BKD [*]	Kidney		Pasteurella atlantica + skyensis O1	Kidney	Heart (only salmon)
Branchiomonas	Gill	Kidney	Piscichlamydia	Gill	Kidney
Costia - necator	Gill	Skin	Piscirickettsia [*]	Kidney	
Costia - salmonis	Gill	Skin	PMCV	Heart	Kidney
EHNV	Kidney		Poxvirus ¹¹	Gill	
Flavobacter [*]	Spleen	Kidney, Gill, Skin	PRV-1 [*]	Kidney, Heart	
Francisella [*]	Kidney	Spleen	PRV-3	Kidney, Heart	
Gyro	Skin	Fin	Pseudomonas anguilliseptica	Kidney	
IHNV	Kidney		Salmoxcellia vastator ²	Heart	Liver, Kidney
IPNV [*]	Kidney		Saprolegnia spp.	Fry	Skin, Gill
ISAV ^{*,6}	Heart	Kidney, Gill	SAV/PDV [*]	Heart	Kidney
ISAV-HPRO [*]	Gill	Heart, Kidney	SmoltTimer [®]	Gill bow	
Lumpfish coronavirus	Fry, Kidney		T. maritimum	Gill	Kidney, Skin
Lumpfish totivirus ²	Fry	Kidney, Spleen	Tenacibaculum spp.	Kidney	Skin, Gill
Lumpsucker virus	Kidney	Liver	Typical furunculosis	Kidney	
Moritella viscosa	Kidney	Skin, Gill	Typical and atypical furunculosis ³	Kidney	
Mycobacterium ^{2,12}	Heart, Kidney	Spleen, Gill	VHSV ^{*,7,10}	Brain	Kidney
Nodavirus [*]	Brain	Milt	Vibrio ang. ⁵	Kidney	
Nucleospora c.	Kidney		Yersinia	Kidney	
Paramyxovirus ²	Gill	Skin			

*Accredited analyses

¹Patent pending

²Analysis under development, validation performed on a limited amount of material

³Detects type 1-23

⁴Detects type 5, prevalent in ballan wrasse

⁵Detects *Vibrio ordalii* og *anguillarum* type O1, O2, O3

⁶For statutory ISA surveillance in Norway: Heart, and in some cases also kidney. ISA free areas (establishing new, reestablishing or expansion of ISA free area): Analysis of both heart and kidney is a requirement. Surveillance for ISAV-HPRO: Gill is recommended

⁷Kidney early phase, brain late phase

⁸Kidney or gill before disease, pseudobranch after

⁹Detects type 6, prevalent in lumpsucker

¹⁰Detects type I-III

¹¹Patent NO/EP3237607

¹²Two analyses to cover more species. Detects *Mycobacterium salmoniphilum* and closely related species and *Mycobacterium marinum* and closely related species



Preparation

Before sampling and submission, you should have access to:

- Sterile tweezers and scalpels
- Chlorine (3-10% mix), optionally gas flare and ethanol (70%), for continuous sterilisation of tweezers and scalpel shaft
- PatoGen sample collection kit
- Wipers
- Stationery
- Stable and clean surface

Sampling

Remember that the fish can be euthanized with an anaesthetic overdose.

Avoid contamination of the tissue sample with vaccine from the abdominal cavity

Research carried out by PatoGen indicates a certain risk that residual of vaccines can be detected in fish vaccinated against PDV, IPNV, Yersinia or Flavobacter. The risk decreases in time, and is minimized by avoiding direct contact with the vaccine in the internal abdomen. For PDV and IPNV vaccinated fish are distinguished from infected fish based on which virus variant that are detected, while for other agents there are not yet good methods to distinguish vaccinated and infected fish. When sampling for analysis of IPNV, Yersinia and Flavobacter, contamination of the kidney sample with the vaccine is avoided by taking the sample via the neck. When sampling for analysis of PDV, contamination of the heart sample with vaccine is avoided by taking the sample with an incision in the heart cavity without opening the abdominal cavity.

Sterile technique

Use sterile techniques when collecting the samples to avoid contamination between the different samples taken from different fish. We recommend to use a new scalpel for each fish. When reusing equipment we recommend to clean it with wipers to remove organic residuals, before bathing it in 3-10 % chlorine, optionally you can bathe the equipment in 70% ethanol and burn off. Tip: Use the inside of the scalpel package to trim the tissue.

Sampling of tissue

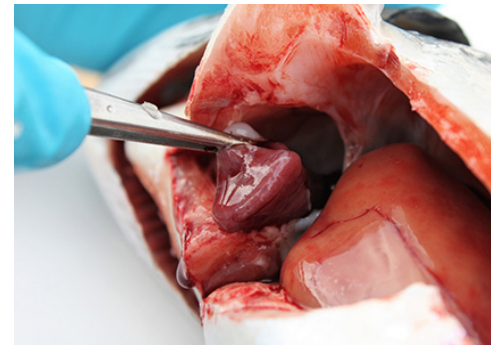
Open the fish with the scalpel so that the relevant organ becomes available. Take the sample, on small fish you can take a larger part of the organs. In case of sampling of gill in addition to other tissues, we recommend taking the gill first (remember to change or secure that the equipment is properly disinfected before you open the fish after sampling of the gill).



Gill



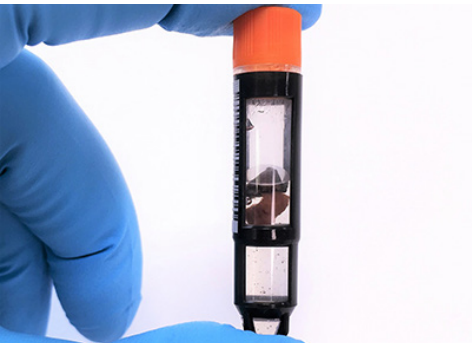
Kidney (head kidney, front part)



Heart (summit of the ventricle)



Trimming of the tissue on the scalpel package



Make sure the tissues are covered by RNAlater



Submission of test tubes in a plastic bag

Trimming of tissue

Place the tissue on the inside of the scalpel package and cut it into two pieces of about 2x2x2 mm (like the head of a match). Make sure that the tissue samples are not too large as it may cause poor preservation. It is important that you take two samples from each organ being analysed (A and B sample).

Transfer of tissue to test tubes

The samples from one fish should be placed on the same test tube, and a maximum of four samples can be placed on each tube. If more than one tissue are being sampled (3,5 etc.), we recommend that the gill is placed on a separate tube. Put gill on the first tube and for example heart and kidney on the next. If more than one tissue are being sampled (2, 6 etc.), the gill can be placed on the same tube as an other tissue. Make sure the tissue is placed in the liquid in the tube, and is not stuck on the edge without being covered in liquid.

Registration of information

Register the sampling in Patolink or note details about the samples on the requisition form following the kit. Contact us if you have any questions regarding access to or use of Patolink. All samplings registered in Patolink gets one day shorter delivery time - 4 working days as opposed to normally 5 working days.

Storage of the samples before shipment

When the test tubes have been used they should be put on ice/cooler continuously. When doing the sampling over multiple days used test tubes can be stored in a refrigerator for a week before shipment. For longer storage it is recommended that the test tubes are frozen, after having been stored in a refrigerator for 24 hours.

Shipment

If the whole kit is not used, the test tubes can be removed and placed in a plastic bag. The rest of the kit can then be used in the next sampling. The samples should be sent to PatoGen with a cooling brick/ice pack. Send the package by express shipment to the address below. Send the tracking number to sample@patogen.no or +47 957 07 910.