

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
<i>Aeromonas salmonicida</i> type 1-23	Kidney	Spleen, heart	<i>Moritella viscosa</i>	Kidney	Skin, gill
<i>Aeromonas salmonicida</i> subsp. sal.	Kidney	Spleen, heart	<i>Mycobacterium</i> ^{2,10}	Kidney, heart	Spleen, gill
<i>Aeromonas salmonicida</i> type 5	Kidney		Nodavirus*	Brain	Spleen
<i>Aeromonas salmonicida</i> type 6	Kidney		<i>Nucleospora cyclopteri</i>	Kidney	
AGD - <i>Paramoeba perurans</i>	Gill		<i>Paranucleospora theridion</i>	Gill	Kidney, heart
<i>Aliivibrio wodanis</i> ²	Kidney	Skin, heart	<i>Parvicapsula pseudobranchicola</i> ⁹	Pseudobranch, gill	Kidney, heart
Atlantic salmon paramyxovirus ²	Gill	Skin	<i>Phocoenobacter skyensis</i> O2	Kidney, heart	Gill
Ballan wrasse birnavirus ¹	Fry, kidney		<i>Phocoenobacter atlanticus</i>	Kidney, heart	
BKD - <i>Renibacterium salmoninarum</i> *	Kidney		<i>Phocoenobacter atl. + sky.</i> O1	Kidney, heart	
Ca. <i>Branchiomonas cysticola</i>	Gill	Kidney	<i>Piscirickettsia</i> spp.*	Kidney	
Ca. <i>Piscichlamydia salmonis</i>	Gill	Fry	<i>Piscirickettsia salmonis</i>	Kidney	
<i>Carnobacterium maltaromaticum</i> ²	Kidney, heart		<i>Piscirickettsia nova</i>	Kidney	
Cod poxvirus ²	Gill		PMCV	Heart	Kidney
<i>Carnobacterium maltaromaticum</i> ²	Kidney, heart		PRV-1*	Heart	Kidney, blood
Costia - <i>Ichtyobodo necator</i>	Gill	Skin	PRV-3	Heart	Kidney
Costia - <i>Ichtyobodo salmonis</i>	Gill	Skin	<i>Pseudomonas anguilliseptica</i>	Kidney	
<i>Flavobacterium psychrophilum</i> *	Spleen	Kidney, gill, skin	Salmon poxvirus ⁴	Gill	
<i>Francisella</i> *	Kidney	Spleen	<i>Salmoxcellia vastator</i> ²	Heart	Liver, kidney
<i>Gyrodactylus salaris</i>	Fin, skin		<i>Saprolegnia</i> spp.	Fry	Skin, gill
IHNV	Kidney		SAV/PDV*	Heart	Kidney
IPNV*	Kidney		SmoltTimer®	Gill arch	
ISAV ^{*,6}	Heart	Kidney	<i>Tenacibaculum maritimum</i>	Gill	Kidney, skin
ISAV-HPR0*	Gill	Heart, kidney	<i>Tenacibaculum</i> spp. ⁷	Kidney	Skin
Lumpfish coronavirus	Fry, kidney		VHSV ^{*,3,8}	Brain	Kidney
Lumpfish totivirus ²	Fry, kidney	Spleen	<i>Vibrio anguillarum + ordalii</i> ⁵	Kidney	
Lumpsucker virus ⁵	Kidney	Liver	<i>Yersinia ruckeri</i>	Kidney	

*Accredited analyses

¹Patent NO347379

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

³Detects genotype I-III

⁴Patent NO346439

⁵Patent NO344051

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-HPR0: Gill is recommended

⁷The analysis does not detect *Tenacibaculum maritimum*

⁸Kidney early phase, brain late phase

⁹Kidney or gill before disease, pseudobranch after

¹⁰Two 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
- Disposable gloves
- 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

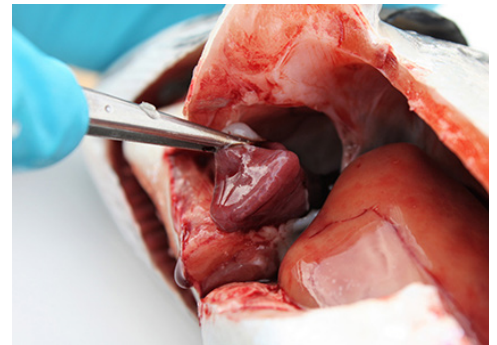
Open the fish with the scalpel so that the relevant organ becomes available. Take the sample, on small fish you can take a larger part of the organs. In case of sampling of gill in addition to other tissues, we recommend taking the gill first (remember to change or secure that the equipment is properly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



Gill



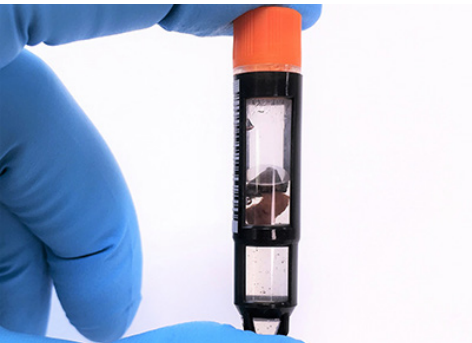
Kidney (head kidney, front part)



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 another 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. Refers to patogen.no for general delivery conditions and otherwise to our standard conditions and information on the order form. For contract customers, other conditions may apply.

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. <https://patogen.no/nb/sending-samples/>