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
Aeromonas salmonicida type 1-23	Kidney	Spleen, heart
Aeromonas salmonicida subsp. sal.	Kidney	Spleen, heart
Aeromonas salmonicida type 5	Kidney	
Aeromonas salmonicida type 6	Kidney	
AGD - Paramoeba perurans	Gill	
Aliivibrio wodanis[2]	Kidney	Skin, heart
Atlantic salmon paramyxovirus[2]	Gill	Skin
Ballan wrasse birnavirus[1]	Fry, kidney	
BKD - Renibacterium salmoninarum*	Kidney	
Ca. Branchiomonas cysticola	Gill	Kidney
Ca. Piscichlamydia salmonis	Gill	Fry
Carnobacterium maltaromaticum[2]	Kidney, heart	
Cod poxvirus[2]	Gill	
Carnobacterium maltaromaticum[2]	Kidney, heart	
Costia - Ichthyobodo necator	Gill	Skin
Costia - Ichthyobodo salmonis	Gill	Skin
Flavobacterium psychrophilum*	Spleen	Kidney, gill, skin
Francisella*	Kidney	Spleen
Gyrodactylus salaris	FIn, skin	
IHNV	Kidney	
IPNV*	Kidney	
ISAV*,[6]	Heart	Kidney
ISAV-HPR0*	Gill	Heart, kidney
Lumpfish coronavirus	Fry, kidney	
Lumpfish totivirus[2]	Fry, kidney	Spleen
Lumpsucker virus[5]	Kidney	Liver
Moritella viscosa	Kidney	Skin, gill

Analysis	Recommended	Others that can be analysed
Mycobacterium marinum[2,10]	Kidney, heart	Spleen, gill
Mycobacterium salmoniphilum[10]	Kidney, heart	Spleen, gill
Nodavirus*	Brain	Spleen
Nucleospora cyclopteri	Kidney	
Paranucleospora theridion	Gill	Kidney, heart
Parvicapsula pseudobranchicola[9]	Pseudobranch, gill	Kidney, heart
Phocoenobacter skyensis O2	Kidney, heart	Gill
Phocoenobacter atlanticus	Kidney, heart	
Phocoenobacter atl. + sky. O1	Kidney, heart	
Piscirickettsia spp.*	Kidney	
Piscirickettsia salmonis	Kidney	
Piscirickettsia nova	Kidney	
PMCV	Heart	Kidney
PRV-1*	Heart	Kidney, blood
PRV-3	Heart	Kidney
Pseudomonas anguilliseptica	Kidney	
Salmon poxvirus[4]	Gill	
Salmoxcellia vastator[2]	Heart	Liver, kidney
Saprolegnia spp.	Fry	Skin, gill
SAV/PDV*	Heart	Kidney
SmoltTimer®	Gill arch	
Tenacibaculum maritimum	Gill	Kidney, skin
Tenacibaculum spp.[7]	Kidney	Skin
VHSV*,[3,8]	Brain	Kidney
Vibrio anguillarum + ordalii[5]	Kidney	
Yersinia ruckeri	Kidney	
Yersinia ruckeri CC1	Kidney	

*Accredited analyses
[1]Patent NO347379
[2]Analysis under development, validation performed on a limited amount of material
[3]Detects genotype I-III
[4]Patent NO346439
[5]Patent NO344051
[6]For statutory ISA surveillance in Norway: heart ventricle (heart valves and endothelium), and in some cases also kidney. ISA free areas (establishing new, reestablishing or expansion of ISA free area): analysis of both heart ventricle and kidney is a requirement. Surveillance for ISAV-HPR0: gill is recommended
[7]The analysis does not detect *Tenacibaculum maritimum*
[8]Kidney early phase, brain late phase
[9]Kidney or gill before disease, pseudobranch after
[10]Detects also 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 contiuous 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.

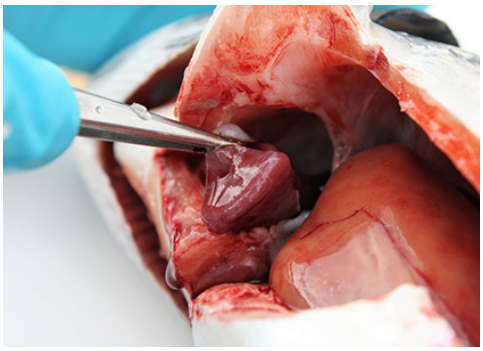
Avoid contamination of the tissue sample with vaccine from the abdominal cavity
Research carried out by PatoGen indicates a certain risk that residual of vaccines can be detected in fish vaccinated against, for example, PDV, IPNV, Yersinia or Flavobacter. The risk decreases in time, and is minimized by avoiding direct contact with the vaccine in the internal abdomen. For IPNV vaccinated fish are distinguished from infected fish based on which virus variant that are detected, while for other agents, however, reliable methods for distinguishing between vaccinated and infected fish are not yet available. 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.

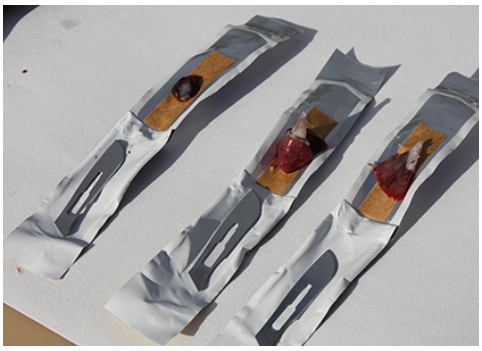
Sampling of tissue
Open the fish with the scalpel so that the relevant organ becomes available. Take the sample, on small fish you can take a larger part of the organs. In case of sampling of gill in addition to other tissues, we recommend taking the gill first (remember to change or secure that the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

Gill

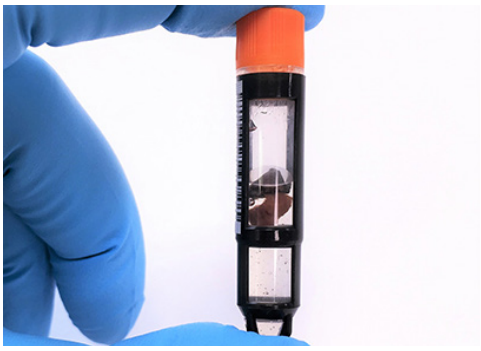
Kidney (head kidney, front part)



Heart (summit of the ventricle)[6]



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).

Tranfer 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 sample submission electronically in PatoLink. Please contact us if you have any questions regarding access to or use of PatoLink, by email at post@patogen.no or by phone at +47 70 11 69 00. Please refer to patogen.no for general terms of delivery and information in the overview of recommended area of use: https://patogen.no/recommended-area-of-use/.

Storage of the samples before shipment
When the test tubes have been used they should be put on ice/cooler continuosly. 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 later. Send the package by express shipment to the address below. Order return label for sending samples here https://patogen.no/sending-samples/

Norway: PATOGEN AS - RASMUS RØNNEBERGSGATE 21 - 6002 ÅLESUND - NORWAY
UK: PATOGEN - Malin House - European Marine Science Park - Dunbeg - PA37 1SZ Oban