Equipment recommended to have available when sampling realtime RT-PCR:

- Styrofoam box and cooling elements
- Tweezers and scalpel
- Clean surface
- Gas burner and ethanol (70%)
- Paper towel

Recommended sampling tube:

Tissue and fry	Barcode tube with RNAlater
Ovarian fluid, ova and milt	Barcode tube with RLT-buffer

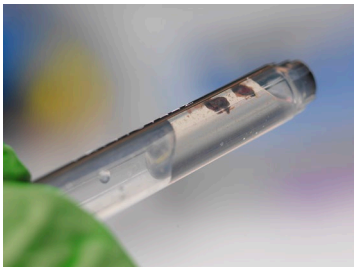
General recommendations

We encourage to register all samples electronically in our customer portal iWise or in APP before shipment. Be sure to mark the shipment with location, location number and order number to ensure traceability upon arrival at our laboratory.

Sampling should be performed using sterile technique to ensure that contamination is avoided. The sterile interior of the scalpel blade packaging can be used as a surface to trim tissues. Contact between head kidney/heart tissue samples and the abdominal cavity should be avoided.

Heart and kidney samples from the same fish can be placed in the same tube. Additional organs from the same fish are placed in separate tubes. Barcode tubes should be placed in a rack after sampling. Empty racks are sent out upon request.

Samples can be stored in the fridge for a few days after sampling but should be frozen at -20°C for longer storage.



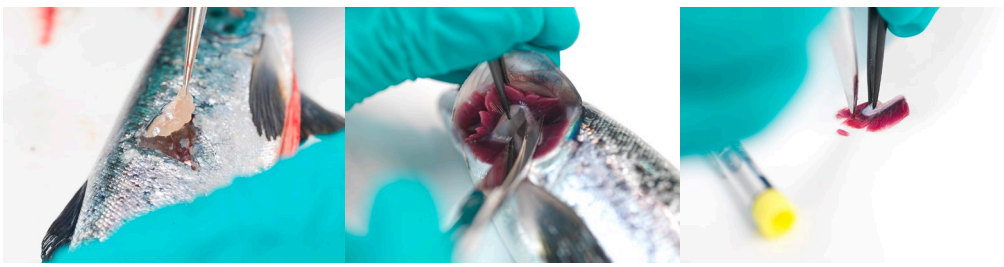
The tissue samples should be the size of a match head, 2x2x2mm. It is important to include two tissue samples of each organ, A- and B-sample. The tissue samples should be properly immersed in the preservation fluid in the sample tubes.

When doing regular screening we recommend sampling moribund and/or freshly deceased individuals until a positive RT-PCR sample is detected. After a pathogen has been detected in moribund/dead fish, screening of random healthy individuals should follow to get an overview of the prevalence in the population.

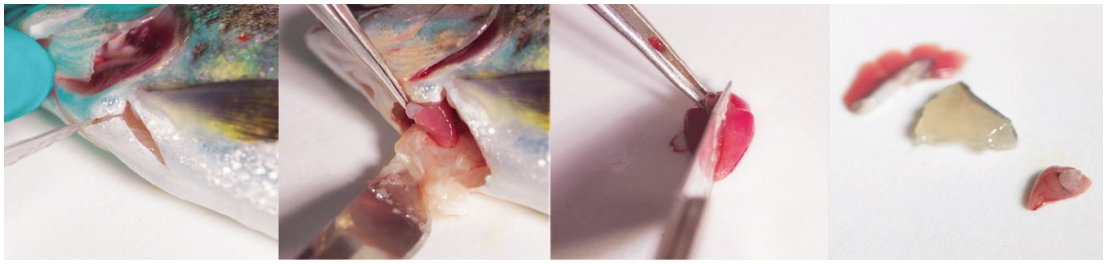
Videos showing sampling techniques are available in iWISE.

Sampling

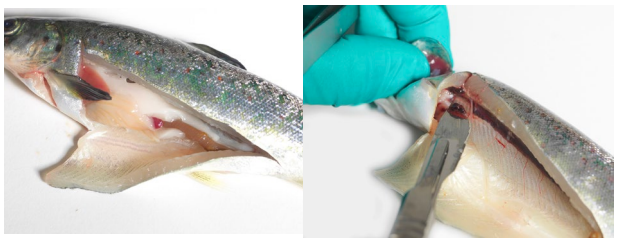
1. Start by sampling skin around wounds.
2. Continue with sampling gill (arch number 2). Cut out tissue with sterilized scalpel and place it on a clean surface. Split tissue into two pieces and place both pieces in sample tube.



3. Sterilize the scalpel blade and tweezers with gas burner before making a cut into the heart cavity. Use the tweezers to pick up the heart by the bulbus area and cut out the heart. Cut off the apex, split the tissue in two pieces and place both pieces in new sampling tube.



4. Remove any tissue residues from the instruments and sterilize them with gas burner before making a cut in the abdominal cavity.



5. The kidney should be the first organ sampled from the abdominal cavity. Identify the kidney by removing the swim bladder. Use gas burner to sterilize the instruments again before extracting a small square from the head kidney. Split the tissue in two pieces. The kidney can be placed in the same sampling tube as the heart tissue.
6. When you have finished sampling one fish, write down any comments by the correct sampling tube (i.e healthy, moribund, dead or injury).
7. Place the sample tubes in a thick envelope or styrofoam box together with cooling elements and information about location and location number. Order form and barcode sheet should also be included if samples are not registered electronically. Send the package with express delivery.

Fry

Alevins	Place the whole alevin in tube
Larger fry	The head is cut just behind the operculum, and if necessary, can be cut in half before being placed in tube. It is important to include gills, heart, and kidney in the section.

Broodfish

Ovarian fluid/milt	Min 0,2 ml and max 1 ml ovarian fluid or milt in each tube.
Ova	One ovum per tube

For ovarian fluid, ova and milt, tubes with RLT-buffer should be used. Do not pool ovarian or milt samples from several individuals in the same tube. Ovarian fluid should preferably be taken from the bucket after stripping, and not directly from the fish.

If anything is unclear – Please contact us!

Ship the samples with express delivery to:

PHARMAQ Analytiq AS
Thormøhlensgate 53D
5006 Bergen
Norway

Overview of pathogens and recommended sampling tissue

Fry, ova, ovarian fluid and milt can be tested for all viruses and bacteria.

Virus	Tissue	Bacteria	Tissue	Parasites	Tissue
Piscine myocarditisvirus (PMCV)	Heart	*Flavobacterium psycrophilum*	Kidney, Gill	Paramoeba perurans	Gill
Piscine Orthoreovirus (PRV)	Heart	*Yersinia ruckeri*	Kidney	Paranucleospora theridion	Gill
Salmon Gill Poxvirus (SGPV)	Gill	*Renibacterium salmoninarum*	Kidney	Parvicapsula pseudobranchicola	Gill
Infectious Pancreatic Necrosis virus (IPNV)	Kidney	*Piscirickettsia salmonis*	Kidney	Ichtyobodo sp. * (Costia)	Gill
Pancreas Disease Virus* (SAV)	Heart	*Branchiomonas cysticola*	Gill	Nucleospora cyclopteri	Gill, Kidney
Infectious Salmon Anemia Virus (ISAV)	Heart	*Clavochlamydia salmonicola*	Gill	Spironucleus salmonicida	Heart
Viral Hemorragic Septicemia Virus (VHSV)	Kidney	*Moritella viscosa**	Wound, Kidney		
Nodavirus (VNN)	Kidney, CNS	*Pasteurella spp.*	Kidney		
Infectious Haematopoetic Necrosis Virus (IHNV)	Kidney	*Pasteurella skyensis*	Kidney		
Atlantic Halibut Reovirus (AHRV)	Liver Kidney	*Aeromonas salmonicida**	Kidney		
Lumpfish Flavi Virus (LFV)	Kidney, Liver	*Tenacibaculum sp.*	Wound, Kidney		
Cyclopterus lumpus Coronavirus (CluCV)	Kidney	*Tenacibaculum maritimum*	Wound, Kidney, Gill		
Cyclopterus lumpus Totivirus (CluTV)	Kidney	Tripple analysis for *Vibrio anguillarum* (analysis for O1, O2α and universal for other variants)	Kidney		
		Vibrio anguillarum O1	Kidney		
		Vibrio anguillarum O2α	Kidney		
		Francisella philomiragia ssp. noatuensis	Kidney		
		Allivibrio salmonicida	Kidney		
		Mycobacterium salmoniphilum	Kidney		

***possiblity for subtyping**