Recommended equipment

- Barcode tube **containing transport medium – *MicrobeVision***
- Box or zip-lock bags for tubes
- Cooling elements
- Sterile scalpels, forceps, and scissors
- Gas burner for sterilising scalpel blades, forceps, and scissors
- Clean surface for dissection; bench protector paper
- Tissue paper
- Disposable gloves
- Disinfection solution, spray with 70% ethanol or 10% chlorine
- Styrofoam box for secure transport
- Absorbing tissues
- Cool packs (frozen)

The sampling package with barcode tubes from PHARMAQ Analytiq contains microbiology submission form. We recommend that you fill in the form as the samples are being taken.
We encourage to register all samples electronically before shipment.

Preferred tissue
For microbiological analysis, the preferred tissue is **kidney**. Other tissues can be relevant, depending on clinical signs. Gills, spleen, heart, skin, and wounds are tissues that can be used for microbiological analysis.

Size of the tissue
The tissue should be larger than for RT-PCR sampling, however small enough to fit into the 2ml tube. Make sure the tissue is completely covered by the liquid.

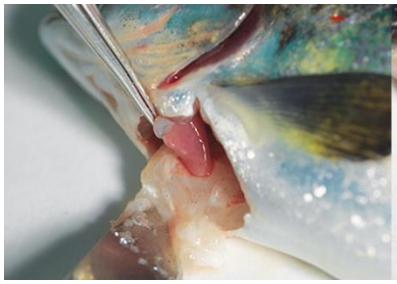
Guidance for sampling
Sampling for microbiology analysis is done in the same manner as for RT-PCR analysis. The main difference is the type of tubes used, and the fluid in them. For Microbiology analysis, 2ml barcoded tubes are used, pre-filled with transport medium – *MicrobeVision*. NB! Please do not use any other sampling container, or medium, than the tubes w/transport medium provided for *MicrobeVision*.

The tissue samples should be as large as possible, without compromising sterility. It is important that the tissue samples are properly immersed in the fluid contained in the sample tubes.

2A **2B** **2C**

01. Gill (arch number 2) and skin should be sampled first (2A, 2B and 2C).
02. Hold the tissue with sterile forceps and, using sterile scissors, cut each sample in two pieces of similar size. (A- and B sample) Put both samples (of the same organ) into a tube containing transport medium. For the heart, sterilise the scalpel blade and tweezers before making a cut in the heart cavity. Use the tweezer to pick up the heart by the bulbus area (3).
04. Hold the heart with sterile forceps and use sterile scissors to cut off the apex and put both samples in a tube containing transport medium.
05. Any residues of tissue should be removed from the instruments, which should then be sterilised before making a cut in the abdominal cavity (4).

06. The kidney should be the first organ sampled from the abdominal cavity. Identify the kidney by removing the swim bladder. Sterilise the instruments again before extracting a small square from the head kidney. Split the extracted square in two and put both samples in a tube containing transport medium (5).



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4

5

07. If necessary, sample the remaining organs from the abdominal cavity, sterilizing the instruments in between organs.
08. When sampling multiple organs from the same fish, use **<u>one</u> tube per organ/piece of tissue**.

09. When sampling is finished the order form should be completed.
10. Make a note on the barcode sheet if additional information regarding the tubes (i.e., cage, wound, healthy) needs to be included.
11. Place all the **<u>sample tubes in the tube rack provided</u>** and place rack in a Styrofoam box, together with order form, barcode sheet, and a cooling element. The samples should be sent using express delivery. (6)

Additional notes:

Sampling for Microbiology analysis should be performed using sterile techniques to ensure that contamination is avoided.
The samples should **NOT** be stored over a longer period before submission (ship within 48 hours). The samples must be kept cold (4°C) the entire time, both at the sampling facility, and during transport. The samples should **NOT** be kept in room temperature nor at -20°C.

NB! When suspecting ***Moritella viscosa*** and/or ***Tenacibaculum*** spp., it is especially important to keep the tubes and the transport medium **cold all the time**, as these bacteria will die at room temperatures.

Make sure that the tubes are not in direct contact with the cooling elements, as to avoid freezing of the tubes and their contents – this could impact bacterial viability!

If anything is unclear – please contact us.

Ship the samples with express delivery:

PHARMAQ Analytiq AS, Thormøhlensgate 53D, 5006 Bergen, Norway

NB! Mark the shipment: **Microbiology**