



For microbiology, the following equipment is recommended:

- Requisition form for microbiology
- Agar plates:
 - **Marine fish:** blood agar w/2% NaCl (BAS) and marine agar (MA) (*Tenacibaculum* spp.)
 - **Freshwater:** blood agar (BA) and Ordal's medium (OM) (for *Flavobacterium* spp.)

Or
- Tubes w/transport medium for samples of tissue
- Sterile inoculation loops (platinum or disposable, plastic loops)
- Scissors, forceps and scalpel (all sterile equipment)
- Gas burner for sterilising inoculation loop, scalpel blades, forceps and scissors
- Surface for dissection; bench protector paper
- Tissue
- Disposable gloves
- Permanent marker (waterproof) for labelling of agar plates
- Disinfection solution, spray with 70% ethanol or 10% chlorine
- Parafilm and Ziplock bag for packaging
- Styrofoam box for secure transport
- Absorbing tissues
- Cool packs (frozen)



Picture 1: Example of how to label the agar plate

NB! All samples should be registered in the customer portal prior to shipment.

Guide for sampling for microbiology samples:

If disease is suspected, sample moribund fish, or fish that clearly shows signs of disease. If there is only dead fish available for sampling, sample newly dead fish that does not show any signs of decomposition.

Sampling for microbiology analysis should be performed using sterile technique to avoid contamination of the samples.



- Agar plates: condensation is removed from the lid by tapping the lid against clean paper.
- Each sample should be clearly marked with type of agar, ID, date and organ, with a permanent waterproof marker (see example in Picture 1).
- The agar plates are labelled at the bottom, as the lid can fall off.
- Use paper tissue to remove remains of tissue from the scalpel blade.
- Use the gas burner to sterilize the incubation loop, scalpel blade, forceps and scissors between different tissues and fish.
- When sampling head kidney and heart, avoid contact with the abdominal cavity.



Picture 2: Streak dilution

Gill/skin samples are done first

a. Gill streak:

- i) the operculum is removed with a sterile scissor.
- ii) sterilize the scissor.
- iii) for small fish; sample the whole gill arch.
- iv) for large fish; sample a 2 cm piece of the gill arch
- v) the gill arch is gently pressed onto the agar of the plate, before removing the gill arch
- vi) the sample is diluted as shown in picture 2.

b. Skin/streak from ulcer:

- i) For *Tenacibaculum* spp. on marine agar (MA): bring the sterile inoculation loop from the exterior rim of the wound, towards the interior rim of the wound, but not under the interior rim. Dilute as seen in picture 2.
- ii) For *Moritella viscosa et al.* on blood agar w/salt (BAS): bring the sterile inoculation loop under the skin of the fish, at the edge of the wound. If using a platinum inoculation loop, burn the loop and cool it off at the insertion point.

Internal organs (kidney, spleen, etc.)

- c. Remove the abdominal wall with a sterile scalpel, pull out the internal organs and remove the swim bladder.
- d. If using a platinum inoculation loop, burn the loop and cool it off by inserting it into the organ.
- e. Make sure to use aseptic technique when inserting sterile inoculation loop into the organ.
- f. Carefully withdraw the inoculation loop to avoid contamination.
- g. Streak out on suitable medium and dilute sufficiently, as seen in picture 2 (one streak per plate).



Shipment:

Seal agar plates with parafilm or similar, and swabs in tubes with transport medium.

- a) Pack in spill-proof secondary container (for example Ziplock bag).
- b) Use cushioning and/or absorbing material between secondary and external packaging.
- c) External packaging should be of a strong and insulating material, for example a Styrofoam box with cool packs.

Organs and whole fish:

- a) Pack individually in sterile Ziplock bags.
 - b) Pack in spill-proof secondary container (e.g., Ziplock bag).
 - c) Use cushioning and/or absorbing material between secondary and external packaging.
 - d) External packaging should be of a strong and insulating material, for example a Styrofoam box with cool packs.
- We prefer streak plates, or tissue in transport medium, rather than whole fish.
 - Samples should be sent immediately after sampling and stored refrigerated until shipment.

Should anything be unclear – please feel free to get in touch!

Samples are shipped with express delivery to:

**PHARMAQ Analytiq AS
Thormøhlensgate 53D
5006 Bergen**