

SAMPLING GUIDE: BACTERIOLOGY SAMPLES

Proper sampling is important to secure optimal quality of the analyses. This guide contains PatoGen's guidelines for sampling and handling of samples for bacteriological examination.

GENERAL ASPECTS

- We recommend plating samples from one individual per dish. In cases where samples are collected from several organs from each individual, a maximum of two organs can be plated on each dish (distributed on 50 % of the dish).
- Mark the dish with the fish ID /letter and note which organs that are collected per individual on the requisition form.
- In cases where PCR, histology and bacteriology samples are taken from the same individual, the fish ID/letter must match on the requisition form.
- The unused agar plates should be stored in a refrigerator until use.
- Always use a sterile wire loop, a sterile disposable plastic loop or sterile cotton swabs when sampling for bacteriology.
- **Agar types that are recommended for use in a routine bacterial sampling:**
 - Freshwater fish and broodstock reared on freshwater: blood agar without added NaCl
 - Freshwater fish reared on water with salinity: blood agar without added NaCl and blood agar with added NaCl
 - Saltwater fish, cleaner fish and broodstock reared on seawater: blood agar without added NaCl and blood agar with added NaCl or marine agar
 - Contact PatoGen if there is need for a special agar (Flavobacterium sp needs Ordal medium and Tenacibaculum sp needs marine agar)
- We always recommend that primary smears are submitted for analysis, but when submitting secondary smears this must be highlighted in the requisition.

SAMPLING

Euthanasia

- Fish shall always be anesthetised in a regulatory manner before euthanasia.
- Euthanasia may be done mechanically, with an overdose of anaesthetic or bleeding.
- Small fish/fry is euthanised with an overdose of anaesthetic before sampling.

Sampling

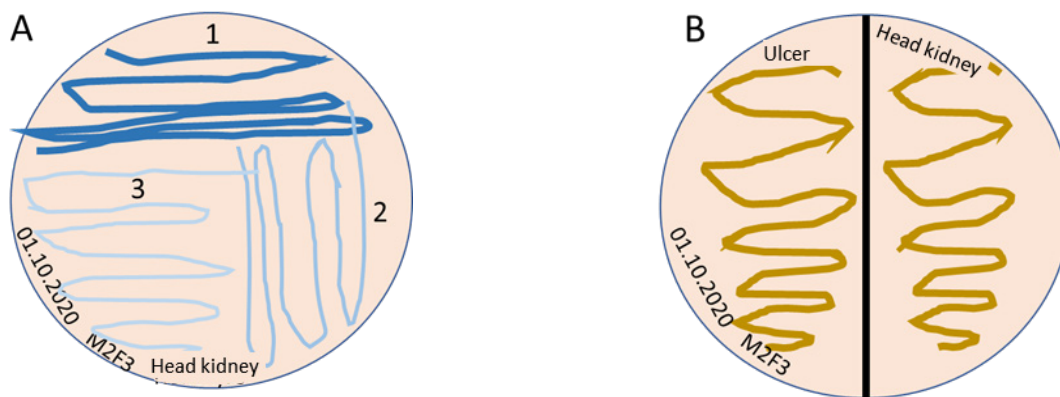
- Remove condensation on the dish lid by gently tapping it on clean paper towels.
- Label around the edge of the bottom (not the lid) of the agar plate with a waterproof marker with at least: date of sampling, pen number, fish number and organ. If samples are collected from two organs from the same fish, these should be plated on two equal halves of the plate, indicated by a center line on the bottom of the plate. Each semicircle must be clearly labeled with the organ that have been sampled, see figure 1.
- The use of aseptic technique when collecting bacteriological samples is important to avoid contamination with environmental or intestinal bacteria.
- Push the loop (wire or plastic) or the sterile cotton swab into/on the organ to be sampled or on the periphery of the pathological finding (wound, abscess etc.).
- Place the loop/swab gently on the agar surface and spread the sample with zigzag movements across the agar surface, see figure 1. It is an advantage that the spreading on the dish is diluted as shown in figure 1A. Perform the first spreading in one sector of the plate(1). For the second spreading, turn the Petri dish 90° on the first and start the spreading at the end of the last streak. Spread the material in the next sector with a back-and-forth movement. (2). Perform this a third time for the final dilution (3).
 - **Sampling of organ (kidney, spleen and skeletal muscle):** Puncture the surface of the organ with a glowing wire loop. Then, let the wire loop cool off before the loop is pushed a bit further into the organ to collect the sample material.
 - **Sampling of skin ulcers:** Open wounds should first be sampled from the exposed area close to the wound margin without flaming the sterile wire loop before plating the sample. Then collect a new sample with a flamed wire loop, wait a little bit on the wound margin for the loop to cool off and then push it into the deeper layers of the skin before plating the sample.
 - **Sampling of fluids (blood/ovarian fluid/milt/ fluids in abdomen or heart cavity):** Dip a sterile loop or swab in the fluid and spread it over the agar surface with a back-and-forth movement as shown in figure 1.

- **Sampling of gill:** Remove up to 2 cm of the second gill arch with a sterile scissor and place the gill filaments on the agar surface applying a soft pressure. Spread the material from the footprint of the gill sample as shown in figure 1.
- **Sampling from the lumen of the intestine:** Sterilise the anal opening with 70% alcohol. Introduce the loop into the anal opening and further in the intestine for sample collection before spreading it on the agar dish as shown in figure 1.

Figure 1: Spreading a sample on a Petri dish.

A: Plating sample from one individual and one organ with dilution.

B: Plating sample from one individual and two organs with no dilution.



Storage

Plated agar dishes should be stored cooled (not above room temperature) until shipment. Avoid freezing of the dishes as this may change the bacterial composition on the dish.

SHIPMENT

- Shipment should be done as soon as possible after sampling.
- If we receive bacterial dishes late on a Thursday or a Friday, the shipment will be stored at PatoGen until Monday before it is sent on to VI, to avoid that the material is left in the mail over the weekend. This will lead to some longer delivery time and can affect the quality of the samples. We encourage therefore to send in the material so that we receive it Thursday morning at the latest.
- The lids on the dishes must be secured with parafilm, tape or a rubber band and packed in a leak proof bag (ex. Ziplock bag). The dishes are padded with paper or bubble wrap before being packed in a Styrofoam box with 1-2 cooling elements. Make sure there are no room for the dishes to move in the box. This shall prevent that the dishes are broken or that the lids open and the samples can be contaminated from other dishes. A bubble wrap envelope can be used if its only 1-2 dishes in the shipment.
- If the bacteria samples are sent in together with PCR samples, the bacteria dishes must avoid direct contact with the ice packs.
- Avoid spillage on the requisition form if possible. It can preferably be placed in its own packaging.
- The package is marked "BIOLOGICAL SUBSTANCE CATEGORY B".
- Send to:

PATOGEN AS - RASMUS RØNNEBERGS GATE 21 - 6002 ÅLESUND - NORWAY

SCOPE OF ANALYSIS ASSIGNMENT AND THE LABORATORY'S DELIVERY TIMES

- The delivery time for bacteriology will depend on how long the bacteria growth takes
- Number of samples beyond 10 individuals is considered a project and delivery time must be agreed with PatoGen. In all cases we will do our best to give you a quick response.