

SAMPLING GUIDE: BACTERIOLOGY

To ensure high-quality analyses, proper sample collection and handling are crucial. This guide outlines PatoGen's guidelines for collecting and handling of samples for bacteriological examination. All samples must be registered directly in PatoLink.

GENERAL ASPECTS

- We recommend spreading samples from one fish per plate. In cases where samples are collected from several organs from each fish, a maximum of two organs can be plated on each dish (distributed 50/50)
- Mark the plate with letter and each half with organ, cage- and fish number. Record all information of the sampling in PatoLink
- To enable PatoGen to link results to each specific fish, the fish- and cage number must be consistently recorded on all materials submitted for PCR, histology, and bacteriology
- Unused agar plates should be stored in the refrigerator until use
- Always use a sterile wire loop, a sterile disposable plastic loop when sampling for bacteriology
- See our guide for information on which agar type to use for different bacteria. Contact PatoGen if there is need for a special agar
- We always recommend that primary smears are submitted, please register whether the spread is primary or secondary in PatoLink

SAMPLING

Euthanasia

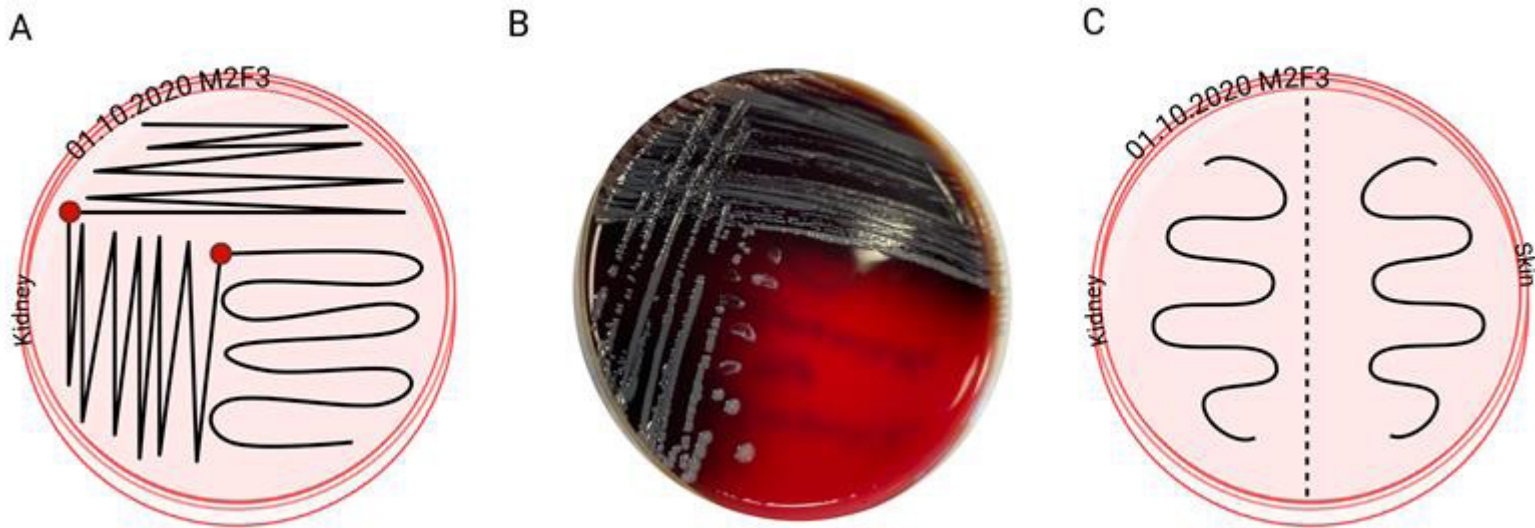
- Fish shall always be anesthetised in a regulatory manner before euthanasia
- Euthanasia may be done mechanically, with an overdose of anaesthetic or bleeding
- Small fish/fry is euthanised with an overdose of anaesthetic before sampling

Sampling

- Remove condens on the plate 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: date of sampling, pen number, fish number and organ. If two samples are collected on the same plate, the plate has to be divided in two equal halves of the back of the plate, both sides need to be marked clearly
- The use of sterile techniques when collecting bacteriological samples is crucial to prevent contamination with environmental or intestinal bacteria
- Push the loop (wire or plastic) into the organ to be sampled or onto the periphery of the pathological finding (wound, abscess, etc.)
- Gently place the loop on the agar surface and spread the sample using zigzag movements across the agar, as shown in Figure 1. It is beneficial to dilute the sample while spreading, as illustrated in Figure 1A.
- Perform the initial streaking in one sector of the plate. For the second streaking, rotate the Petri dish 90° and start streaking at the end of the previous streak. Spread the sample in the next sector using a back-and-forth movement. Repeat this process a third time for the final dilution. The start of each dilution is indicated with a red dot in the figure
- **Sampling of organ (kidney, spleen and skeletal muscle):**
 - Puncture the surface of the organ with a glowing wire loop or scalpel. Allow the wire loop to cool before gently pushing it further into the organ to collect the sample material, then transfer it to the agar plate
- **Sampling of skin ulcers:**
 - Open wounds should first be sampled from the exposed area near the wound margin without flaming the sterile wire loop before plating the sample. Then, take a new sample using a flamed wire loop, allow it to cool briefly on the wound margin, 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:**
 - Sterilize the anal opening with 70% alcohol. Insert the loop into the anal opening and further into the intestine to collect the sample before spreading it on the agar plate, 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: Shows the bacterial growth with dilution technique.
- C: 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 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 petri dishes late on a Thursday or a Friday, the analysis will start on the following Monday. This will lead to some longer delivery time and can affect the quality of the samples. We encourage therefore to send the material so that we receive it no later than Thursday morning
- The lids on the petri dishes must be secured with parafilm 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 petri dishes to move in the box. This shall prevent that the petri dishes are broken or that the lids open and the samples can be contaminated. 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 petri dishes must avoid direct contact with the ice packs
- The package is marked "BIOLOGICAL SUBSTANCE CATEGORY B" and with PG-number

Send the samples in Norway:

Fürst Medisinsk Laboratorium AS
Søren Bulls vei 25
1051 OSLO

Send the samples in UK:

PatoGen Ltd. (UK)
Suite 9, Malin House
European Marine Science Park
Dunbeg, Oban PA37 1SZ

SCOPE OF ANALYSIS ASSIGNMENT AND THE LABORATORY'S DELIVERY TIMES

- The delivery time for bacteriology will depend on the growth of the bacteria
- Larger orders must be agreed upon with PatoGen before sending

A filmed version of the sampling guide can be found here: <http://patogen.no/how-to-carry-out-sampling-from-the-kidney-for-bacteriology/>