

# SAMPLING GUIDE: BACTERIOLOGY

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. PatoGen recommend to register all samples 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 the fish number, cage number and organ. Record all information of the sampling in PatoLink or in PatoGens requisition form
- To allow PatoGen to link answers on each particular fish, the fish number and cage number must be harmonized on all material submitted to PCR, histology and bacteriology
- The unused agar plates should be stored in a refrigerator until use
- Always use a sterile wire loop, a sterile disposable plastic loop 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
  - Freshwater fish reared on water with salinity: blood agar
  - Saltwater fish, cleaner fish and broodstock reared on seawater: 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, please do always register primary or secondary spread in PatoLink or in the requisition form

## 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

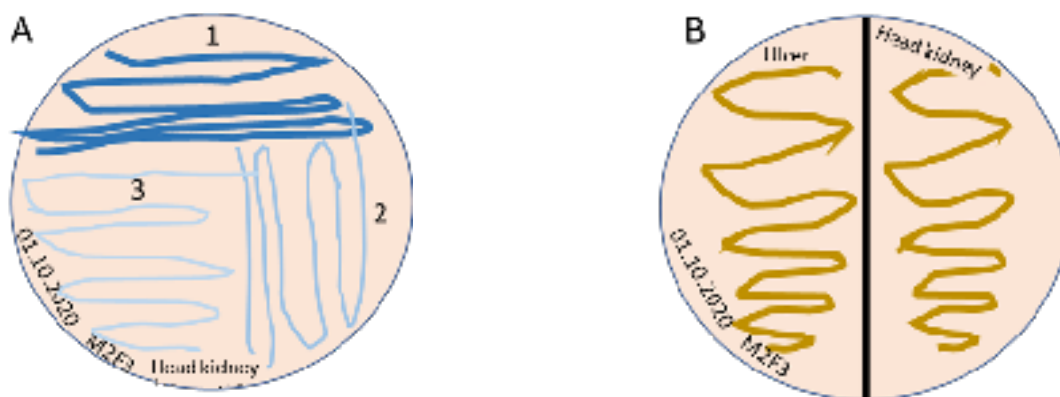
- Remove condens on the plate lid by gently tapping it on clean paper towels
- Label around the edge of the bottom (not the lid) of the agar plate with a waterproof marker with: date of sampling, pen number, fish number and organ. If two samples are collected on the same plate, the plate has to be divided in two equal halves of the back of the plate, both sides need to be marked clearly
- The use of aseptic technique when collecting bacteriological samples is important to avoid contamination with environmental or intestinal bacteria
- Push the loop (wire or plastic) into/on the organ to be sampled or on the periphery of the pathological finding (wound, abscess etc.)
- Place the loop 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 plate 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 or a scalpel. 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 deep er 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 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
- 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 the samples in Norway:

**Først 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 shall be agreed with PatoGen prior to sending