



# **ICTHIOCHECK**

## **SAMPLING PROTOCOL**

### **PRELIMINARY CONSIDERATIONS**

Vaccines are an extraordinary tool for preventing diseases. As they are very specific, they require a good diagnosis of the condition in order to confront the agent causing the problem. At HIPRA, it is essential that we know which pathogen(s) is(are) present so that we can recommend the most suitable vaccines or prepare a specific autovaccine.

This kit is designed to obtain and send good microbiological samples in the best conditions possible. With them the DIAGNOS department will be able to recover and isolate the pathogen.

The success of the sample taking, shipping and isolating process depends greatly on its planning. An incorrect selection, haste or mistakes made during sampling and shipping can lead to erroneous or failed diagnostics, with all the efforts made being in vein. That is why this process is a critical point for making correct decisions on the prophylactic measures that will have to be taken.

# **WHAT SHOULD BE TAKEN INTO ACCOUNT BEFORE USING THE ICTHIOCHECK KIT?**

As mentioned previously, **planning is key** to obtaining good samples.

Therefore, the first thing is to make sure that the kit includes all the content in appropriate conditions to complete the process.

**A fire source is required in order to work in the most aseptic environment possible;** a Bunsen burner or blowtorch is the best option.

**The cold accumulator must be put in the freezer beforehand.**

**Check the expiry date of the culture media and/or swabs.**

## The box includes:

- Inoculating loops
- Scalpel blade
- Culture media and/or swabs
- Gloves
- Mask
- Cold accumulator
- Sample submission form
- Zip-seal bags
- Laboratory filter paper
- Parafilm® M



## **SELECTING THE MOST APPROPRIATE SAMPLE**

In order to have a greater chance of isolating the bacteria responsible for the problem, first we must **sample a batch of fish that is at the start of the disease process, i.e. in the first 2-3 days in which symptoms start to manifest**, thus ensuring that the process is recent and active.

## **WHAT NOT TO SELECT**



**Do not select batches that are 4-5 days into the process,** because it is likely that they are affected by secondary infections.



**Do not select batches of medicated fish either;** the use of antibiotics seriously inhibits bacteria growth and renders the isolating efforts pointless.

Selecting the batch is the time for choosing the most appropriate fish for sampling: You must always collect fish that clearly present symptoms that are most characteristic of the pathological process. They are usually found swimming with difficulty or dying.

## **WHAT NOT TO COLLECT**



**Fish that are already dead:** They can be found in an advanced stage of decomposition



**Fish with different symptoms to the rest of the group.**



**Fish that present many lesions on the skin and fins, as they probably have secondary infections.**

## **COLLECTING AND STORING THE FISH UNTIL THE KIT IS USED**

If possible, these fish will be kept alive until they are slaughtered and the samples are taken, since carcasses spoil quickly. It is best to combine these two actions:

Slaughter and take samples immediately after, thereby reducing the processing time. Three or four fish in one batch is sufficient.

If the fish cannot be kept alive, once slaughtered they must be stored in a plastic bag which is surrounded by ice or in a container at 4°C (never frozen) until the sampling takes place. The time between the slaughtering and cold storage must be minimal, particularly in juveniles and small fish.



Storage of fish in cold conditions for up to 24 hours.



More than 24 hours in cold conditions or frozen.



# **OBTAINING A GOOD SAMPLE WITH THE ICTHIOCHECK KIT**

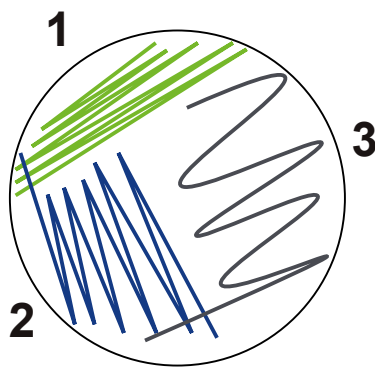
- 1** Clean and disinfect the work area (with alcohol or bleach).
- 2** Light the Bunsen burner or blowtorch.
- 3** Arrange the content of the ICTHIOCHECK kit. Use the gloves and mask provided.
- 4** Place the fish to be sampled on the work surface, on top of the absorbent paper and carry out an external examination.
- 5** If the fish presents external lesions, such as ulcers, lumps, areas of necrosis on the skin or fins, proceed to the sampling.



Using the sterile scalpel, cut into the area of the lesion and insert the swab into the incision, rotate 360°, close and label adequately (organ, animal identification and inoculation date). If using an inoculating loop, proceed using **the streak plate technique**, following the indicated pattern changing the loop for each groove made. Adequately label the plate (organ, animal identification and

inoculation date) and leave to rest with the lid turned down avoiding contamination due to condensation.

When finished, seal the plates with Parafilm® M



*Streak plate technique  
in three grooves*

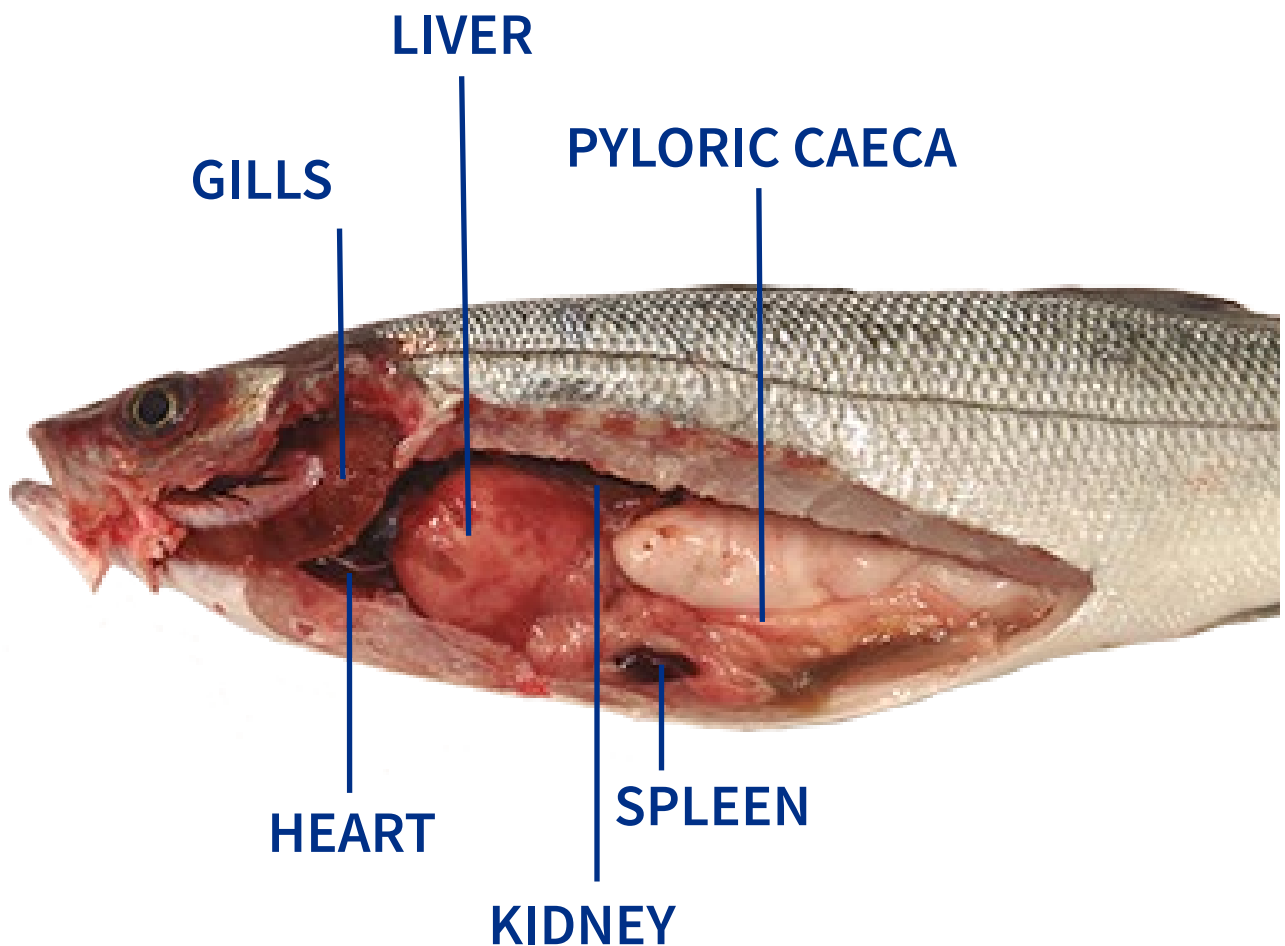
**6** Once the process has been completed, or if there are no external lesions present, clean/disinfect the abdomen of the fish.

Using the sterile scalpel (new, or passed through the flame) make a cut as indicated in the picture until the abdominal organs are exposed.

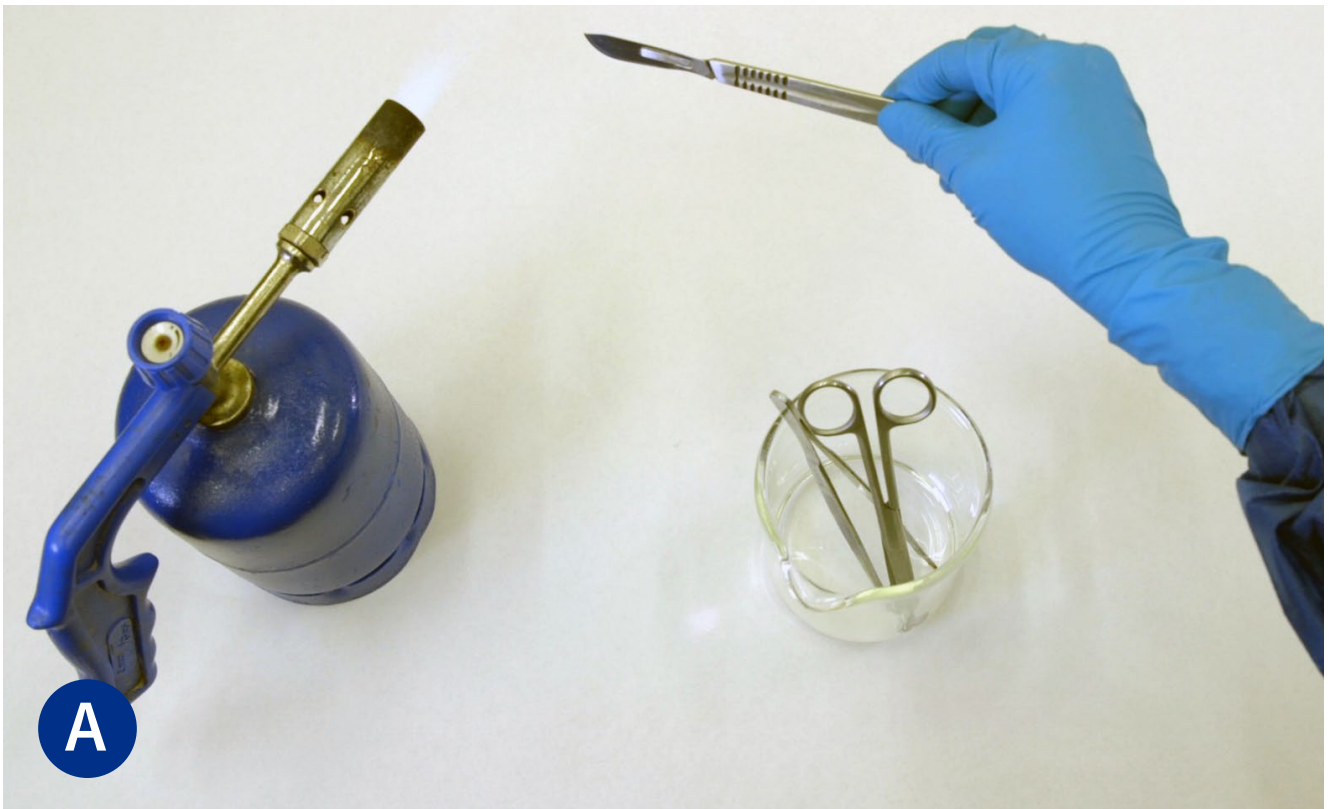


It is important to be careful to avoid external contaminations, making a clean cut avoiding rupturing the intestine. Once used, the scalpel must be disinfected, or use a new one instead, adequately disposing of the previous one.

**7** Locate the liver, spleen and kidney and examine their appearance; if a clear abnormality is detected, select the most appropriate organ for sampling. If not, first select the kidney, followed by the spleen and liver.



8 Disinfect the material used for each sample using the flame (A) inserting the inoculating loop in the selected organ (B) and proceeding to the **streaking technique** (C). Label appropriately. If using swabs, insert the tip into the organ, rotate 360°, close and label appropriately.





**9** Adequately dispose of the slaughtered fish (in accordance with standards).

## **WHAT DO I DO ONCE THE SAMPLE HAS BEEN TAKEN?**

The samples must be individually deposited in the zip-seal bags and all together in the box with the cold accumulator (previously frozen) without being in direct contact with the plates/swabs\*.

Fill out the sample submission form for DIAGNOS which is included and contact HIPRA. We will provide and manage the import permit necessary for sending the samples.

When sending the shipment, make sure that there is nothing moving inside, seal the box properly and label the outside with the labels provided.



The box containing the samples must be stored in the fridge and the accumulator in the freezer until the kit is sealed and shipped.

You will be notified of the results when available.



\* Triple packaging system