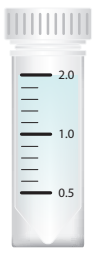
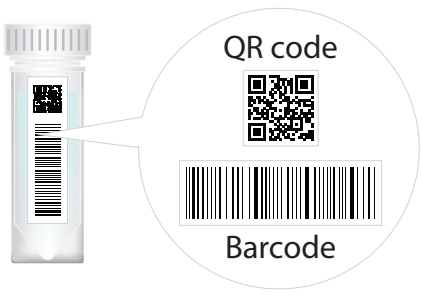
Quick fish sampling guide for disease diagnostics

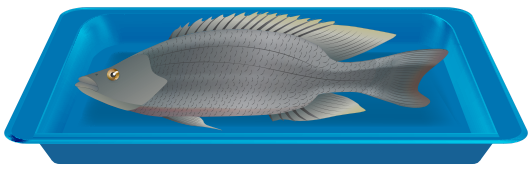
Microbiome sampling guide

FISH MICROBIOME



2 ml





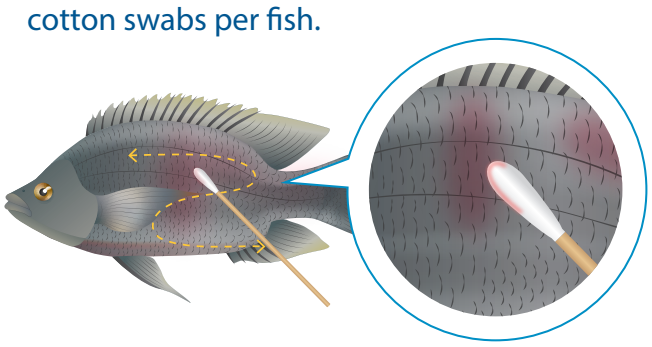
- Prefill 2 ml tubes with molecular grade 95%–100% ethanol (EtOH).
- Pre-label tubes using a solvent resistant marker pen, or stick a preprinted barcode/QR code label with information on the following:
  - date of sampling
  - fish/specimen number
  - specimen type (skin/gill/water)
  - date of sampling.

For handwritten labels, use abbreviated code (e.g. 210112_F1_S): 21 for 2021; 01–12 for the month (e.g. 01 for January); 01–31 for the day (e.g. 12 for January 12) F1 for fish 1; S for skin specimen.

- Place freshly killed fish (blow on the head) on a clean surface.

Microbiome sampling from skin and lesion/ulcer

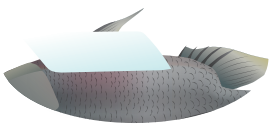
1 Swab the skin with 2x sterile polyester or cotton swabs per fish.



Make three long body swipes while twisting swab to maximize mucus collection along the body.

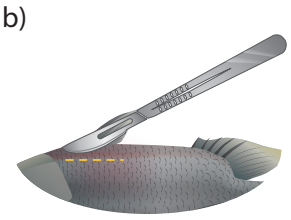
If there is more than one lesion/ulcer per fish, only swab one per fish.

a)



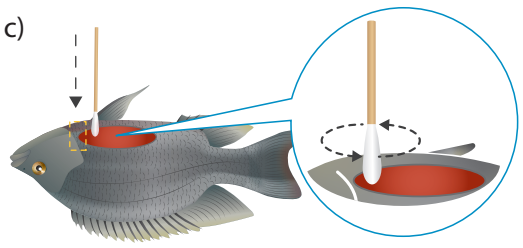
Clean lesion/ulcer superficially with a clean wipe.

b)



Make a sharp incision.

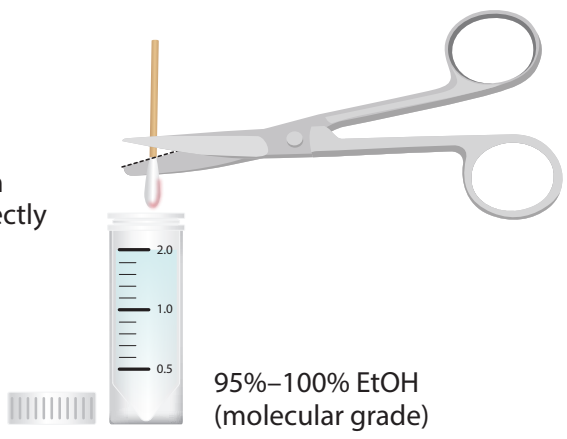
c)



Insert and rotate swab to coat with material.

2

Cut polyester or cotton swab tip at margin directly into the 2 ml tube.



95%–100% EtOH (molecular grade)

3

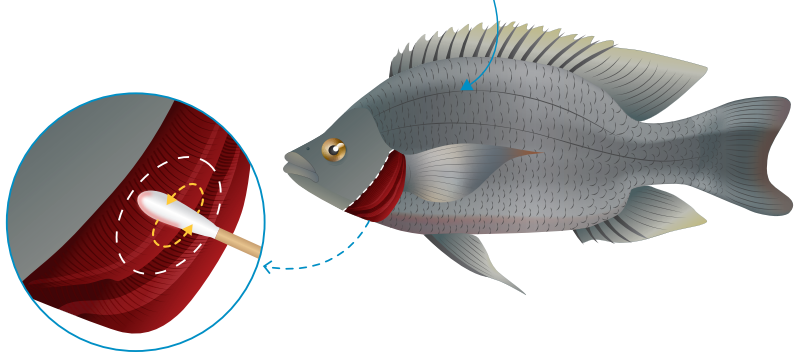
Close tube tightly

Two tips/skin/fish per tube

Microbiome sampling from gills

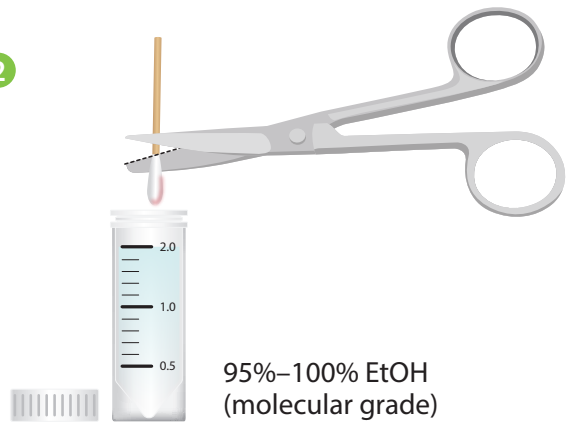
To remove operculum

1



Remove the fish operculum to expose the gills.
Rub and twist a sterile cotton or polyester swab to collect mucus from between the gill racks and filaments (at least 3 gill racks per swab for each fish). Repeat with one more swab.

2



95%–100% EtOH (molecular grade)

Snip off swab tips into pre-filled tubes with 95%–100% molecular grade ethanol.

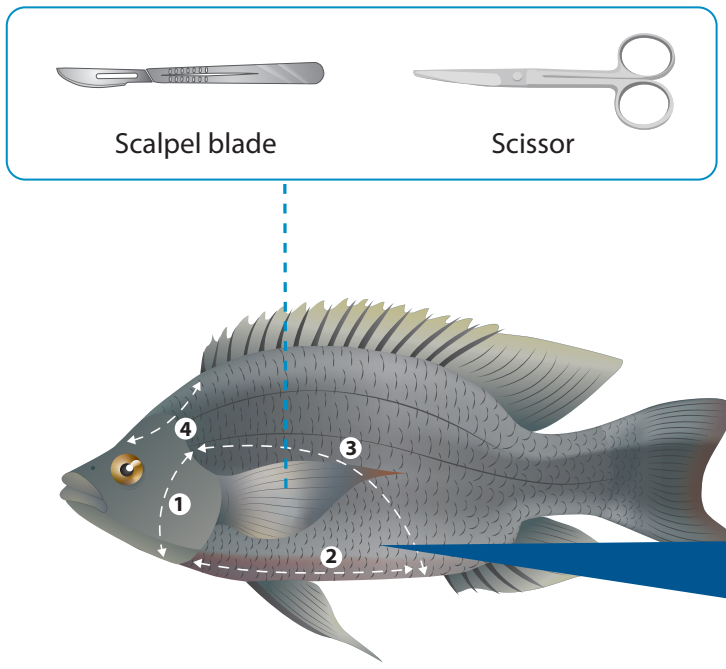
3

Close tube tightly

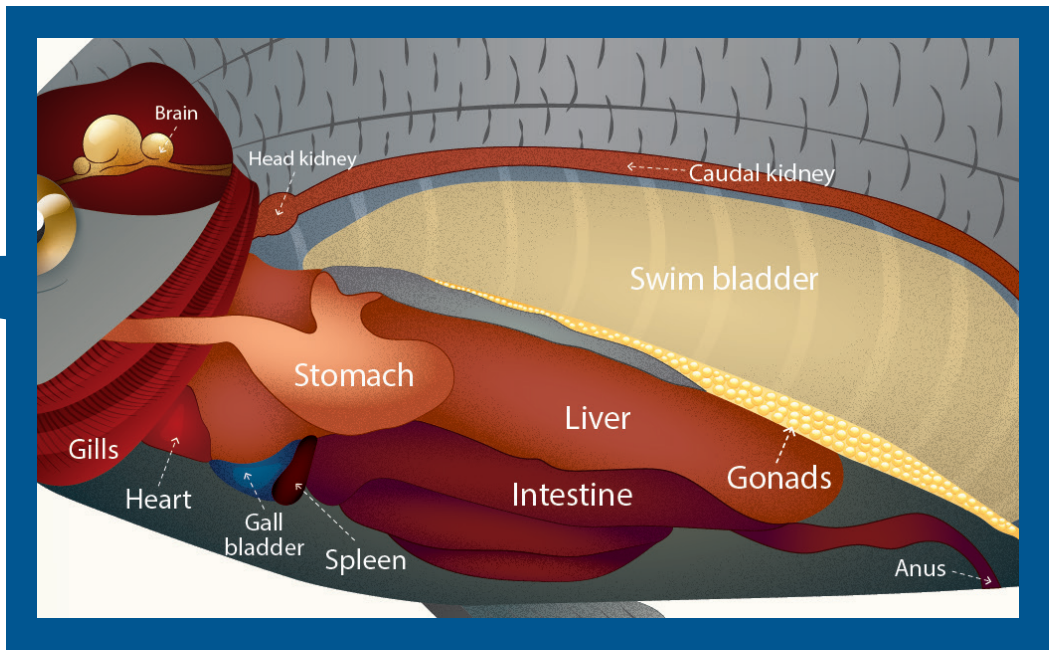
Two tips/gills/fish per tube

Microbiome sampling from internal organs

1 Dissect as per arrows to expose internal organs

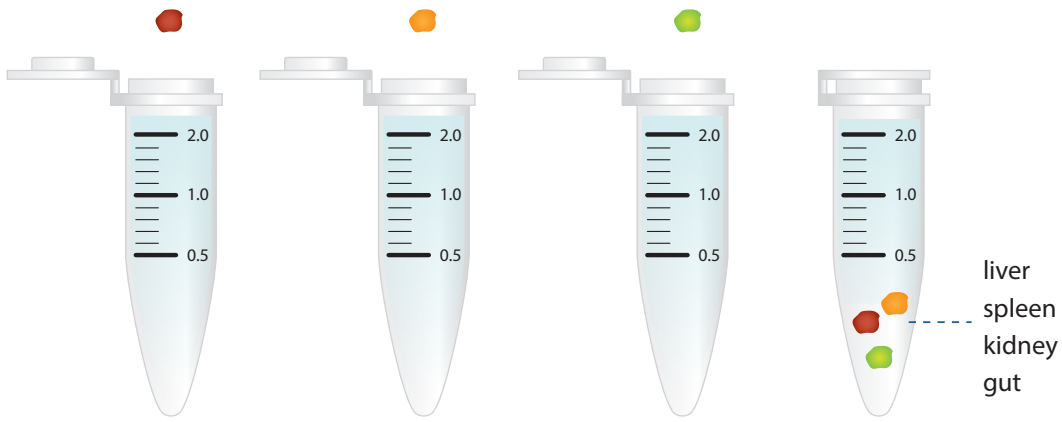


- Dissect the fish to expose internal organs.
- Collect specimen (5x5 mm) of each target organ (spleen, liver, kidney, gut).



- If specimens for spleen, liver, gut or other internal organs have already been collected under molecular diagnostic protocol, then the same sample can be used for microbiome analysis. If not already collected then collect, here (only if required by the specific study protocol).

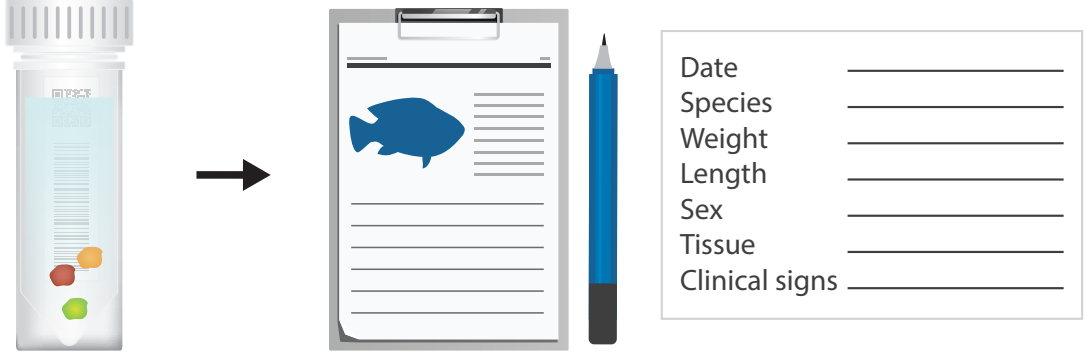
2 Place specimens (individual or pooled) into pre-filled tubes of 95%–100% molecular grade ethanol and seal.



Note

This will depend on each study: individual organ or combined organs can be sampled per tube. Combined organs are used for community level microbiome diversity analysis or broad pathogen screening.

3



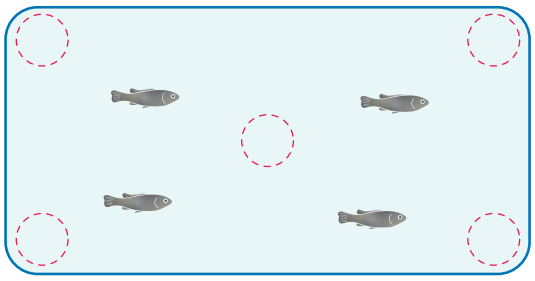
Record all relevant fish/specimen details on the fish health examination sample record form.

4

Transport tube(s) at ambient temperature back to the lab.

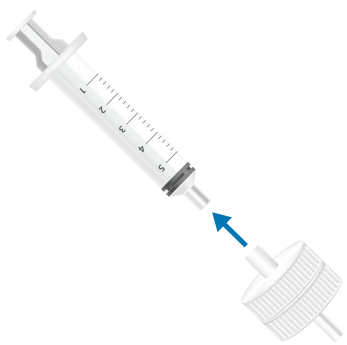
WATER MICROBIOME

1 Collect water samples from evenly distributed locations.



Ideally, sample 5 locations in the pond/cage/lake (1–3 samples per location; 5–15 total).

2



Attach 50 ml syringe (screw) to filter holder.

3

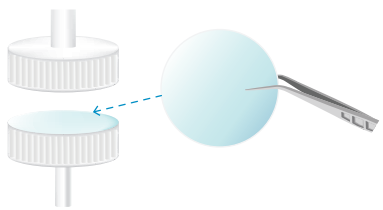
Fill the syringe (attached to the empty filter holder) with the water sample.

4

*Note: Repeat steps 1–8 for every new sample from a different location.

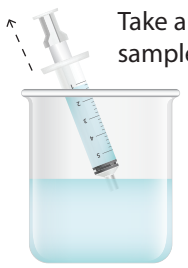
Push the plunger to rinse the empty filter holder with the water sample. Repeat the rinsing (steps 3 and 4) twice.

5



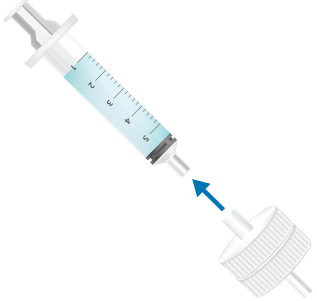
Detach and open the filter holder. Place a 47 mm polycarbonate filter inside and then close it tightly.

6



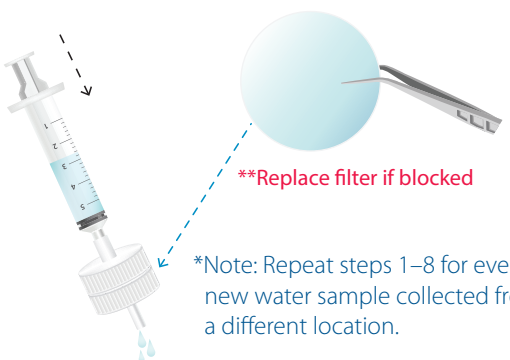
Fill the rinsed syringe with the water sample before reattaching the filter holder.

7



Reattach the 50 ml syringe (screw) to the filter holder unit (containing the filter).

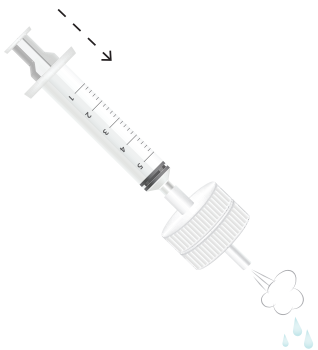
8



*Note: Repeat steps 1–8 for every new water sample collected from a different location.

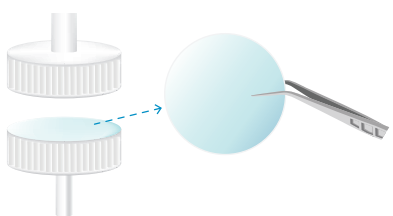
Push the sample water through the filter. Refill and repeat until 200 ml sample water has been filtered.

9



After the 200 ml water is filtered, push air through the filter unit to remove excess sample water.

10



Open the filter holder and remove the filter with the tweezers.

11

Using forceps/tweezers, fold the filter into 2 and 4.

12

Place folded filter into tube prefilled with 95%–100% ethanol.

13

Close/seal tubes, place in a transportion box and send to the lab.

In partnership with

www.worldfishcenter.org

This work was undertaken as part of the CGIAR Research Program on Fish Agri-Food Systems (FISH) led by WorldFish, the CGIAR Inspire Challenge project on *Rapid Genomic Detection of Aquaculture Pathogens*, the Norwegian Agency for Development Cooperation (Norad) project *Increased Sustainability in the Aquaculture Sector in sub-Saharan Africa, through Improved Aquatic Animal Health Management*, and the Feed the Future (FtF) Innovation Lab for Fish (FIL) project *Improving Biosecurity: A Science-Based Approach to Manage Fish Disease Risks and Increase the Socioeconomic Contribution of the Nigerian Catfish and Tilapia Industries.*

The programs are supported by contributors to the CGIAR Trust Fund, Norad, and the FtF FIL through the United States Agency for International Development (USAID).