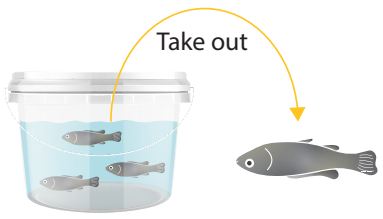
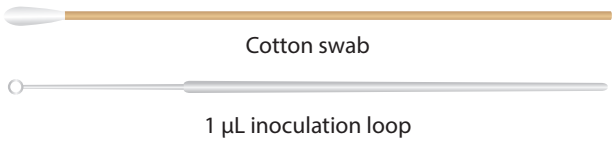
Quick fish sampling guide for disease diagnostics

Bacteriology sampling guide

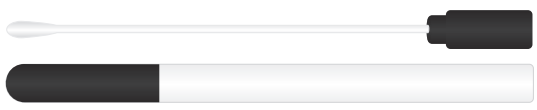
1 Euthanize fish according to standard operating procedure.



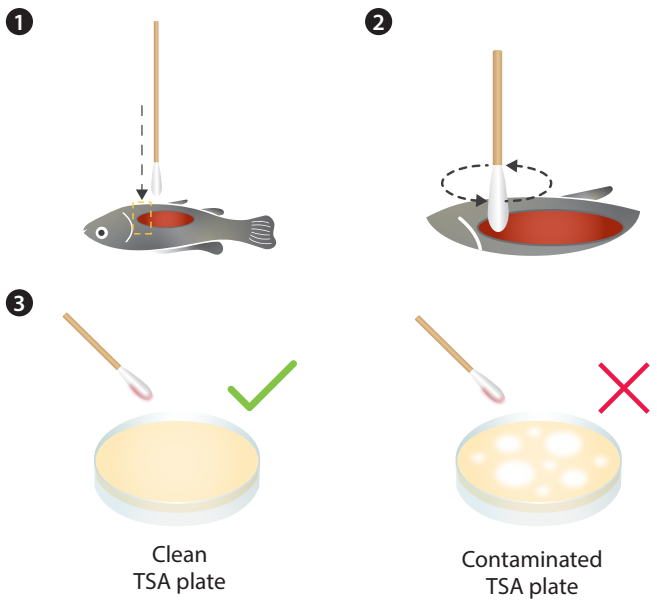
3a Sampling from ulcer:
1. Clean surface of the ulcer with an ethanol wipe.
2. Select one ulcer per fish. Expose edge of ulcer by removing scales or sharp incision.
3. Insert sterile cotton swab or 1 µL inoculation loop directly into and behind the ulcer.
4. Inoculate biological materials from cotton swab onto selective or non-selective agar plate (e.g. Tryptic soy agar TSA).



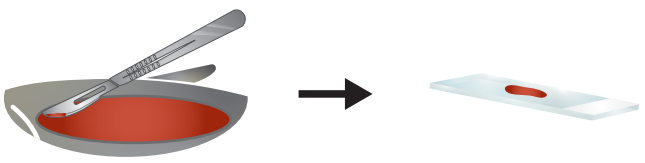
Alternatively, sampling can be done using bacteriology transportation swab(s) (Transwabs) using the same technique as described in 3a. Transwabs need to be transported chilled and plated onto non-selective or selective media within 48 hours.



Charcoal absorbs toxins and is better for fastidious organisms.



Prepare smear slide from target tissue or lesion/ulcer.

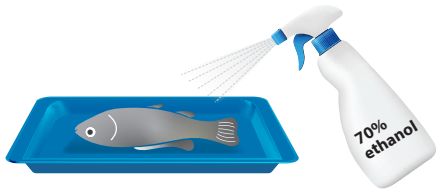


Ulcer being scraped with scalpel blade.

Scraped material being placed on slide then air dry 3–5 min.

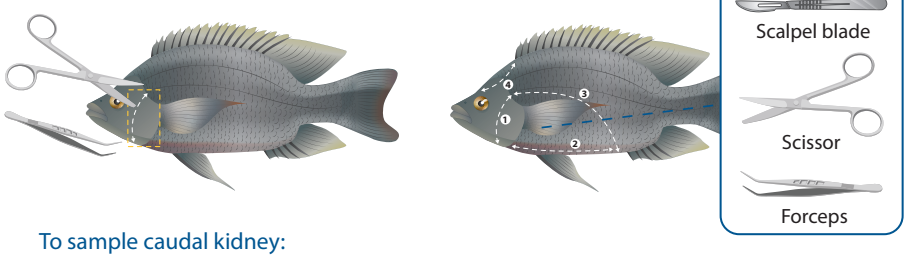
Pack and transport without cool pads.

2 Place fish on a clean surface and spray with 70% ethanol. Leave to dry.



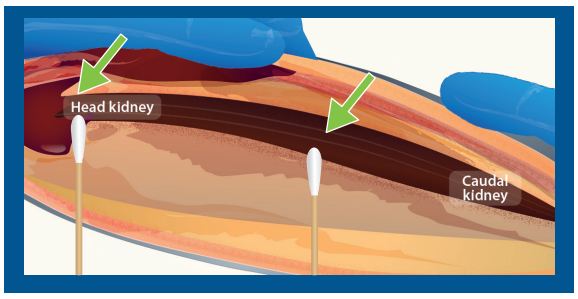
3b Sampling from kidney (head or caudal):
1. Cut away the operculum.
2. Make a ventral incision from anus toward the gills.
3. Finish the triangle opening to expose the abdominal cavity.

Caution: Avoid perforating the gut or any other organs.

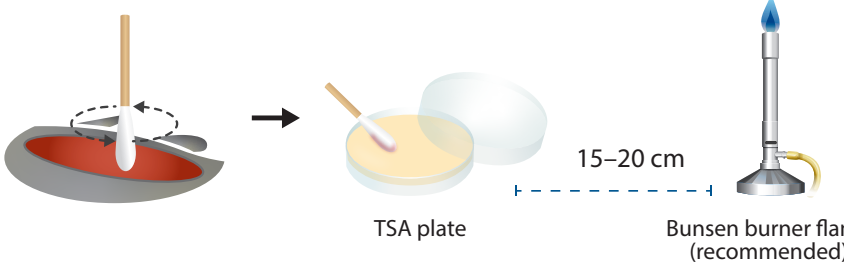


To sample caudal kidney:
- Pull out viscera with flat side of a clean scalpel.
- Puncture membrane to expose the caudal kidney using swab tip.

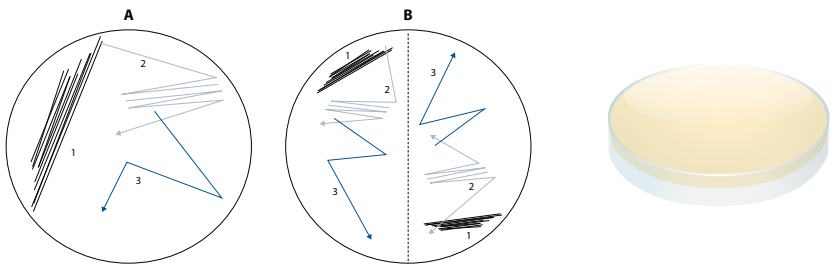
To sample head kidney:
- Remove gills or other organs using forceps, scissors or scalpel.
- Puncture membrane to expose head kidney using swab tip.



Rotate swab tip to coat with inoculation material.



Inoculate with agar side facing down near the flame (15–20 cm). If using plates in the field, inoculate the plate in a side-to-side motion, zig-zagging down the plate. If using transwabs, when back at the lab streak plates following patterns below. On suspicion of a bacterial disease, use one plate per fish (A) or, during routine screening, one plate split in two for two fish (B). In the lab, to maximize dilution, use a new sterile loop after stroke one and two.



Seal agar plates with parafilm. Place upside down below 30^{0}C (as per revised protocol); return to the lab and incubate for 12–72 hours to check for bacterial colony forming units growth.

In the presence of clinical signs or depending on the targeted bacterial disease, sampling for bacteriology can be done from other organs (e.g. brain, eye, liver, heart).

4 Onsite or back in the lab, perform Gram stain from bacterial culture growing on agar or fish tissue imprints (kidney, brain etc.). For fish <5 cm, perform Gram staining from histological material.

Tissue imprints

Tissue imprints can also be Gram-stained, e.g. kidney, but not ulcer smears (as almost certainly mixed bacterial species are present).

Examine under microscope using 100x oil immersion objective to determine morphology and color of Gram-positive (purple) and/or Gram-negative (pink) bacteria.

*With immersion oil for gram stain observation (bacteriology)

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