MT Aqua Biotechnology and Diagnostics

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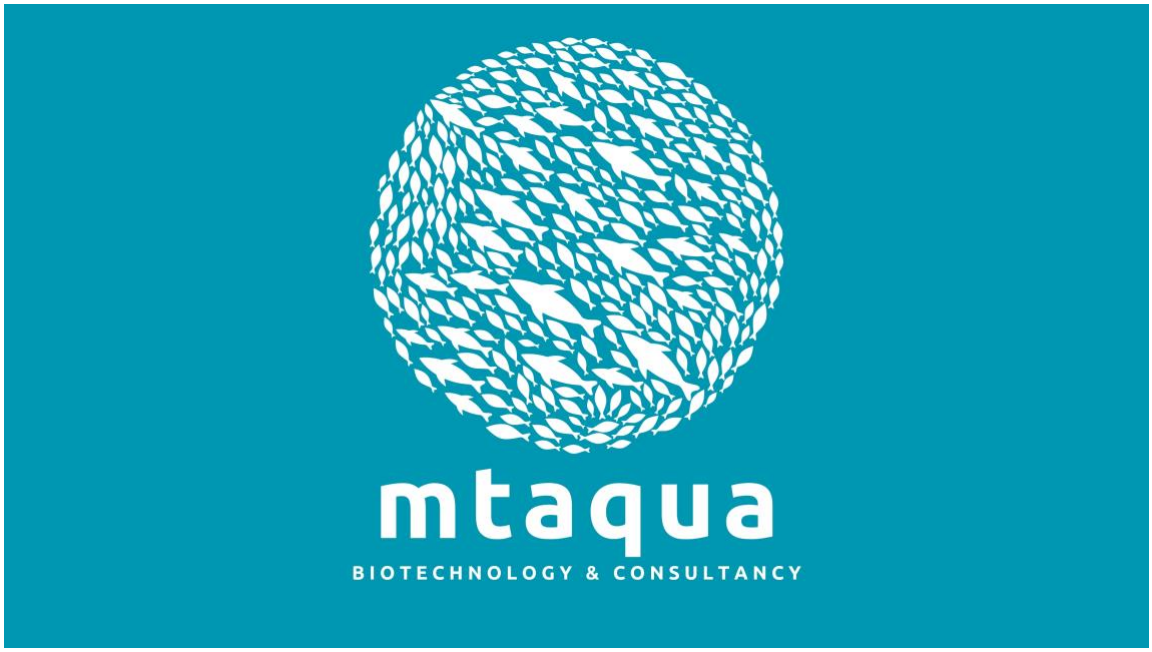

SAMPLING PROCEDURE MANUAL

WRITTEN BY: DR. METİN İLEMİN

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SAMPLING PROCEDURE MANUAL

1. INTRODUCTION AND CLINICAL HISTORY

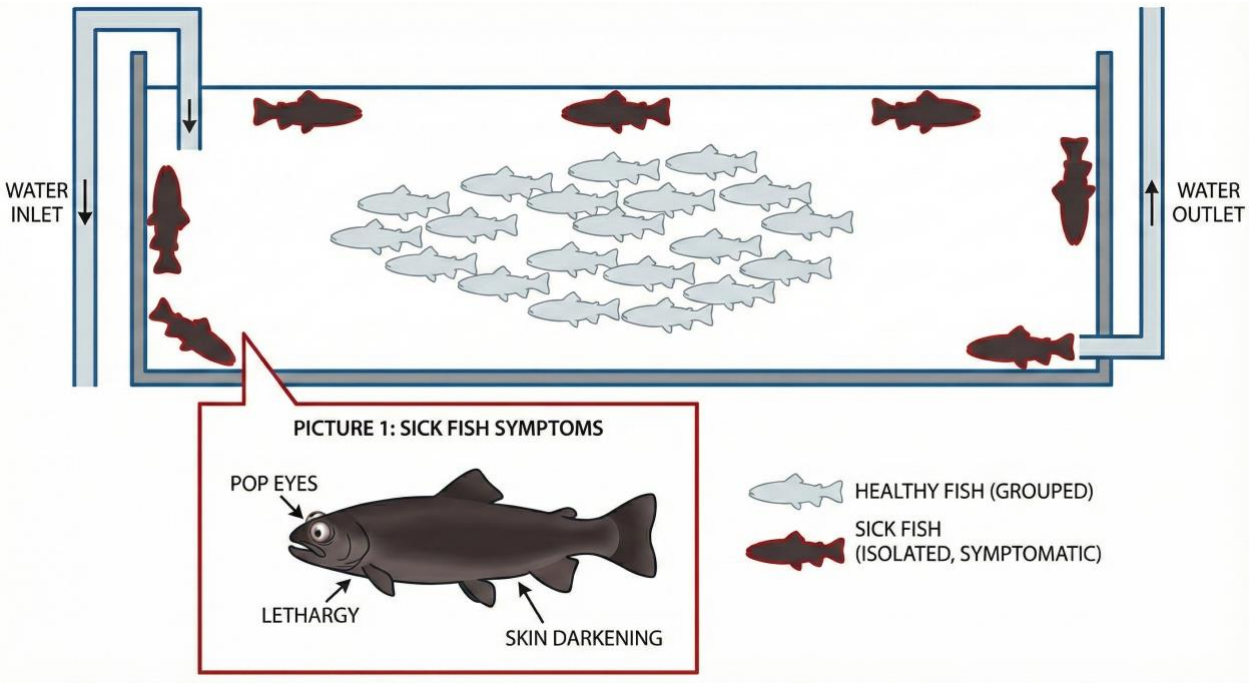
Diagnosing a fish disease requires more than just observing clinical signs. **Many diseases share common symptoms** (e.g.,darkening, exophthalmia/pop-eyes, ascites, hemorrhage, splenomegaly, changes in organ color/shape). A complete diagnosis relies on combination of physical examinations, sampling, and environmental data.

Key Clues for Diagnosis: Observation of fish behavior, reflexes, lesion description, necropsy, **bacteriology**, and **parasitological examination**.

Fundamental Context: Record information on:

- **Transportation** and **handling** history.
- **Previous treatments** (medicated fish should be avoided if possible).
- Possible **stressful events** (heavy rain, predators, power outages, etc.).
- Current **water parameters**.

2. SAMPLE SELECTION: CHOOSING THE RIGHT FISH



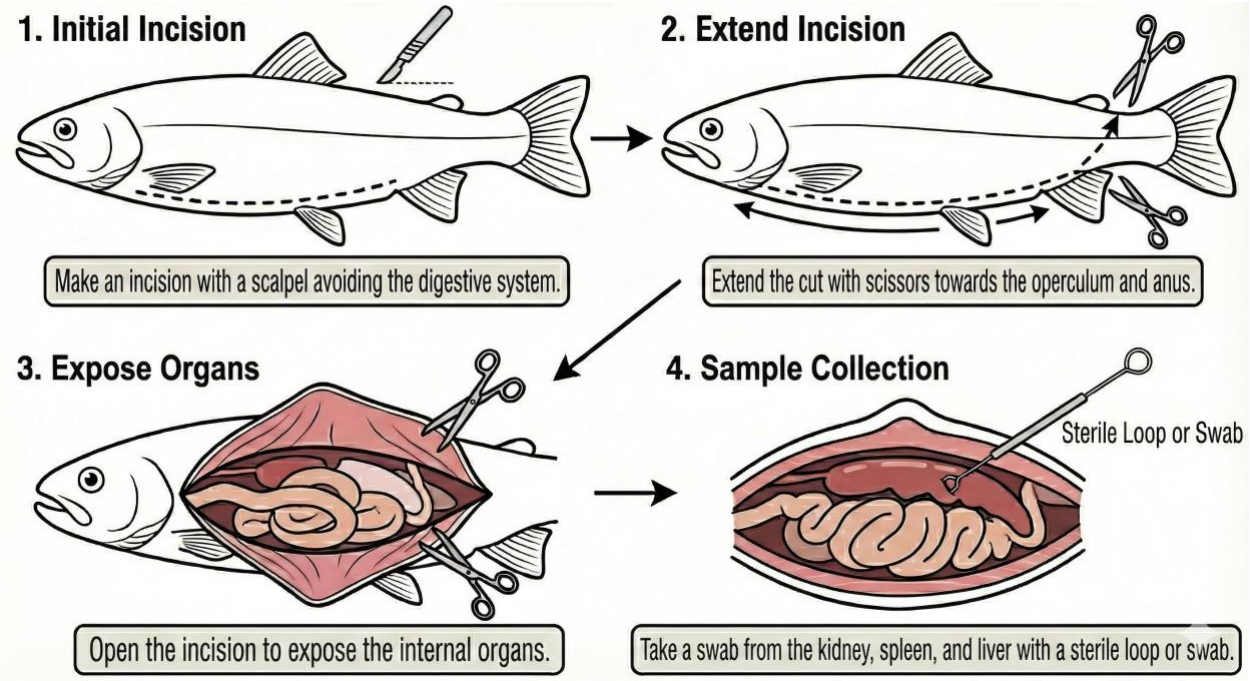
The quality of the sample is must for an accurate diagnosis.

Select: Sick fish showing any disease symptom or **moribund fish** (those near death).

- Sick fish are often easy to spot: they may be lethargic, separated from the group, or found near the sides, surface, or inlet/outlet pipes.
- **Avoid: Never sample dead fish**. Tissue condition, chemistry, and ectoparasites rapidly deteriorate or detach post-mortem, leading to a false picture of the disease state.

3. BACTERIOLOGY SAMPLING

NOTE: Aseptic procedures must be used for all bacteriology samples to prevent contamination. Samples for bacteriology must be taken **before** any other samples (e.g., PCR, histopathology) to avoid cross-contamination from one organ to another.



Procedure

1. **Preparation:** Place the selected fish on its **right flank**.
2. **Incision:** Using a **sterile scalpel** (new or cleaned with alcohol/bleach), make an incision in the **abdominal area**. **Carefully avoid perforating the digestive system.**
3. **Extending the Cut:** Use a pair of **sterile scissors** to extend the incision:
   - Forward to the **operculum**.
   - Backward to the **anus**.
4. **Exposing Organs:** Using the sterile scissors, **carefully cut away the musculature** overlaying the internal organs to expose the intestine and the underlying organs (kidney and spleen).
5. **Swabbing the Organs:** Using a **sterile bacterial swab**, gently push in and insert the tip into the **kidney**.
6. **Repeat:** Perform the **same swabbing procedure** for the **spleen**.
7. **Packaging: Close the tap/cap of the sterile swab** securely and prepare the sample for **immediate shipment** to the diagnostic laboratory following their instructions.

4. FTA CARD SAMPLING

FTA cards capture and preserve DNA/RNA directly in the field by binding cells onto a chemically treated fiber matrix. The card instantly lyses and inactivates pathogens while stabilizing nucleic acids at room temperature, eliminating the need for cold-chain transport. This makes sampling easy, safe, and reliable even for non-expert farm staff. Once in the lab, a small piece from the card is enough for high-quality PCR analysis, providing a fast, practical, and contamination-resistant solution for aquaculture diagnostics.

5. FTA CARD USAGE AND CAPACITY

FTA cards are specialized paper that **absorbs and fixes genetic material** (nucleic acids) to the cellulose upon contact with tissue. The nucleic acids are immobilized and preserved, allowing for sample transport at **room temperature**.

• Each FTA card contains **4 circles or wells**.
• Up to **3 samples** can be pooled in each well.
• Total capacity: **12 samples** per card (4 wells 3 pooled samples).
Pooling allows multiple fish to be treated as a single sample:
• **Small Fish (Fry or Fingerlings):** 5 to 10 fish may be pooled together
• **Larger Fish:** Tissues from a maximum of **5 fish** may be pooled.

Prior to shipment, **always complete the sample submission form** and notify the recipient laboratory of the impending submission, following their advice regarding labeling and shipping dates.

6. SAMPLING METHOD

Use the same procedure described above for bacteriology. Collect the sample using a foam applicator or a sterile swab, then apply the material from the internal organs onto the FTA card. Alternatively, you may directly excise a small piece of the organ and gently imprint it onto the FTA Card.

7. SHIPMENT

FTA cards make sample transport and shipment significantly easier and simpler due to several key features:

• **No Biohazard Classification:** The cards actively **inactivate the pathogen**, ensuring the sample is **not categorized as a Biological Sample Type B**. This crucial feature eliminates the need for extensive paperwork, such as import licenses.
• **Temperature Stability:** Samples can be transported at **ambient (room) temperature**.
• **Extended Shelf Life:** The shipment window is long, allowing transport for **up to 4 weeks**.
• **Simple Mailing:** You can use **normal mail** for shipment by simply packing the cards securely in an envelope.