



Sampling and Processing of Aquatic Organisms for AMR Surveillance

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Objectives

To be able to understand

- ❖ Pre-collection preparations for AMR surveillance
- ❖ Required background information for AMR surveillance
- ❖ Sampling and transportation of aquatic organisms for AMR surveillance
- ❖ Fish and shrimp necropsy for AMR surveillance
- ❖ Organ collection from fish and shrimp and enrichment for AMR surveillance

Pre-collection preparation

Sample collection plan

- ❖ Species to be collected
- ❖ Sample collection location
- ❖ Specimen quantity required
- ❖ usually, large quantity is required

Sample collection plan is vital for ensuring that data is valid, reliable, and useful

Inform laboratory before sample arrival

- ❖ Species
- ❖ Numbers
- ❖ Size classes
- ❖ Transported samples e.g. on ice, live, whole or tissue sample etc.

The Laboratory can be better prepared with a clear sample processing plan

Background information

Include all relevant supporting information with submitted samples

Relevant information	Description
Geographical information	Location, GPS coordination, location type e.g., farm, wet-market etc.
Environmental condition	Description of surrounding environment, water quality changes, unusual weather events etc.
Species	Species of the specimen, age, age category, specimen number, code of species
Health status	Diseased, healthy, symptoms
Collection details	Name of person collected, collection date, time, seasons
Transport condition	Describe the transport condition
Specimen quality	Quality of the sample upon arrival
History	History of health problem, treatment etc.

Ensure samples are labeled with necessary details using waterproof marker

Aquatic organism collection from farm/wet market

Live sample collection from fish farm

Select appropriate sampling tools: You may need appropriate nets/traps to catch fish. Specific types of fish may need specific tools

Sterilize equipment: Ensure all equipment, including nets, containers, and other sampling tools, are properly sterilized before use

Fill in the data form: Fill the background information

Choose the right time: Collect fish during cooler parts of the day (morning or evening) when fish are less stressed

Quick transfer: Transfer the captured fish immediately into clean, sterilized containers, such as plastic bags or tubs, with enough water from the same source to keep the fish alive

- ❖ **Avoid contamination:** Always wear gloves and use sterile tools to prevent contamination
- ❖ **Eco-conscious sampling:** Collect only the number of fish needed for the study and avoid disrupting the pond's ecosystem
- ❖ **Ethical concerns:** Consider the welfare of the fish and follow ethical practices, including humane euthanasia

Fish Euthanasia

Physical techniques

Pithing, spinal cord dislocation, or decapitation generally are acceptable methods, provided the procedure is performed quickly and accurately

Immersion techniques

Fish can be euthanized by exposure to relatively high concentrations of anesthetics e.g. Buffered MS-222, Benzocaine, Clove oil

Two-step euthanasia

Two-step euthanasia may be required: first, anesthesia to induce loss of equilibrium, followed by a physical or chemical method to induce brain death

The procedure should be quick, painless, and stress-free, with an initial effect on the central nervous system to ensure immediate insensitivity to pain

Aquatic organism collection from farm/wet market

Fresh sample collection procedure from fish farms/wet market:

- ❖ Wash your both hands with soap before wearing the PPE
- ❖ Wear appropriate PPE before entering the market with necessary precaution
- ❖ Wash the gloved hands with 70% ethyl alcohol before and after each sampling
- ❖ Fill in the sample collection data form.
- ❖ Collect fresh fish and directly package in zipper/plastic bag.
- ❖ Label each bag with a unique Sample Identifier Code using a waterproof and cold resistant permanent marker.
- ❖ Keep the bags containing the samples in the sample transportation box (ice box)
- ❖ Fill the box with ice packs.
- ❖ Disinfect hands and instruments after each sample, change gloves, and then collect the next sample.
- ❖ Place all completed forms in a zipper bag, store in the sample box,

Necessary aseptic measures should be ensured during sample collection.

Aquatic organism collection from farm/wet market

Sample collection materials

- Disposable apron set or lab coat
- Face mask
- Rubber boots or waterproof shoes
- Hand sanitizer
- Paper towel (tissue paper)
- Ice box
- Clean plastic box
- Waste bin
- Scoop net and cast net/seine net
- Record keeping materials (notebook and pen)
- Test tubes for sample collection



Fish farm



Wet market



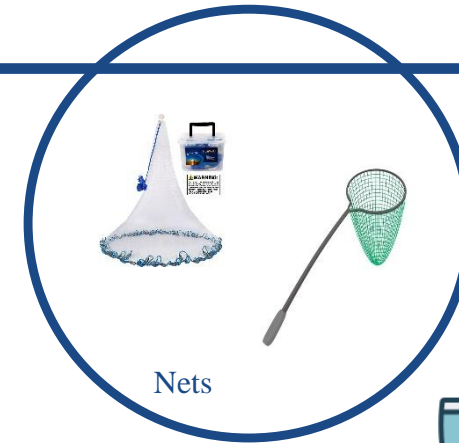
Hat

Mask

Gloves

Apron

Shoe



Nets



Paper towel



Rubber boots



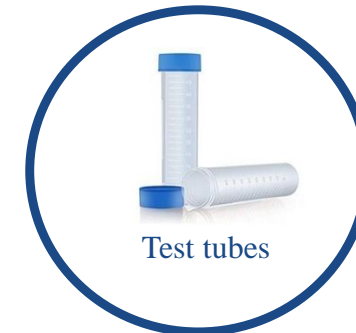
Clean plastic bag



Hand sanitizer



Ice box with ice packs



Test tubes



Waste bin

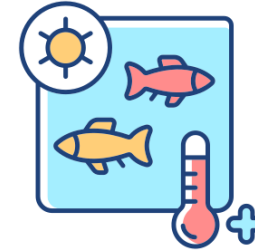


Sample identification

Aquatic organism collection from farm/wet market

Major cautions in sampling and processing

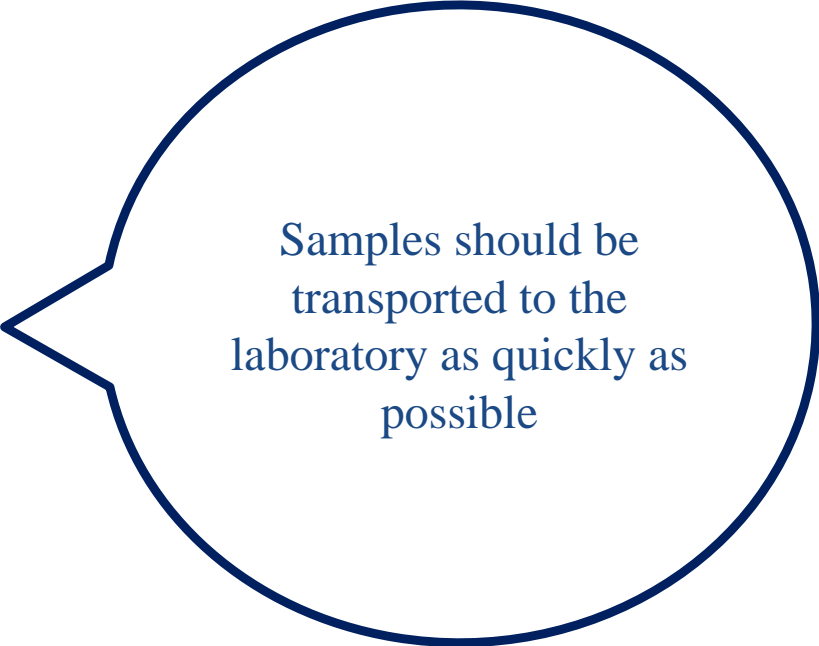
- Temperature control
- Cross contamination
- Hygiene
- Sample integrity
- Equipment and environment



Sample transportation

- ❖ Dead specimen transportation
- ❖ Live specimen transportation

- Transport time should ideally not exceed 12 hours
- Laboratory should analyze within 24 hours of sampling
- Samples should be kept at low temperatures but not freezing or on ice/ice packs



Samples should be transported to the laboratory as quickly as possible

Sample transportation

- ❖ Dead specimen transportation
- ❖ Live specimen transportation

Dead specimen transportation

- ❖ Small fish: transport whole on ice or frozen gel packs in individually sealed bags
- ❖ Large fish: aseptically remove the viscera, place in sterile individual containers, and ship on ice or with frozen gel packs

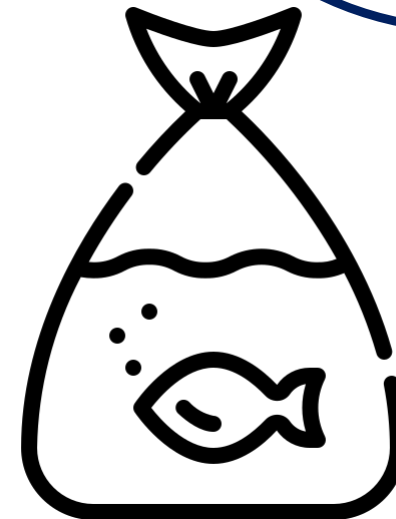
Intact or live specimens are preferred; dissected tissues quickly undergo autolysis, especially in tropical climates
bacteriology

Sample transportation

Live specimen transportation

- ❖ Collection should occur just before shipping to minimize mortality and transportation time
- ❖ Inform the laboratory about the estimated time of sample arrival
- ❖ Pack fish in double plastic bags with one-third water, two-thirds air/oxygen, and seal tightly (rubber band)
- ❖ Place inside styrofoam box or cardboard box lined with styrofoam
- ❖ A 60 x 180 cm plastic bag is suitable for a maximum of four 200–300 g fish.

Label containers with transportation instructions e.g. temperature range, "DO NOT FREEZE," and contact details



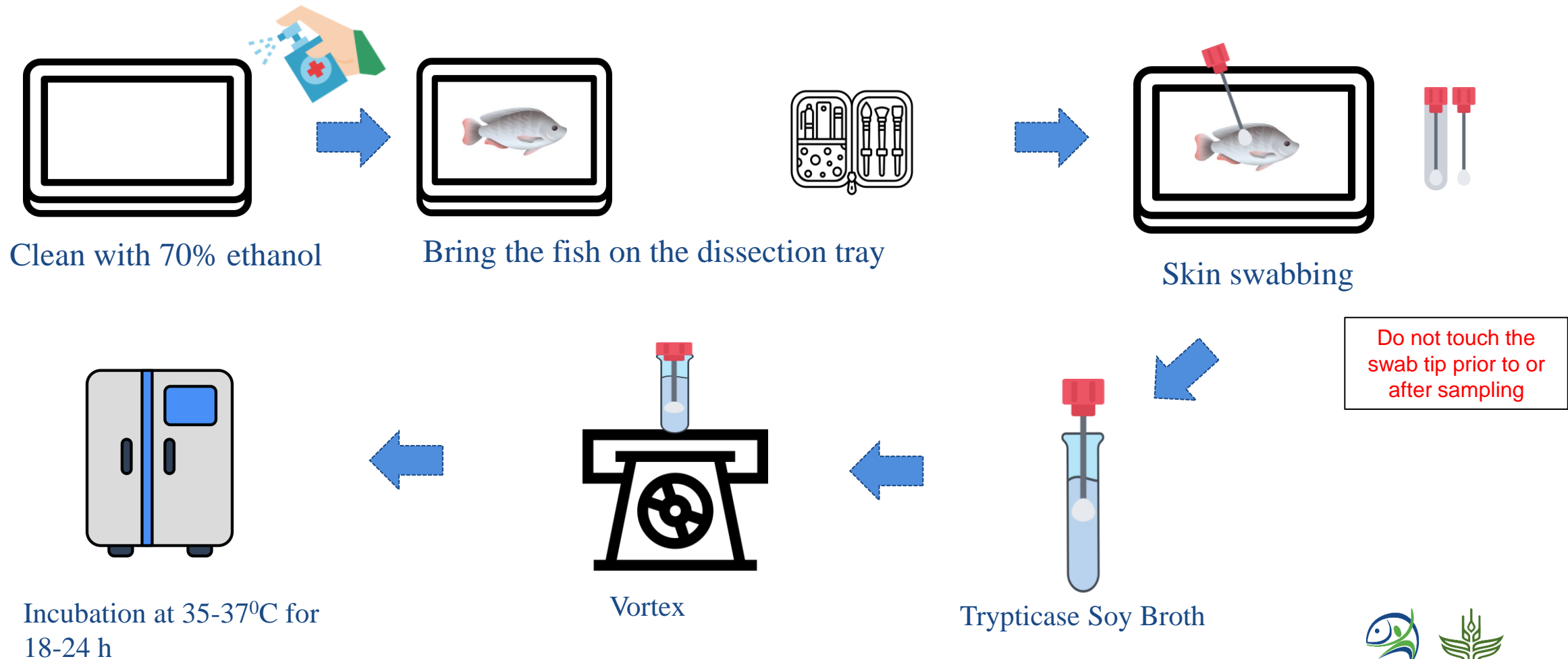
Sample rejection (after arriving the lab):

The following points should be considered for sample rejection:

- ❖ If the tube/container/plastic bag containing the sample is not properly labelled.
 - a. Type of aquatic animal sampled
 - b. *Kind of environment sampled*
 - c. Type of sample
 - d. Date of sample collection
 - e. Sample number
- ❖ If a sample bag has a leaks; or remains empty.
- ❖ If the same label is found on more than one tube/container/plastic bag.
- ❖ If external side of a sample container is contaminated by any organic materials, feces, soil or any other environmental dirt
- ❖ If samples are collected without maintaining any cool-chain and sent to the laboratory after 24 hours of collection.

Fish necropsy for AMR surveillance

Fish sample processing in laboratory: Skin swab



Fish necropsy for AMR surveillance

Fish sample processing in laboratory: gills, muscle and intestine

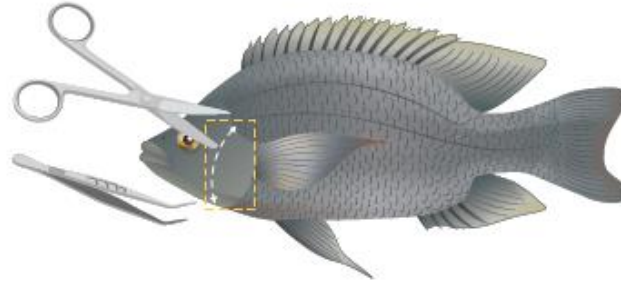
- Disinfect the outer surface of fish by wiping properly using gauze pad soaked with 70% ethanol.
- Place the fish on the dissection table so that the right side of the fish remains in contact with the table surface



Fish necropsy for AMR surveillance

Gills

- Collect gills from both sides of head



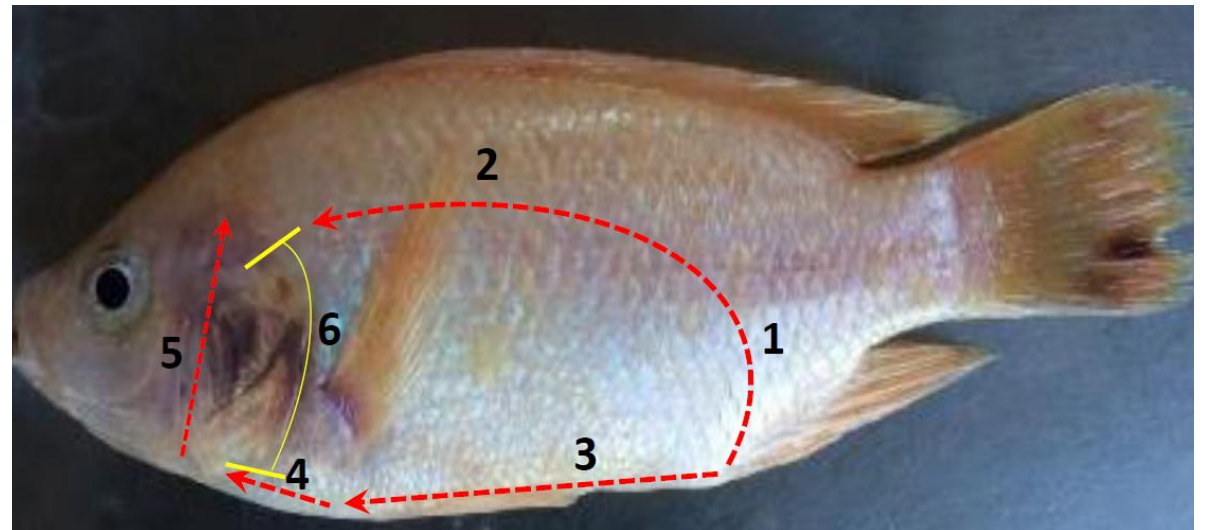
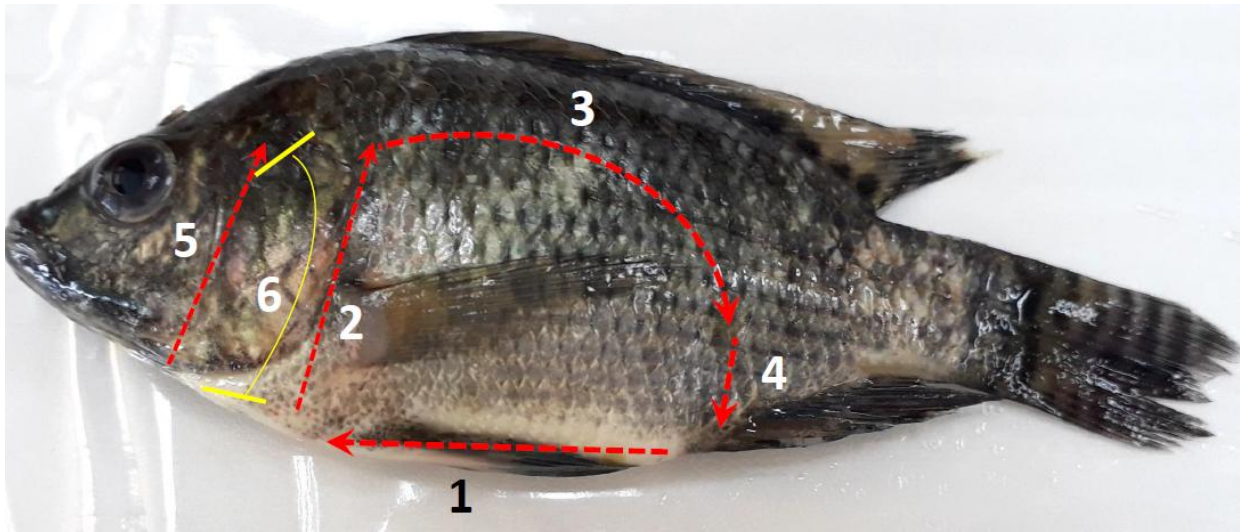
Muscle

- Using scalpel and forceps, carefully remove the skin of body part between dorsal and caudal fin (above the lateral line) of left side of the fish.



Fish necropsy for AMR surveillance

Dissect fish for internal organs



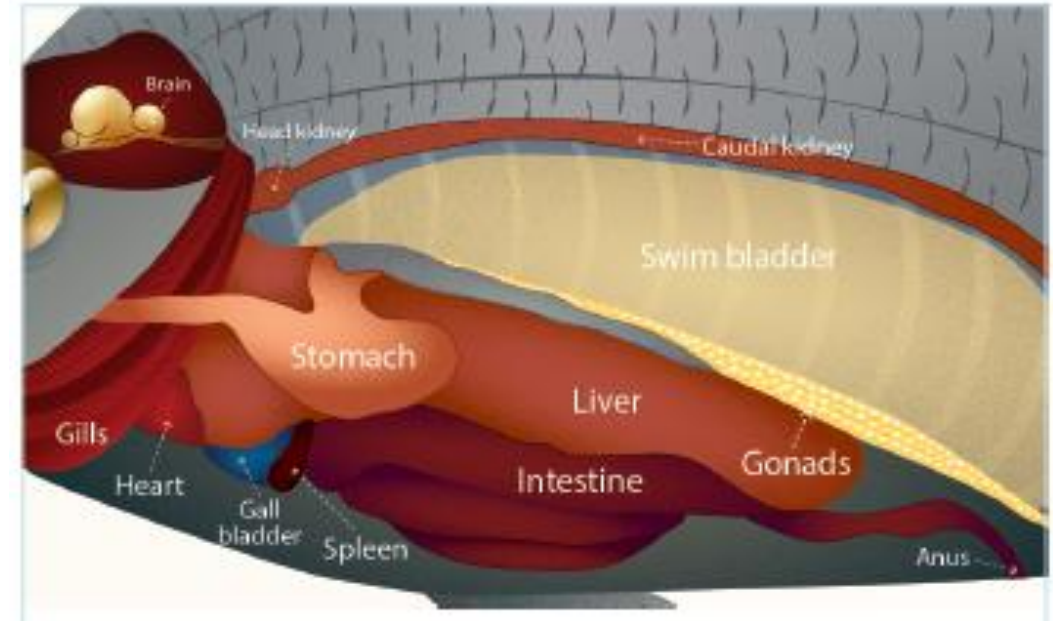
Fish necropsy for AMR surveillance

Dissect fish for internal organs

Intestine

- Pull back the gut using sterile forceps.
- Collect both ends (foregut and hindgut part) of the gut and collect

Do not cut or pierce the
GI-tract



Fish necropsy for AMR surveillance

Body part: kidney

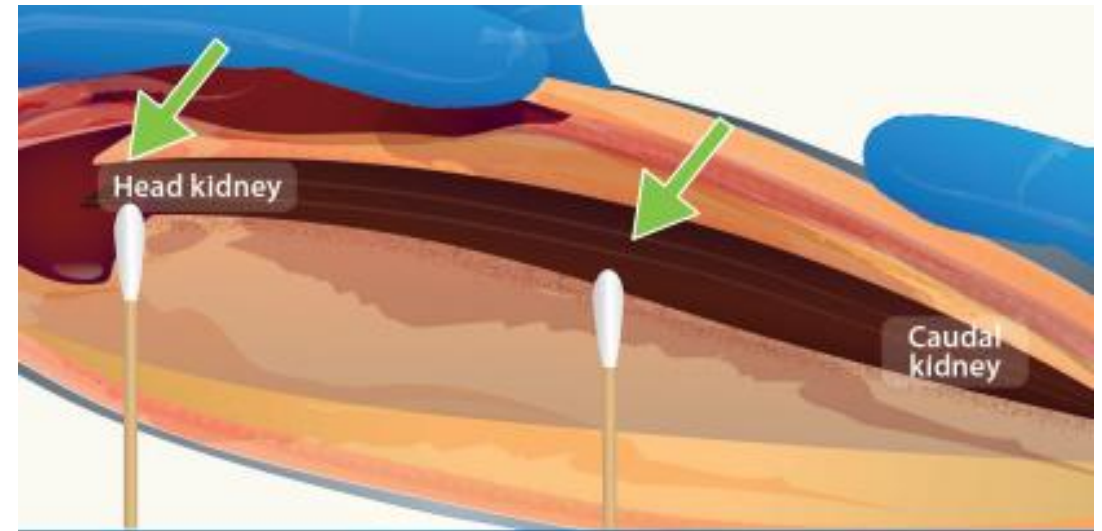
Caudal kidney

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

Head kidney

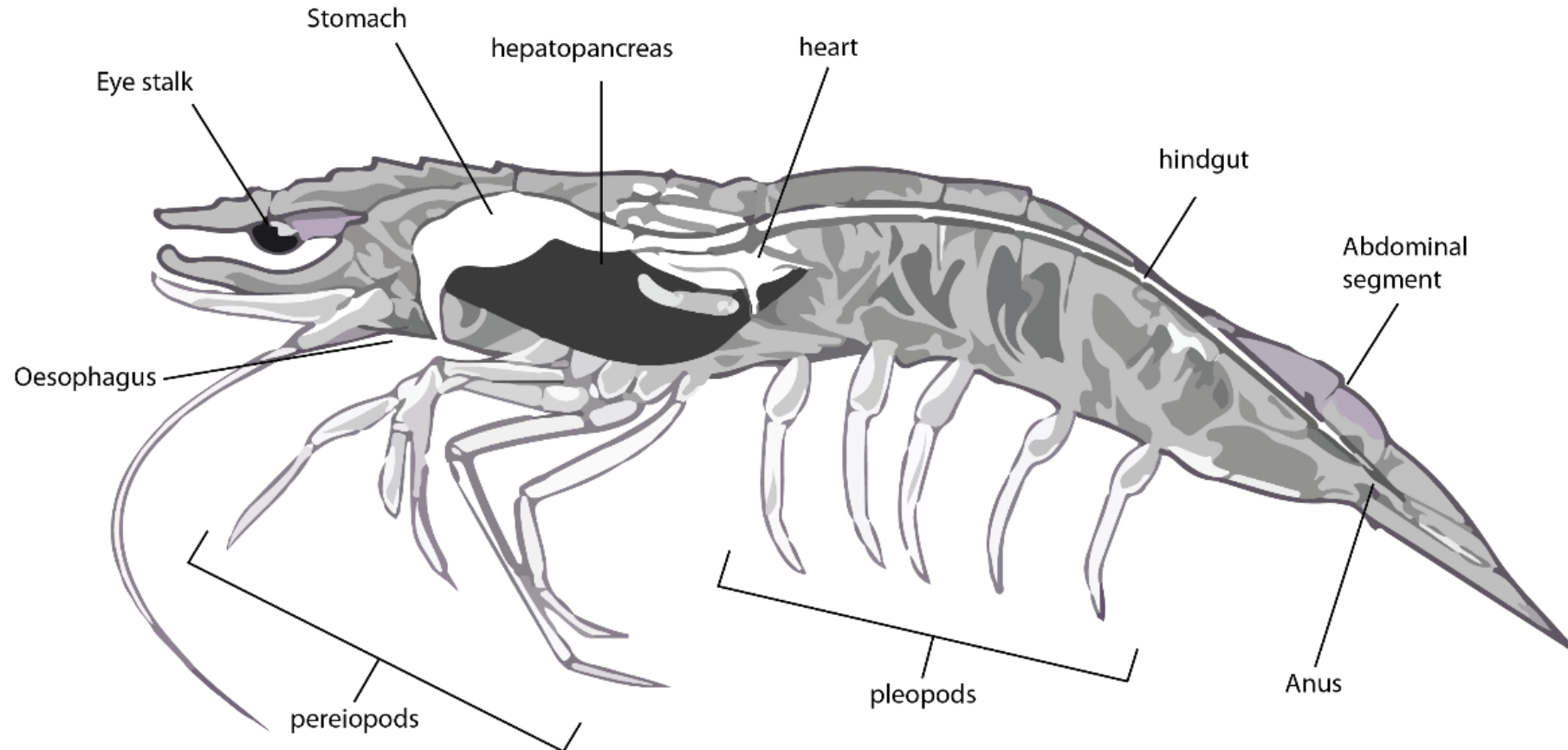
- Remove gills or other organs using forceps, scissors or scalpel.
- Puncture membrane to expose head kidney using swab tip.

Avoid perforating the gut or any other organs



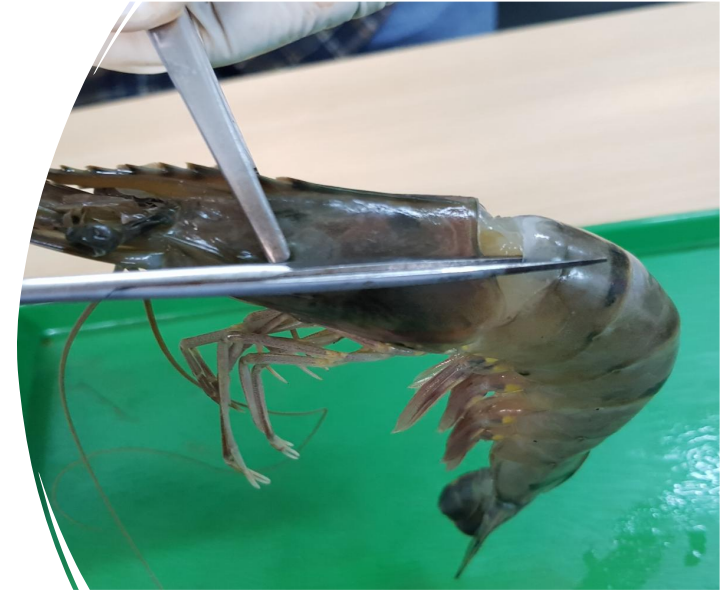
Shrimp necropsy for AMR surveillance

Internal organs of shrimp



Shrimp necropsy for AMR surveillance

- ❖ Clean any debris or mud from body surface using sterile tissue paper. Disinfect the outer surface by wiping with 70% EA soaked gauze pad
- ❖ Make an incision over the head using sterile scissors or scalpel. Using forceps remove the intestine from hepatopancreas to anus
- ❖ Aseptically extrude the gut content into a sterile falcon tube



Shrimp necropsy for AST profiling

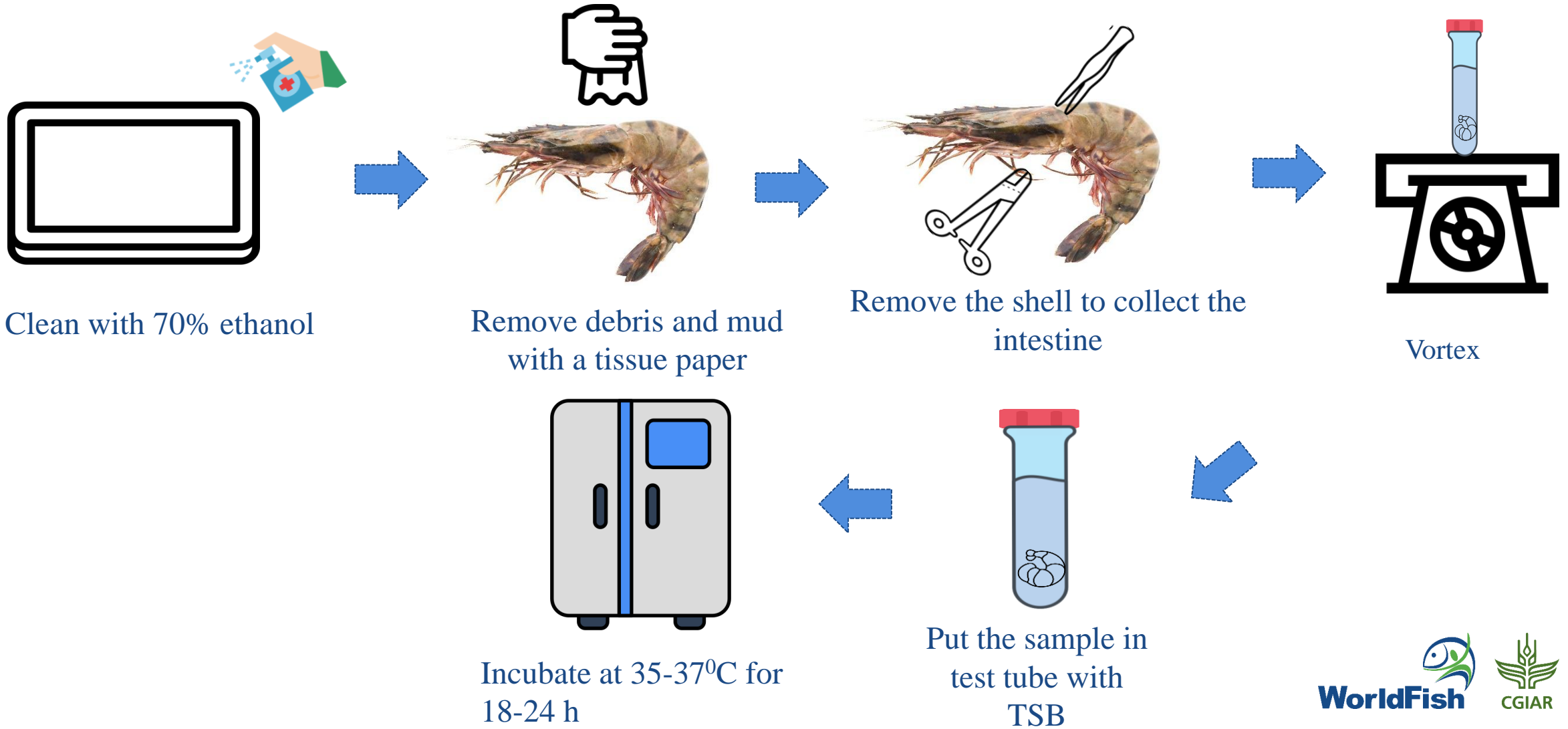
Body part: Muscle

Using sterile forceps remove the shell aseptically. Collect meat in a stomacher bag



Shrimp necropsy for AST profiling

Sample processing in lab (Intestine)



Enrichment

- ❖ Add 25 g of collected sample (tissue, gills, gut, meat) to 225 ml sterile trypticase soy broth in a stomacher bag
- ❖ If you focus on specific organ, place the collected organ samples in separate stomacher bags
- ❖ Homogenize using stomacher for 1-2 minutes.
- ❖ Hold 60 minutes at room temperature; and, then, incubate at 35-37⁰C for 18 hours, aerobically



Thank You

