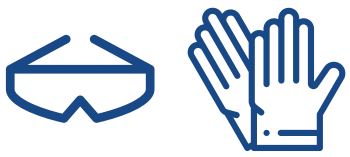
Sampling materials for fish disease diagnostics

Guide for color indications

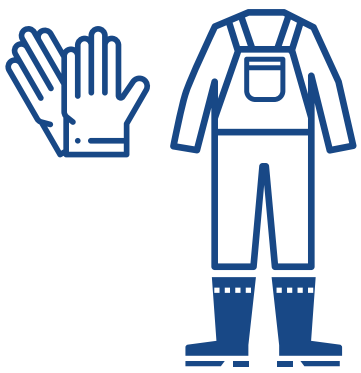
- Protocol 1: Wet-mount microscopy
- Protocol 2: Microbiome
- Protocol 3: Blood
- Protocol 4: Bacteriology
- Protocol 5: Molecular
- Protocol 6: Virology
- Protocol 7: Histology

Personal protective equipment (PPE)

Disposable latex or vinyl gloves (for everyone)



Rubber/Disposable latex gloves and safety glasses (for fish processors)

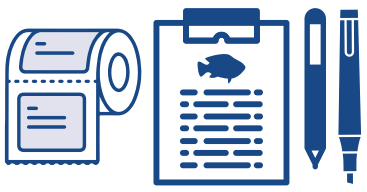


Long sleeves, rubber gloves, boots and overalls (for fish collectors)

*All items required for all 7 protocols

Data collection and recording supplies

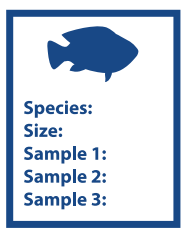
Field guide, this protocol and any other important documents for fish sampling, clinical signs identification etc.



Clipboard, field notepad, labels, pencil, solvent-resistant permanent marker

Farm visit identification form

Camera or smartphone



Fish health examination and sample records forms

Study plan

*All items required for all 7 protocols

Cleaning supplies

Spray or squirt bottle filled with suitable disinfectant (e.g. normal grade 70% ethanol)

Plastic beaker (50–100 ml) to disinfect instruments in 70% ethanol

Garden pump spray bottle filled with deionized water to rinse instruments

Plastic biohazard clinical bags for waste disposal

*All items required for all 7 protocols

Sampling materials: consumables, reagents, media and tools

Post-mortem sheet, clean tissue paper or aluminium fold to create a clean surface to sample the fish

Paper towels (enough for each fish and to clean surfaces and instruments)

Cooler, ice or ice packs, strong resealable plastic bags to pack and transport samples

Box, transportation container, strong resealable plastic bags to pack and transport samples

Anesthetic (e.g. MS-222, benzocaine, clove oil)

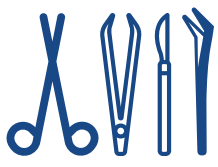
Disinfectant: 70% ethanol (normal grade)

Fixative: 95%–100% ethanol (molecular grade)

Fixative: RNA stabilization solution (e.g. RNA later) to preserve RNA viruses such as tilapia lake virus (TiLV)

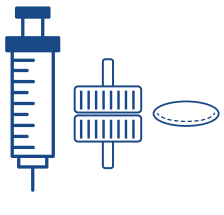
Fixative: 10% neutral buffered formalin (NBF)

Portable Bunsen burner to create a sterile environment around the sampling area and to sterilize dissection instruments between fish

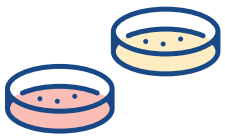


Sterile dissection kit (scissors, scalpel, forceps, tweezers, etc.)

Microcapillary tube



Water filtration kit:
50 ml syringe, filter holder and filter

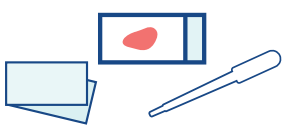


Trypticase soy agar (TSA) plates (or any other bacteriological agar as per study requirement)

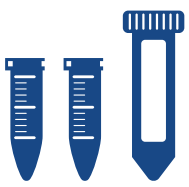
Sterile single-use loops, plain cotton swabs or reusable metal loops

Bacteriological transportation swabs (if no TSA plates to be used for field sampling)

Syringes, needles of a size appropriate to the size of fish and volume of blood to be collected



Microscope slides, cover-slips for slides, plastic pasteur pipette



Sterile tubes of a size appropriate to the volume of specimens to be preserved (e.g. 1.5 ml, 2 ml, 5 ml, 15 ml or 50 ml tubes) for microbiome, blood or molecular sampling

50 ml fix pots/tubes or smaller tubes of a size appropriate to the volume of specimens to be fixed for histology

Viral transporationt media

Compound light microscope with 4x–100x objectives

Bench-top micro-centrifuge

TRIzol reagent

Wright/Giemsa/Diff quick stain

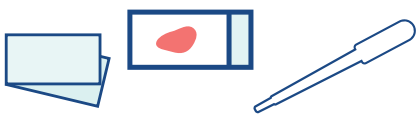
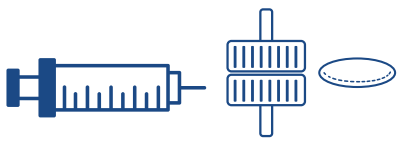
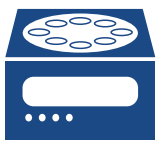
Gram stain reagents consisting of crystal violet, Lugol's iodine, 95% ethanol & safranin red

Checklist: Table of sampling materials by protocol

Materials	Wet-mount microscopy	Fish+Water Microbiome	Blood	Bacteriology	Molecular	Virology	Histopathology
aluminium fold	✓	✓	✓	✓	✓	✓	✓
Sterile dissection kit	✓	✓		✓	✓	✓	✓
Anaesthetic			✓	✓	✓	✓	✓
Paper towels	✓	✓	✓	✓	✓	✓	✓
Cooler storage and transportation			✓			✓	
Normal storage and transportation	✓	✓		✓	✓		✓
Portable Bunsen burner		✓		✓			
Sterile tubes		✓	✓		✓	✓	✓
95%–100% ethanol (molecular grade)		✓			✓		
70% ethanol (normal grade)	✓			✓			✓
Viral transportation media						✓	
RNA stabilization solution					✓		
Trypticase soy agar (TSA), blood agar, etc				✓			
Loops and cotton swab*		✓		✓			
Bacteriology transport swab**				✓			

* For bacteriology either inoculation loop or cotton swab can be used. For microbiome sampling using cotton swab only.

** If using bacteriology transportation swab to collect bacteriology sample onsite, there is no need to use inoculation loop or cotton swab.

Materials	Wet-mount microscopy	Fish+Water Microbiome	Blood	Bacteriology	Molecular	Virology	Histopathology
 10% NBF							✓
 Histology pot							✓
 Microscope slides, cover slips and plastic pasteur pipette	✓		✓	✓			
 Syringes and needles			✓				
 Microcapillary tube			✓				
 Anticoagulant (e.g. 10% EDTA, heparin)			✓				
 Water filtration set		✓					
 Compound light microscope with 4x–100x objectives	✓						
 Tabletop centrifuge			✓				
 TRIzol reagent			✓				
 Wright/Giemsa/Diff quik stains			✓				
 Gram stain reagents	✓			✓			

In partnership with

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*.

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