

Sampling materials for fish disease diagnostics

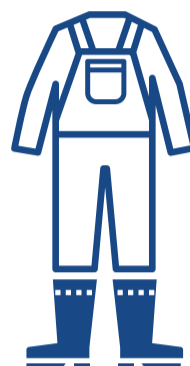
Guide for color indications

- 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)



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



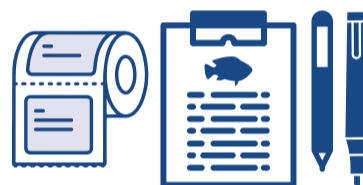
Rubber/Disposable latex gloves and safety glasses (for fish processors)

*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.



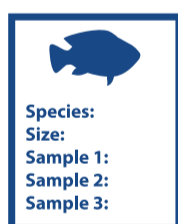
Clipboard, field notepad, labels, pencil, solvent-resistant permanent marker



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




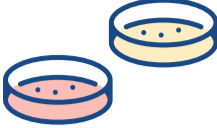







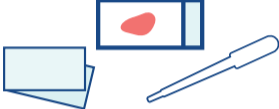

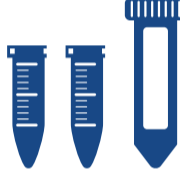



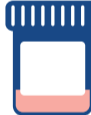








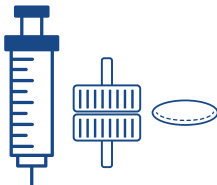

Garden pump spray bottle filled with deionized water to rinse instruments




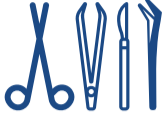













Plastic biohazard clinical bags for waste disposal

*All items required for all 7 protocols













Sampling materials: consumables, reagents, media and tools

	Post-mortem sheet, clean tissue paper or aluminium foil to create a clean surface to sample the fish		Trypticase soy agar (TSA) plates (or any other bacteriological agar as per study requirement)
	Paper towels (enough for each fish and to clean surfaces and instruments)		Sterile single-use loops, plain cotton swabs or reusable metal loops
	Cooler, ice or ice packs, strong resealable plastic bags to pack and transport samples		Bacteriological transportation swabs (if no TSA plates to be used for field sampling)
	Box, transportation container, strong resealable plastic bags to pack and transport samples		Syringes, needles of a size appropriate to the size of fish and volume of blood to be collected
	Anesthetic (e.g. MS-222, benzocaine, clove oil)		Microscope slides, cover-slips for slides, plastic pasteur pipette
	Disinfectant: 70% ethanol (normal grade)		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
	Fixative: 95%–100% ethanol (molecular grade)		50 ml fix pots/tubes or smaller tubes of a size appropriate to the volume of specimens to be fixed for histology
	Fixative: RNA stabilization solution (e.g. RNA later) to preserve RNA viruses such as tilapia lake virus (TiLV)		Viral transportation media
	Fixative: 10% neutral buffered formalin (NBF)		Compound light microscope with 4x–100x objectives
	Portable Bunsen burner to create a sterile environment around the sampling area and to sterilize dissection instruments between fish		Bench-top micro-centrifuge
	Sterile dissection kit (scissors, scalpel, forceps, tweezers, etc.)		TRIZOL reagent
	Microcapillary tube		Wright/Giemsa/Diff quick stain
	Water filtration kit: 50 ml syringe, filter holder and filter		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	✓			✓			

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