Quick fish sampling guide for disease diagnostics

Blood sampling guide

Choose the appropriate size of syringe and needle* based on fish size.

Fry, juvenile fish (<5 cm): 1 cc syringe | glass capillary

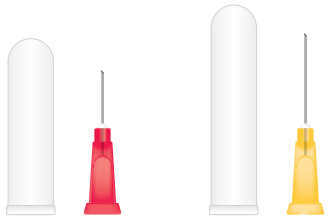
1 cc, 30G for juvenile fish

For very small fish/fry cut the tail off and touch a hematocrit capillary tube onto the blood droplet that forms to draw it up.

Hematocrit glass capillary tube

Juvenile fish (5–10 cm) (<50 g): 1 cc syringe | 29G–25G needles

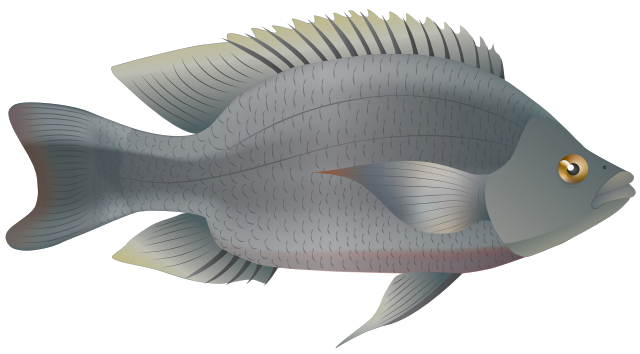
1 cc



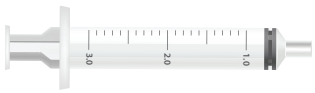
29 G

25 G

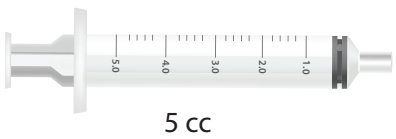
Adult fish: 1 cc, 3 cc or 5 cc syringe | 29G–19G needle



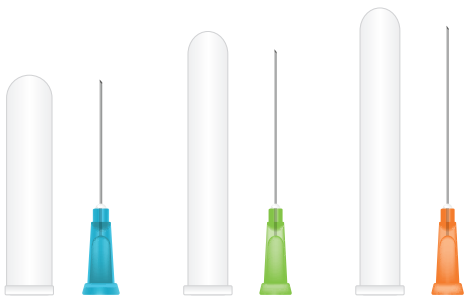
1 cc



3 cc



5 cc



23 G

22 G

19 G

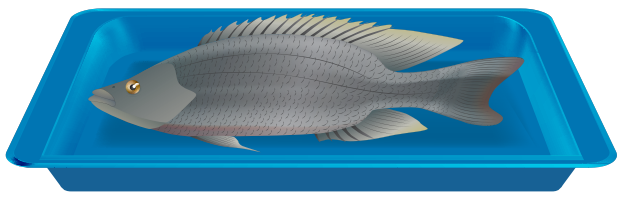
*Note: The higher the number next to (G), the smaller the needle size (e.g. 30G smallest, 19G largest).

For plasma sampling only, rinse both syringe and needle with 10% EDTA.

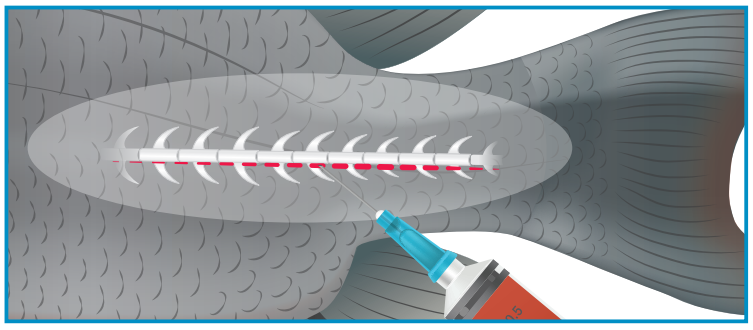
How to prepare 10% EDTA solution

- Add 1 g of EDTA to 10 ml of sterile H_2O
- Vortex
- Let it dissolve completely

Note: EDTA will not go into solution until pH is adjusted to 8.0.



Place anesthetized fish on a clean surface.
For live bleed, it is important to put the fish onto a wet surface to avoid removing scales and mucus.

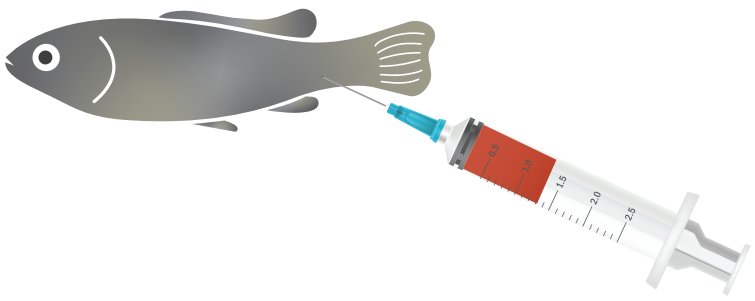


Carefully insert the needle into the caudal vein.

- Withdraw the following amount of blood (when possible) based on the different type of samples and methods:
  - Serum isolation: collect a minimum of 200 µL of blood per fish (ideally > 500 uL for larger fish)
  - Plasma isolation: collect a minimum of 200 µL of blood per fish (ideally > 500 uL for larger fish)
  - DNA extraction: collect a minimum of 100 µL of blood per fish
  - RNA extraction : collect a minimum of 100 µL of blood per fish
  - Blood smear: only a few drops are necessary

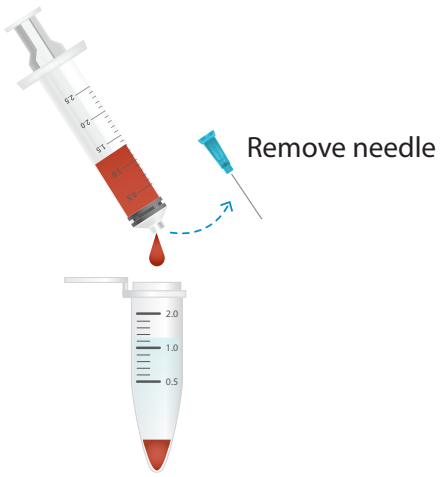
Blood for DNA extraction (e.g. PCR, sequencing)

1



Within 3 min of capture/anaesthesia, withdraw a minimum of 200 µLof blood (ideally >500 µL for larger fish)

2

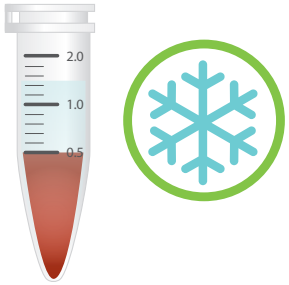


Dispense blood into 2 ml sterile tube prefilled with 1 ml of 95% molecular grade EtOH

3

Transport to lab with ice or cool packs

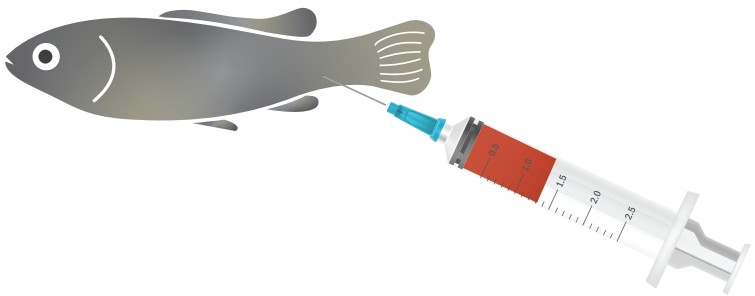
4



Proceed with DNA extraction immediately or store at 4^{0}C for a few hours, or at either –20^{0}C or –80^{0}C (long term storage)

Blood for RNA extraction (e.g. RT-PCR, RT-qPCR, sequencing)

1



Within 3 min of capture/anaesthesia, withdraw a minimum of 200 µL of blood (ideally >500 µL for larger fish)

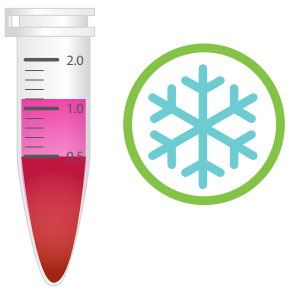
2

Dispense blood into 2 ml tube with 1 ml TRIzol reagent

3

Transport to lab with ice or cool packs

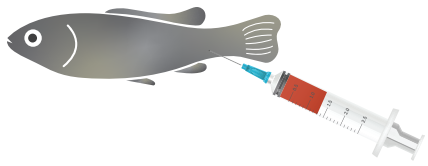
4



Proceed with DNA extraction immediately or store at 4^{0}C for a few hours, or at either –20^{0}C or –80^{0}C (long term storage)

Blood for serum isolation

1



Within 3 min of capture/anaesthesia, withdraw a minimum of 200 µL of blood (ideally >500 µL for larger fish)

2

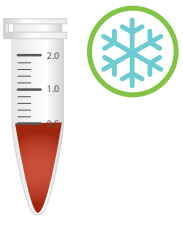
Remove needle

Dispense blood into 2 ml tube

3

Transport to lab on ice or cool packs

4



Allow blood to coagulate for a minimum of 2–4 hours at room temperature or at 4^{0}C overnight

5

Centrifuge tubes at 7000 x *g* for 15 min

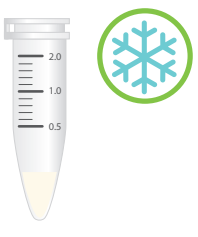
6

Collect serum (upper clear supernatant layer). But do not disturb red blood cells at the bottom

7

Transfer serum to new sterile tube

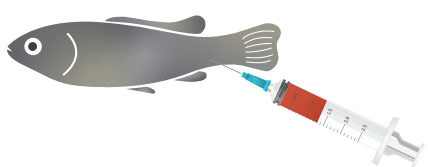
8



Proceed with analytical protocol or store at either –20^{0}C or –80^{0}C for future analysis

Blood for plasma isolation

1



Within 3 min of capture/anaesthesia, withdraw of minimum of 200 uL of blood using a syringe and needle coated with 10% EDTA (ideally >500 uL for larger fish)

2

Remove needle

Dispense blood into 2 ml tube

3

Transport to lab on ice or cool packs

4

Store on ice (minimum 1–2 hours)

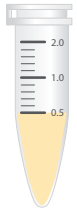
5

Centrifuge tubes at 7000 x *g* for 15 min

6

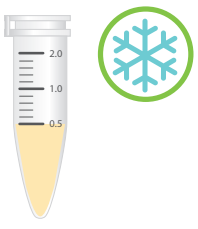
Collect plasma (upper clear supernatant layer).
But do not disturb red blood cells at the bottom
(Note: Blood cells can be kept for future immunocompetence studies)

7



Transfer plasma to new a sterile tube

8



Proceed with analytical protocol or store at either –20^{0}C or –80^{0}C for future analysis

Note

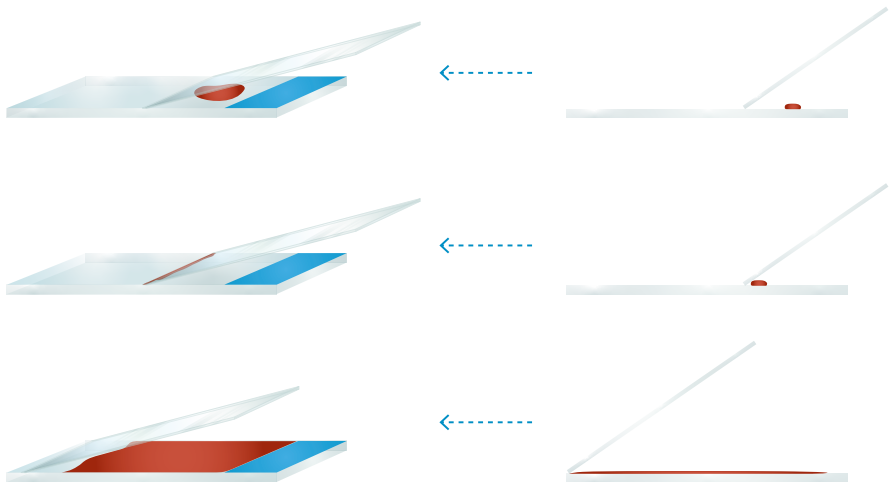
EDTA works for some species, though not so much for others. It can impact molecular analyses, meaning PCR inhibition is strong at high concentration. Heparin works for some species, though not for others. There is no problem with PCR inhibition, but biosecurity is an issue as it is mostly extracted from cows

Blood smear preparation

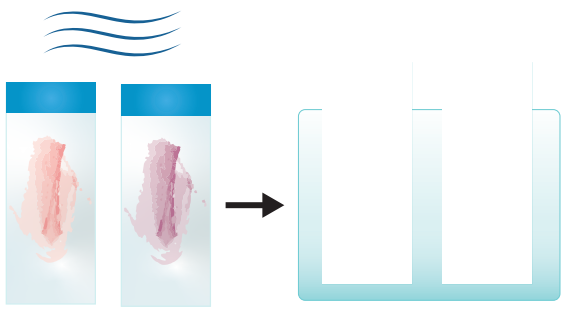
Step 1

Place a few blood drops on a slide

- Use a cover slip to smear the blood across half the slide
- Prepare two slides per fish each with different blood concentrations



Step 2

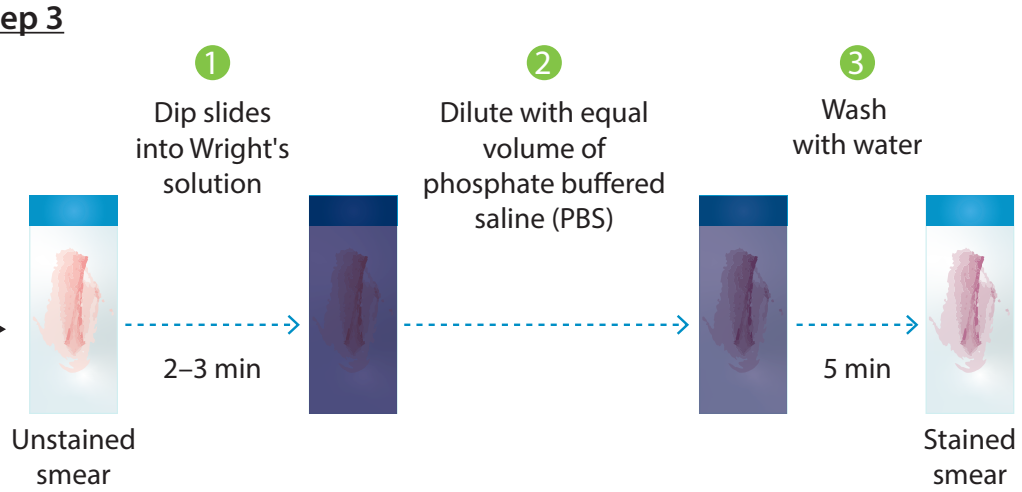


Dry slides upright for 10–30 min

Dip slides into methanol for 20 sec

Methanol dip

Step 3

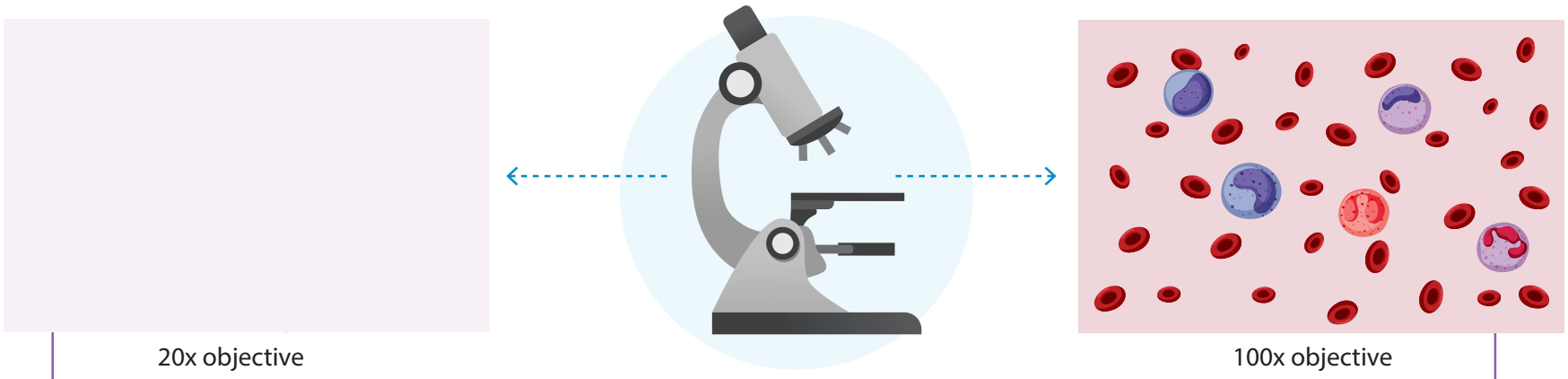


Unstained smear

Stained smear

Stain with Wright, Giemsa or Diff Quick

Step 4



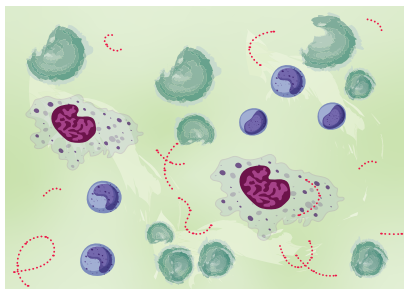
20x objective

Observe under a light transmission microscope

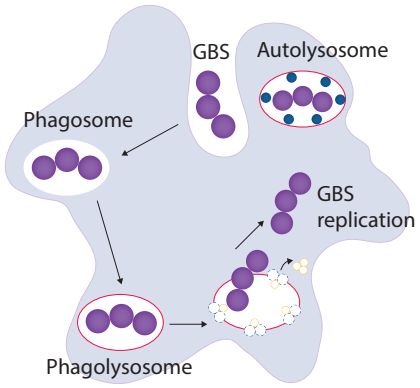
100x objective

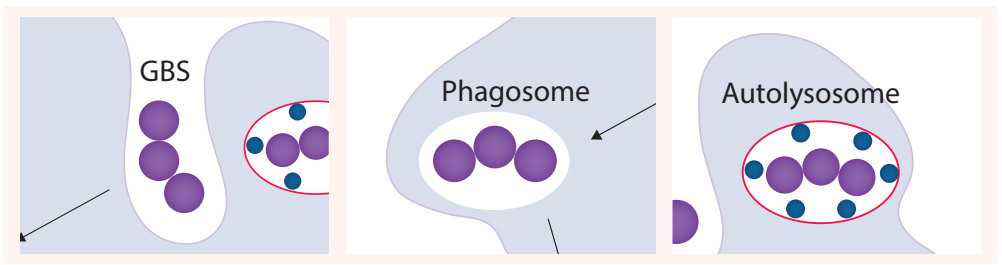
Lymphocyte | Monocyte | Eosinophil | Basophil | Neutrophil | Macrophage

Example of a stained blood smear seen under the microscope using different objectives (top 2 images), and examples of fish white blood cells or leucocytes (row of images below)



Cocci spherical-shaped bacteria (e.g. Group B *streptococcus,* GBS*)* outside leucocytes and inside macrophage phagosome





Internalization of the bacteria | Inside the phagosome | Destruction of bacteria (autolysosome)

Phagocytosis process of Group-B *streptococcus* by a macrophage

In partnership with

WorldFish · CGIAR Research Program on Fish, Led by WorldFish · Cefas · University of Exeter · Veterinærinstituttet · The University Of Queensland Australia, Create Change · Mississippi State University · University of Ibadan, Ibadan, Nigeria

www.worldfishcenter.org

This work was undertaken as part of the CGIAR Research Program on Fish Agri-Food Systems (FISH) led by WorldFish, the CGIAR Inspire Challenge 2019 project on *Rapid Genomic Detection of Aquaculture Pathogens* and the Feed the Future Innovation Lab for Fish project on *Improving Biosecurity: A Science-Based Approach to Manage Fish Disease Risks and Increase the Socioeconomic Contribution of the Nigerian Catfish and Tilapia Industries.* FISH is supported by contributors to the CGIAR Trust Fund.