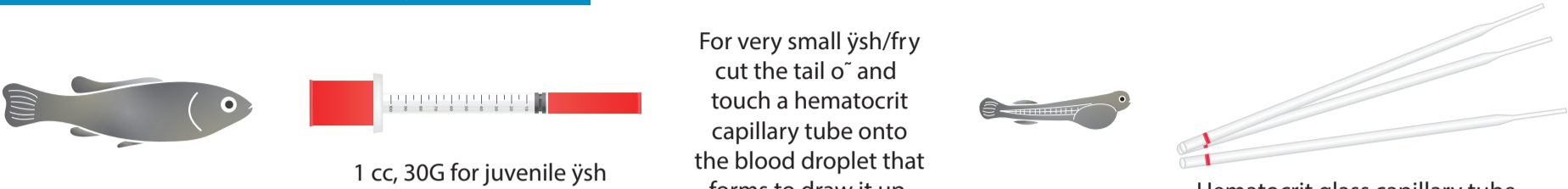


Quick fish sampling guide for disease diagnostics

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

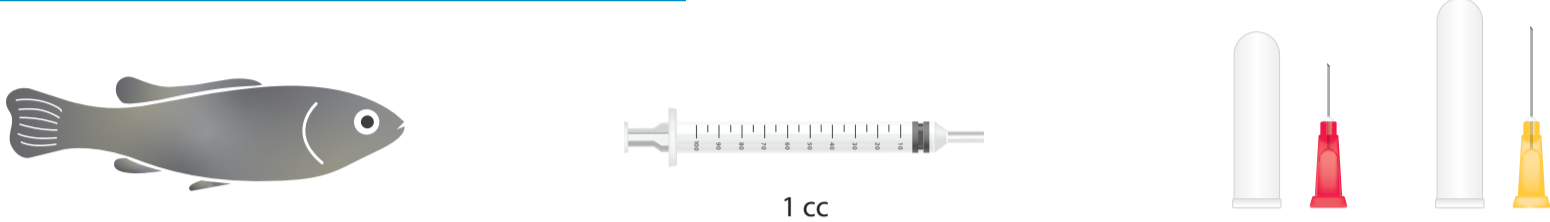


1 cc, 30G for juvenile fish

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

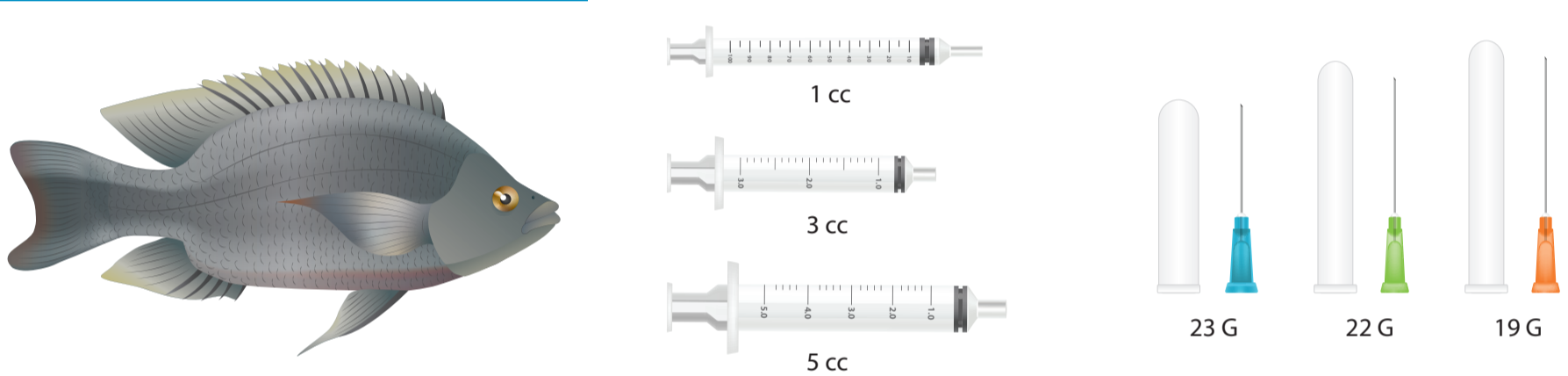
Juvenile fish (5–10 cm) (<50 g): 1 cc syringe | 29G–25G needles



1 cc

29 G 25 G

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



1 cc

3 cc

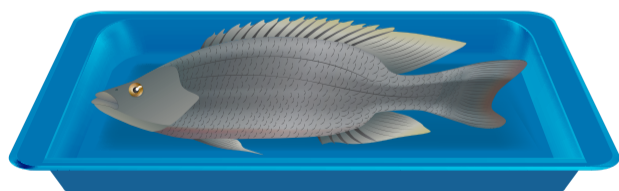
5 cc

23 G 22 G 19 G

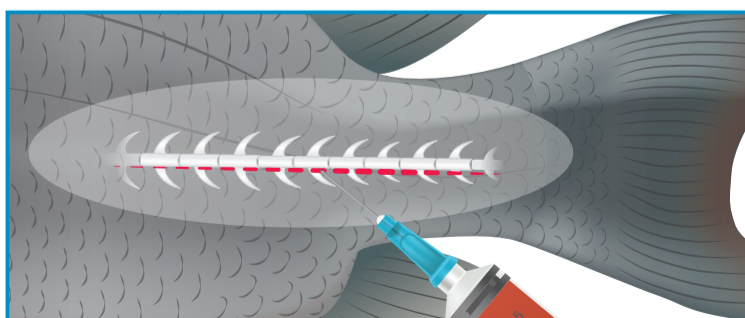
*Note: The higher the number next to (G), the smaller the needle size (e.g. 30G smallest, 19G largest).



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



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.

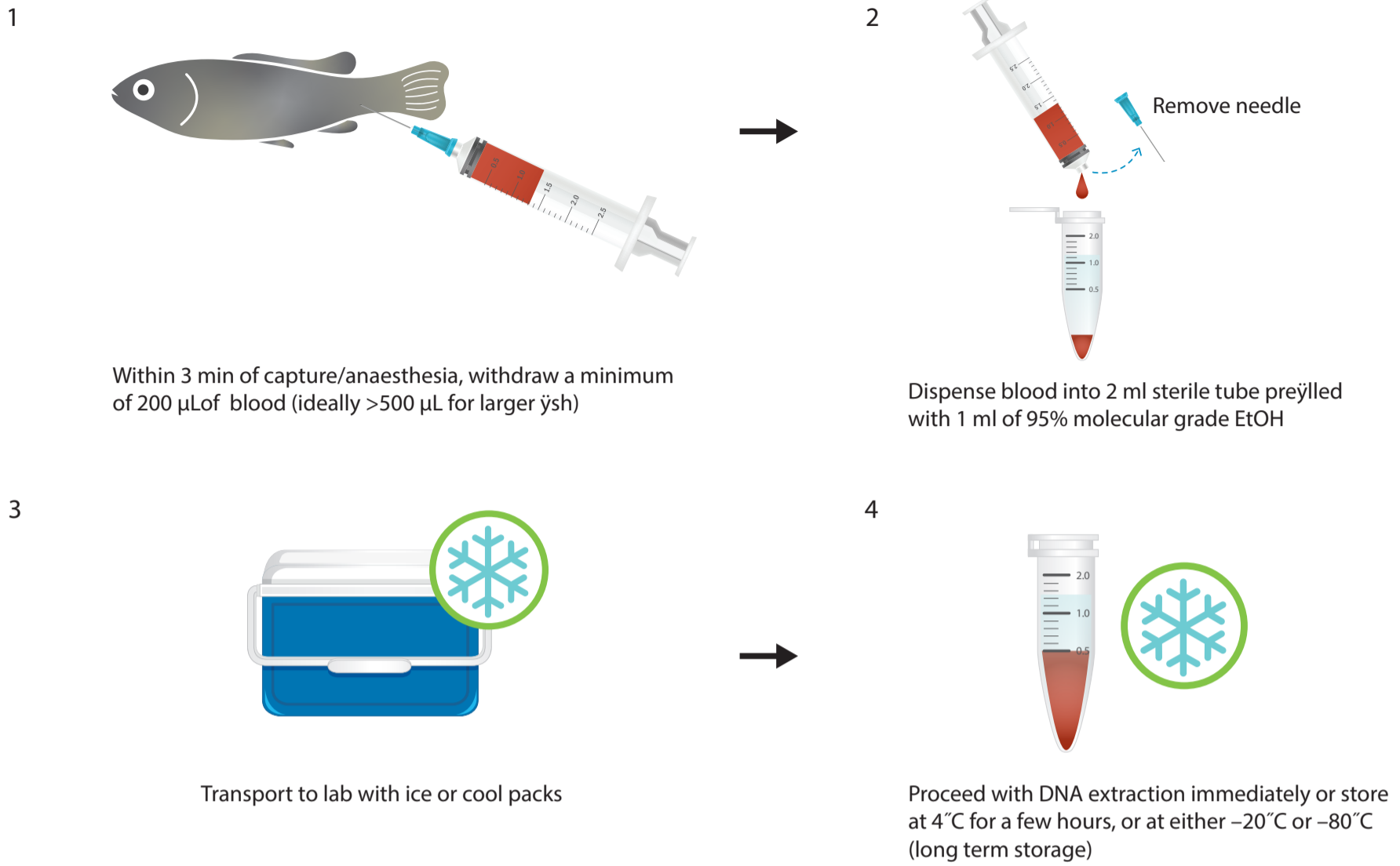
How to prepare 10% EDTA solution

- Add 1 g of EDTA to 10 ml of sterile H₂O
- Vortex
- Let it dissolve completely

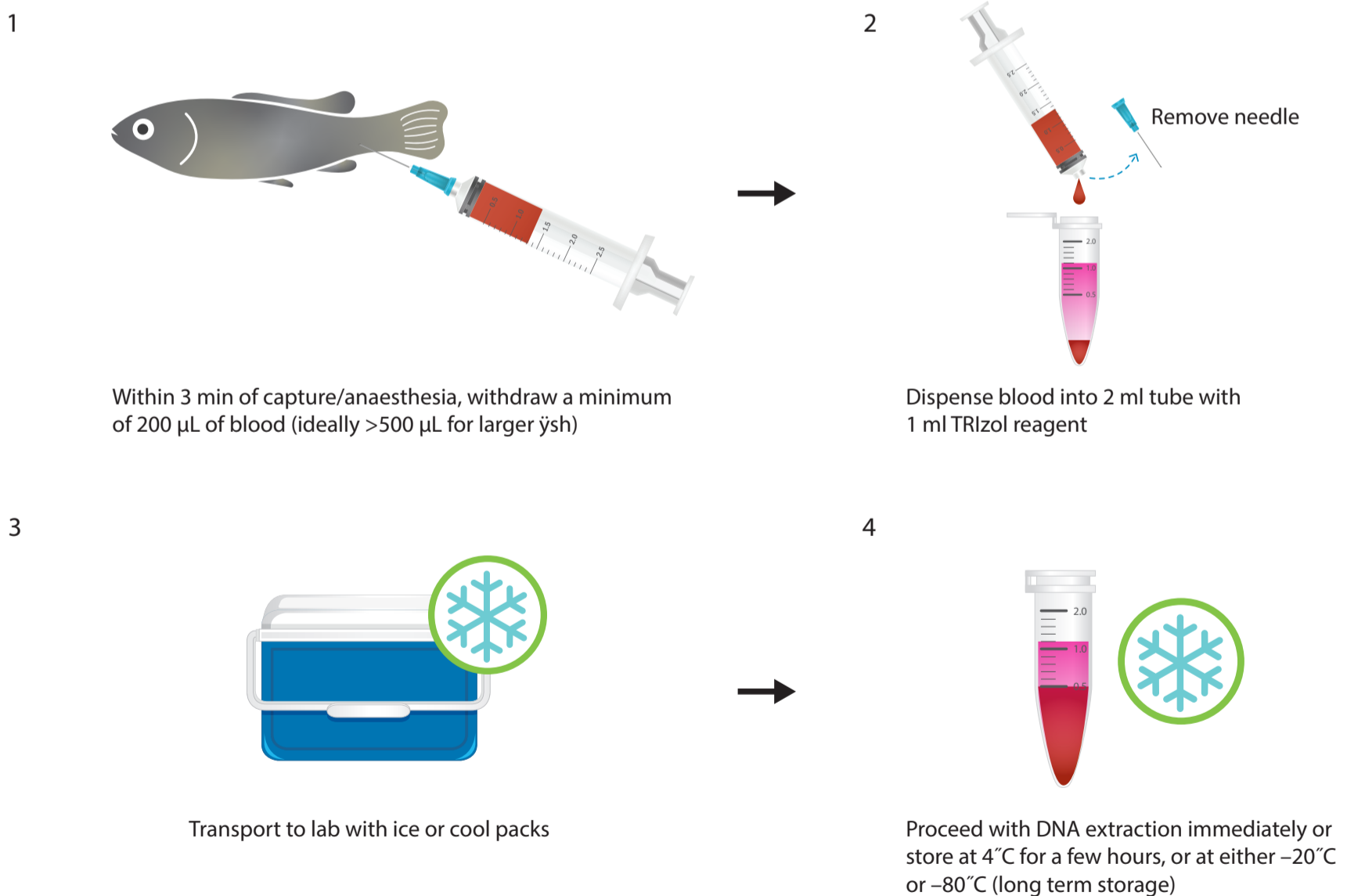
Note: EDTA will not go into solution until pH is adjusted to 8.0.

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

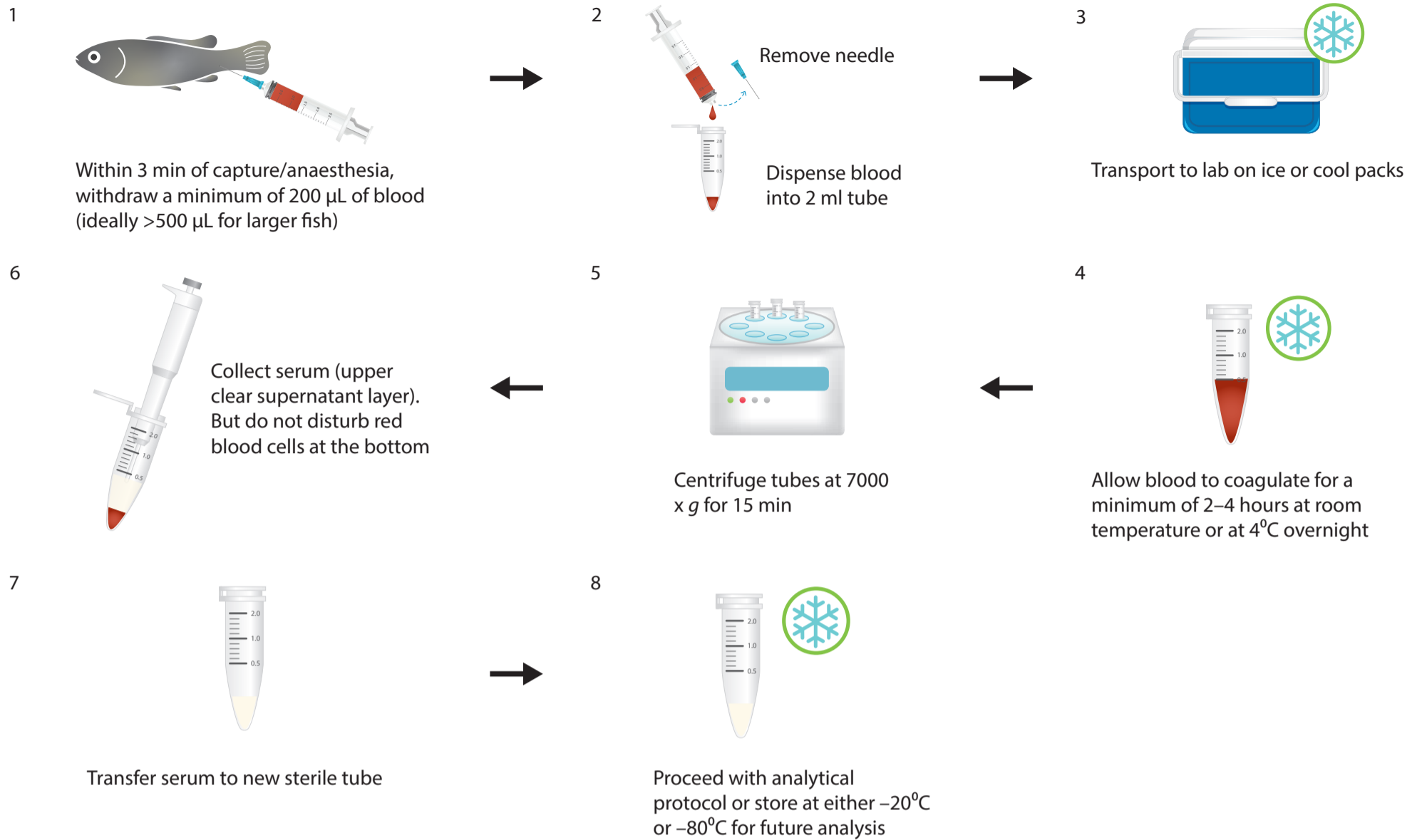
Blood for DNA extraction (e.g. PCR, sequencing)



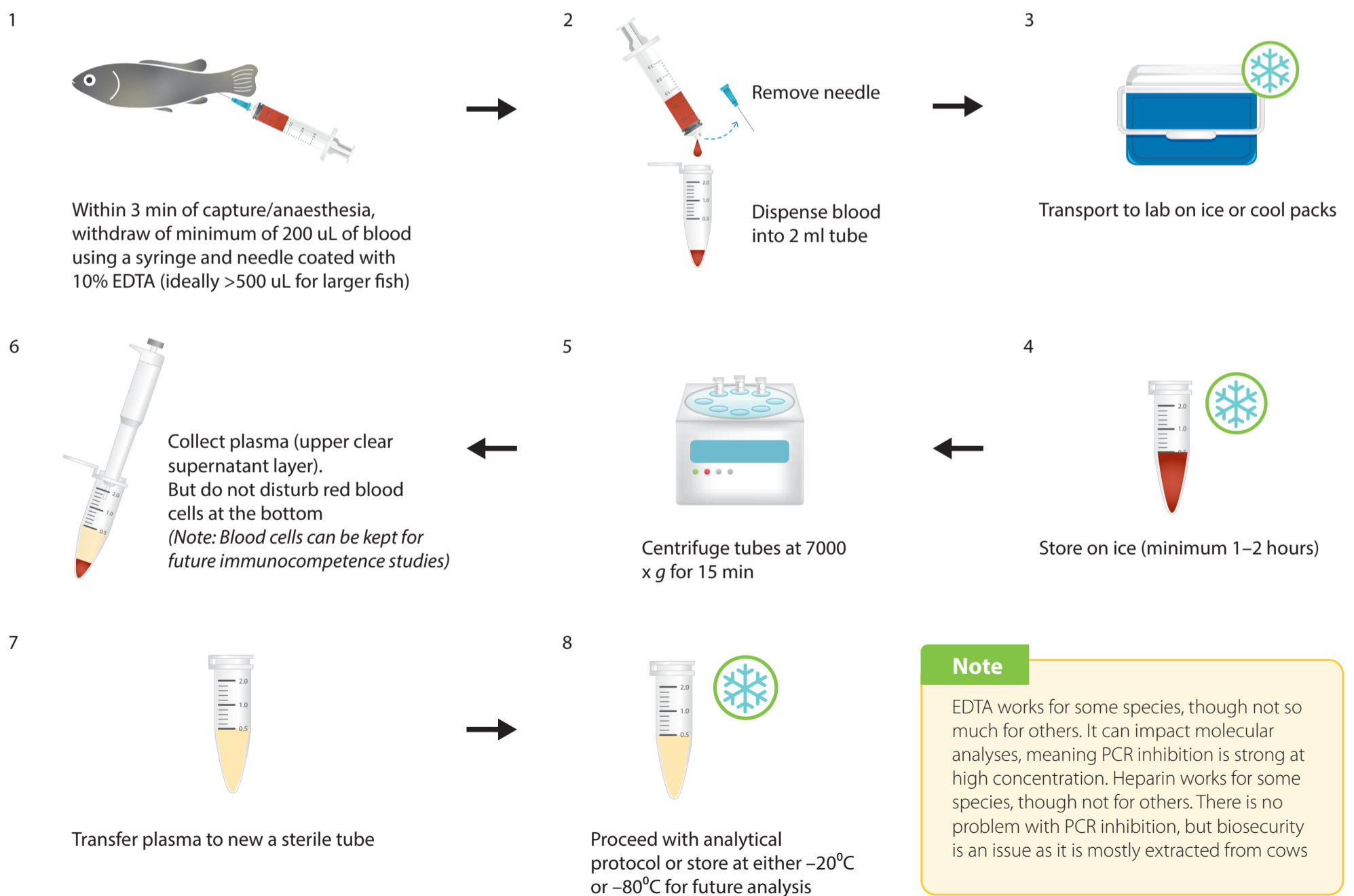
Blood for RNA extraction (e.g. RT-PCR, RT-qPCR, sequencing)



Blood for serum isolation

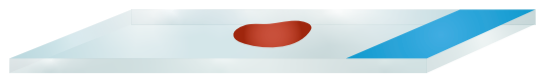


Blood for plasma isolation



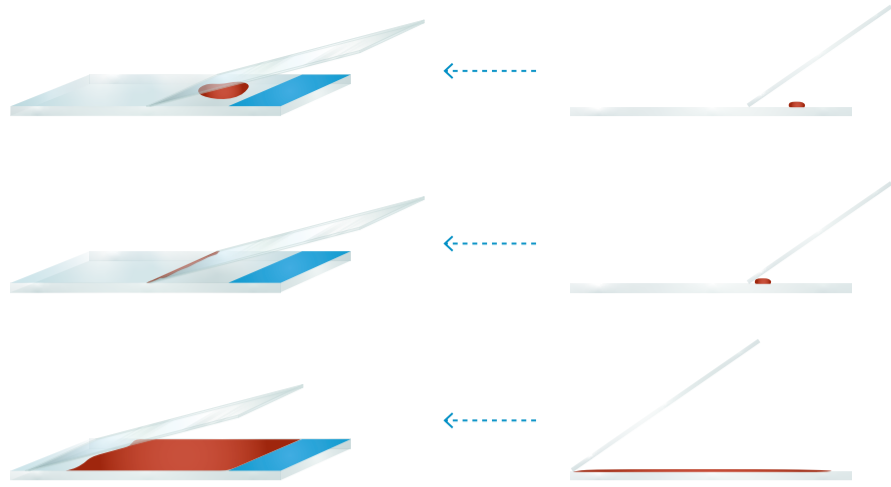
Blood smear preparation

Step 1

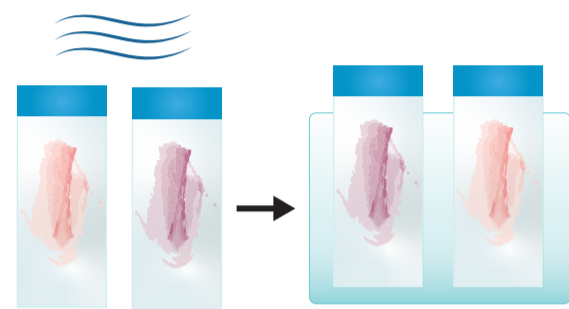


Place a few blood drops on a slide

- 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

1

Dip slides into Wright's solution



Unstained smear

2–3 min

2

Dilute with equal volume of phosphate buffered saline (PBS)



Stain with Wright, Giemsa or Diff Quick

3

Wash with water

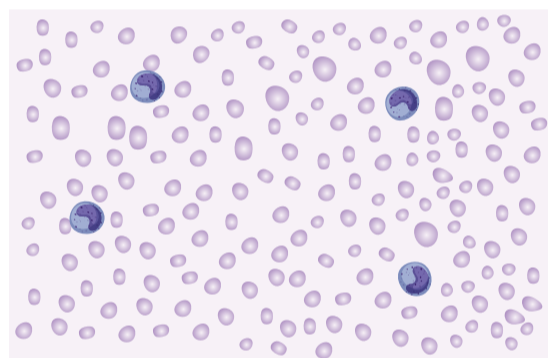


5 min

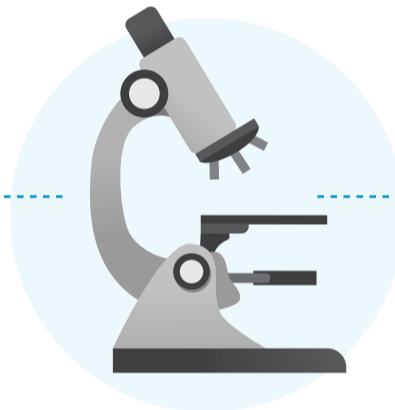


Stained smear

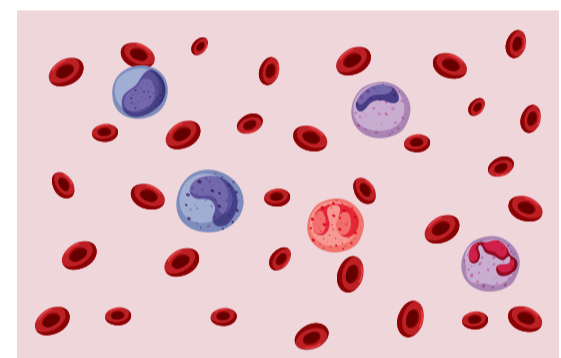
Step 4



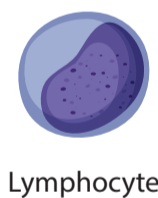
20x objective



Observe under a light transmission microscope



100x objective



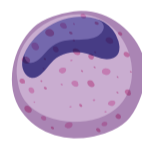
Lymphocyte



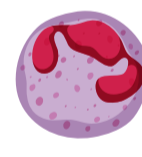
Monocyte



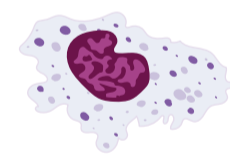
Eosinophil



Basophil

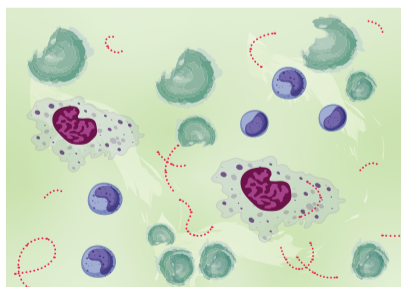


Neutrophil

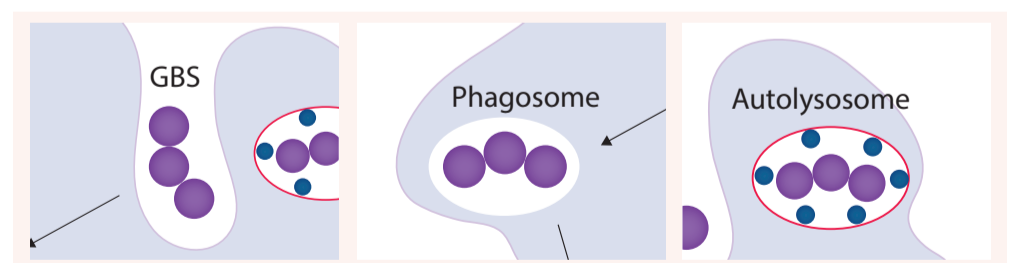
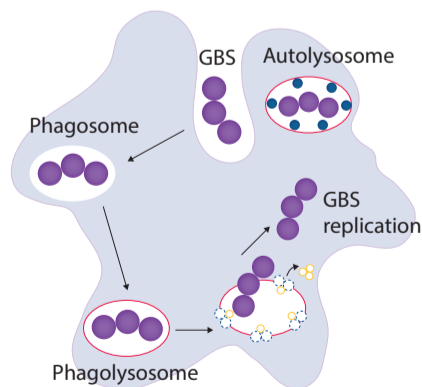


Macrophage

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

