

Blood Collection

Protocol for the fighting fish *Betta splendens*

Reagents and Equipment

- (Option 1 and 2) Heparin powder (Sigma #H4784)
- (Option 1 and 2) Sodium Chloride (Sigma #S9888)
- (Option 1) Syringes (BD 1mL Syringe Ref. 301320)
- (Option 1) Needles G27/G30 x 1/2 inch (Terumo needles)
- (Option 2) Heparinized capillary tube (Kimble 41B2501)

Option 1 – Collection with a syringe

1. Swap the needle in the syringe with a G27/G30 needle.
2. Coat syringes with heparin at 20 U/mL in 0.9% NaCl (leave some inside) and place them on ice before starting the experiment (approx. 1h).
3. Discard the remaining heparin inside the syringe and place the anesthetized fish on a silicon bed and hold it in place using a gauze:
 - a. insert the needle under the scales of the mid-portion of the tail just below the lateral line at a 45° angle (Fig. 1);
 - b. ease the needle toward the vertebral column until you reach the base of the column;
 - c. withdraw the needle a fraction of a millimeter and obtain the blood sample;
 - d. remove and transfer the blood to an 0.5 mL tube using the help of a P10 pipette if needed.
 - e. discard the needle in a sharps container;
 - f. return the fish to a recovery bath or its home tank;
4. Centrifuge the blood samples at 7.5 rpm for 10 min (RT).
5. Following centrifugation, transfer the plasma to a new tube and store it at -20°C.

Option 2 – Collection with a pipette or heparinized capillary tube

1. (pipette only) Heparinize the tip of a P20 (heparin at 20 U/mL in 0.9% NaCl).

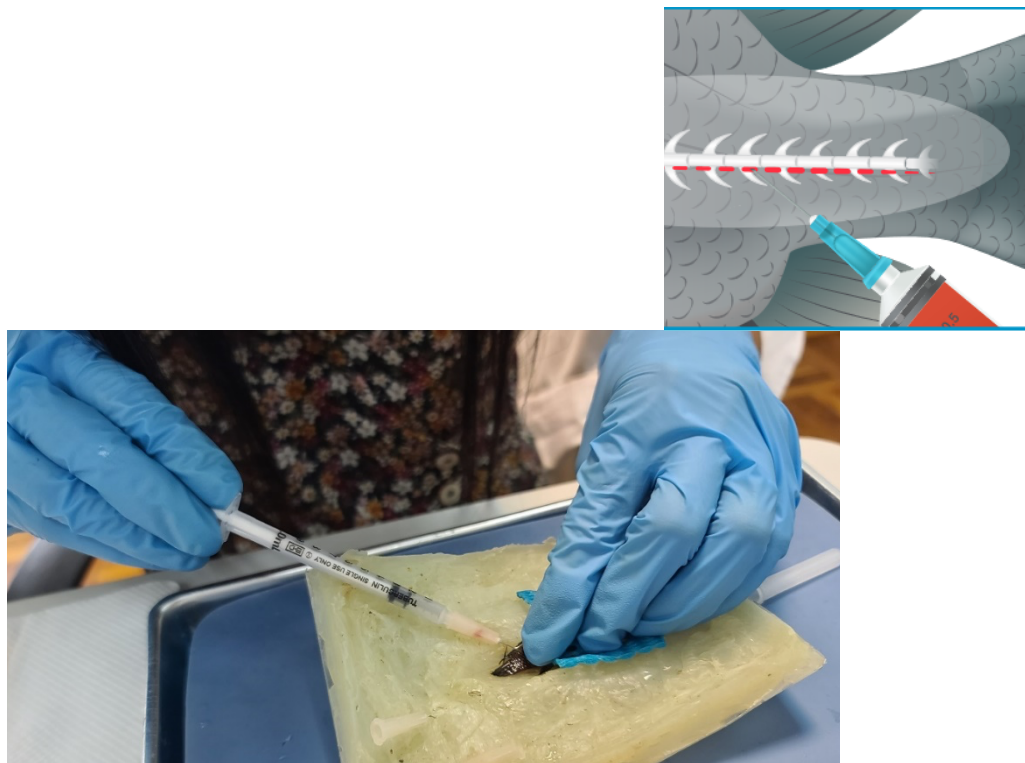


Fig. 1 – Blood sampling using a lateral approach in the fighting fish and schema of the location of the caudal vein.

2. Place the fish on top of a glass petri dish and use a piece of gauze to hold the fish in place, leaving the tail accessible. Using a steel blade, make a diagonal incision between the anal fin and the caudal fin (tail ablation approach).
3. Quickly collect the blood with the P20/capillary tube and transfer it to a 0.5 mL Eppendorf tube.
4. Centrifuge blood (RT) for 10 minutes at 7.5 rpm.
5. Following centrifugation, transfer the plasma to a new tube and store it at -20°C.

Solutions:

Heparin (Sigma #H4784; ≥ 180 USP units/mg), stored in the fridge

- Stock solution of heparin 200 U/mL in 0.9% NaCl \rightarrow 11.1 mg heparin powder in 10 mL of 0.9% NaCl. Aliquot 1 mL per Eppendorf and store at -80°C.

- Working solution of heparin 20 U/mL in 0.9% NaCl → 1 mL of heparin 200 U/mL plus 9 mL of 0.9% NaCl (14 mL falcon), keep in the fridge for one month.

0.9% Sodium Chloride (NaCl) - 100 mL

- Dissolve 0.9 g NaCl (MW 58.44) in 80 ml MiliQ water, and bring the total solution volume to 100 ml.
- For long-term storage sterilize by autoclaving.

Version 31.05.2022, Sara Cardoso