

Algoma University Animal Care Committee	
AU #0034 Blood sampling of non-anesthetized fish (adapted from Carleton University's SOP FAR-04)	
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1. Purpose

In fisheries and aquatic sciences research, blood sampling is commonly used to examine the physiological status of fish. In certain situations, anesthesia cannot be used because it alters the blood chemistry of the fish being sampled. For instance, some hormone (e.g., cortisol) and metabolite (e.g., lactate) concentrations change quickly in response to stressors such as handling and anesthesia. In these circumstances, blood should be sampled immediately after capture without anesthesia. In addition, sometimes there is an interest in measuring post-release behaviour of blood sampled fish (e.g., fish with telemetry devices; behavioural trials). In those instances, the use of anesthetics would alter behaviour. Finally, some jurisdictions do not permit the release of fish into natural waterbodies immediately following anesthetic use (e.g., the US Food and Drug Administration requires a 21-day holding period for any fish anesthetized with MS-222 prior to release). This protocol is designed to outline ways to minimize stress on fish when researchers need to non-lethally obtain a blood sample from fish without using anesthesia.

2. Policy

CCAC guidelines and regulations

3. Responsibility

It is the responsibility of the Principal Investigator to ensure that this SOP is followed and that those handling fish are appropriately trained to do so.

4. Training Required

Animal Research Ethics
 Handling/training in the relevant species
 Bio-methodology training in the relevant technique and species

5. Materials Required

Nitrile surgical gloves
 Heparin (lithium)
 Sharps disposal container
 25-gauge syringe needles (smaller sizes may be needed depending on species and age class)
 1 ml syringes (or other sizes as needed)

Vacutainers (various sizes – 3 to 10 ml depending on size of fish – typically green top lithium heparin)
 Vacutainer needles (21G x 1.5")
 Vacutainer cups
 Padded trough or flow through container to blood sample fish
 Cooler containing water-ice slurry

6. Procedures

General Guidelines

- a. Only caudal puncture is acceptable when blood sampling a fish without anesthesia. Do not attempt to take blood from the heart, dorsal aorta, or by tail ablation.
- b. Do not remove more than 10 percent of the overall blood content of a fish. To determine the maximum amount of blood that can be taken based on body mass see Table 1 below.
- c. Fish weighing less than 300 grams are blood sampled using a 1ml syringe. Fish weighing more than 300 grams are typically blood sampled using a vacutainer needle. Both sampling techniques are explained in more detail below.

Table 1. Maximum amount of blood that can be taken from a fish, based on body mass.

Body mass (g)	Maximum Blood sample (ml)
50	0.175
100	0.350
150	0.525
200	0.700
250	0.875
300	1.050
350	1.225
400	1.400
450	1.575
500	1.750
550	1.925
600	2.100
650	2.275
700	2.450
750	2.625
800	2.800
850	2.975
900	3.150
950	3.325
1000	3.500

A. Fish less than 300 grams

1. Put on nitrile surgical gloves (note – wet hands are sufficient for most species)
2. Attach a 25-gauge needle to the end of a 1 ml syringe
3. Draw approximately 0.9 ml of heparin into the syringe and expel it back into the heparin container. This step can be done up to 12 hours before syringes are used for sampling.
4. Hold fish securely ventral side up in a trough or container that has a constant supply of fresh water. Keep the fish fully submerged. Water temperature should be the same as location that the fish was taken from (e.g., housing tank, natural environment). Some fish can be blood sampled without any immobilization (e.g., Lake Sturgeon, Bluegill), while other species (e.g., salmonids) require electroimmobilization.
5. With the caudal fin raised out of the water while keeping the head and gills of the fish fully submerged, insert the syringe needle on a 30 to 40 degree angle into the ventral midline posterior to the anal fin (see Figure 1). The caudal vessels are directly ventral to the spine. If the needle touches the spine slightly, withdraw the needle.
6. Slowly raise the plunger of the syringe to remove blood from the fish.
7. To minimize stress and air exposure, release fish immediately after sampling.
8. Invert syringe approximately 10 times to keep the blood from coagulating.
9. Expel the blood into a micro-centrifuge tube or similar container for storage or centrifuging.
10. Dispose of the used syringe in a sharps container.
11. Dispose of nitrile gloves.
12. Prepare and store blood sample (details of centrifuging and storage will vary with study goal and are beyond the scope of this SOP).



Figure 1. Image of non-lethal blood sampling using a needle and syringe. Note that the fish is being held in supine position in a padded water-filled trough.

B. Fish more than 300 grams

1. Put on nitrile surgical gloves.
2. Screw a vacutainer needle onto the tip of a vacutainer.
3. Place the coloured rubber end of a blood collection tube into the cup end of the vacutainer. Do not apply pressure.
4. Hold fish securely ventral side up in a trough of container that has a constant supply of fresh water. Keep the fish fully submerged. Water temperature should be the same as location that the fish was taken from (e.g., housing tank, natural environment).
5. With the caudal fin raised out of the water while keeping the head and gills of the fish fully submerged, insert the vacutainer needle on a 30 to 40 degree angle into the ventral midline posterior to the anal fin (see Figure 1). The caudal vessels are directly ventral to the spine. If the needle touches the spine slightly, withdraw the needle.
6. Depress the rubber end of a blood collection tube into the vacutainer to start collecting blood. Make sure you select the appropriate blood collection tube for your research. The tube top colour indicates what additives (such as anticoagulants, sodium fluoride, etc.) are inside the tube. For most purposes the tubes with the green tops are appropriate.
7. Remove the vacutainer from the needle prior to removing the needle from the fish.
8. To minimize stress and air exposure, release fish immediately after sampling.

9. Invert syringe approximately 10 times to keep the blood from coagulating.
10. Dispose of used vacutainer needle in a sharps container.
11. Dispose of nitrile gloves.
12. Prepare and store blood sample (details of centrifuging and storage will vary with study goal and are beyond the scope of this SOP).



Figure 2. Image of non-lethal blood sampling using a vacutainer. Note that the fish is being held in supine position in a padded water-filled trough.

REVISION HISTORY		
Revision #	Revision Date	Summary of Changes
1	March 18, 2025	ACC accepted with no revisions