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Fish eRNA: water sampling and filtration through Sterivex filter unit

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We use this protocol and it's working

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Abstract

The objective of this protocol is the **sampling and the filtration of water samples through 0.45 µm Sterivex™ filter units** with the necessary precautions for **environmental RNA (eRNA) analysis**.

This protocol is used **upstream to molecular biology analysis (e.g. qPCR, metabarcoding, ddPCR) to specifically target fish eRNA**.

eRNA is easily degraded, so special precautions must be taken. These include: limit RNase contamination by working with **decontaminated equipment**, proceed **very quickly to filtration after sampling**, and **freeze the samples very quickly after filtration**, either in liquid nitrogen or in a freezer at -80°C. Preservation buffers are available to stabilise RNA at room temperature, but are not used in this protocol.

This protocol allows approximately 0.5 L to 5 L to be filtered through a Sterivex™ filter unit, depending on the characteristics of the water sample and the chosen filtration method (syringe or vacuum pump).

Guidelines

- **Water sampling**
- **Water filtration** : two options are possible, one with a syringe and the other with a vacuum pump.
- **Sample preservation** : two options are possible, one with liquid nitrogen and the other with a -80°C freezer.

Materials

- **Materials**

  - Decontaminated collection bottle (capacity from 1 L to 5 L according to water sample)

  - A cooler with ice packs

  - Field sheet + pen

  - Marker pen OR pre-printed label for sample identification

  - A -80°C freezer

  - Vacuum pump and associated filtration equipment (optional)

  - Decontaminated sampling container (e.g. Niskin bottle) if sampling at depth (optional)

  - A container to store liquid nitrogen (optional)

- **Consumables**

  - Gloves : 2 pairs per sample

  - Sterivex cartridge (Sterivex™ GP pressure filter units, 0.45µm; Millipore) : 1 cartridge per sample

  - Sterile Syringe with Luer lock (Syringes BD Plastipack Luer Lock Vol 50-60mL*)* : 1 syringe per sample

  - Caps for Sterivex (Luer-Lock male & female : outlet & inlet) : 2 per sample (to close the Sterivex after filtration)

  - Sterile bag (or tube) to store the Sterivex after filtration

Troubleshooting

Safety warnings

If liquid nitrogen is used, precautions must be taken:

Handling liquid nitrogen (-196°C) without protection is particularly dangerous. The risk of severe burns is ever-present. Handling products stored in liquid nitrogen involves the risk of both cryogenic burns and anoxia (reduced oxygen levels in the work area).

Burns can have serious consequences, especially if they affect the eyes or face. It is essential to protect yourself against 2 types of burns: splash burns and contact burns.

In addition to wearing suitable gloves, goggles or a visor, it is advisable to wear suitable clothing (apron and protective gloves).

Appropriate gloves should be worn when handling samples in a -80°C freezer.

Before start

- Prior to sampling, the **bottles/containers and filtration material used for eRNA must be decontaminated.** The following protocol may be used:

Protocol

NAME

Hydrogen peroxide decontamination of eDNA dedicated material

CREATED BY

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Preview

- Store all materials in clean bags or containers to reduce the risk of contamination.

- Wear gloves during the whole procedure

- During the handling try to reduce exposure to sunlight if possible.

WATER SAMPLING

1h

1
- Wear gloves and open the bottle without touching the inside of the cap or the neck of the bottle.
- Several options are available:

-**Surface sampling** :Collect by hand sub-surface water (10-20 cm below the water surface) and close the bottle carefully.

-**Depth sampling** : If water is collected deeper using a sampling container (e.g. Niskin bottle), it must also be decontaminated before sampling. Transfer the contents of the tank into the bottle to minimise the risk of contamination.

- After sampling, store the bottle in a clean container to avoid contamination.
- Use a cooler if samples need to be transported.

Note : As eRNA degrades very rapidly even at room temperature or at 4°C, the time between sampling and filtration should be as short as possible. Ideally, filtration should be performed immediately or, if this is not possible, within one hour of sampling.

WATER FILTRATION

1h

2
- Proceed rapidly to water filtration in order to minimise any potential RNA damage during transport. Place the water at 4 °C in the dark for max. 1 hours until filtration.
- There are **two filtration protocols**, one manual **using a syringe**, and the other **using a vacuum pump**. Both protocols are described below:

2.1 **Filtration procedure with a syringe :**

Set up is simple and does not necessarily require bench space (can be done in the field). Prepare the filtration material: gloves, Sterivex cartridge, caps and syringe.

- Put on gloves and remove the syringe and Sterivex from the blister pack (handle carefully, avoiding touching either end of the filter to avoid contamination).
- Gently homogenise the water sample & fill the syringe (60ml) with the water sample (check that the neck of the bottle is larger than the diameter of the syringe).

- Connect the syringe to the Sterivex (Figure 1)

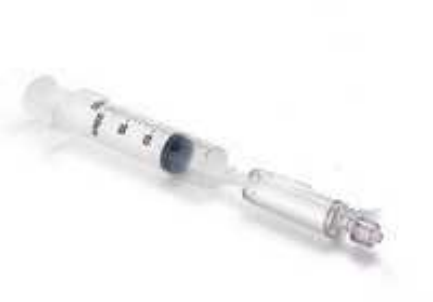

Figure 1: Sterivex cartridge attachment to the syringe

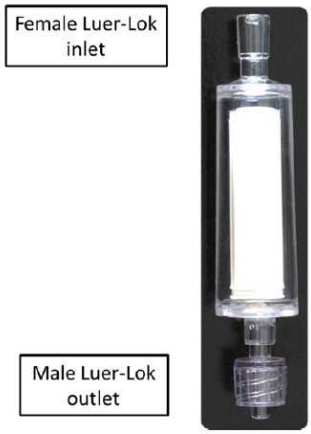

Figure 2: Sterivex cartridge Luer-Lock tips identification

- Filter (slowly) the water sample through the Sterivex unit.
- Remove the syringe from the Sterivex to refill the syringe with water and filter through the same Sterivex.
- Continue the filtration (using the same syringe and Sterivex) to filter a maximum volume of water and note the final volume of water filtered (e.g. by subtracting the remaining volume from the initial volume of water). Typical filtered volumes range from 0.5L to 5L: the volume of water filtered will depend on the microbial load and turbidity of the water sample.

Note: It is recommended that filtration does not exceed 30 minutes to limit the risk of degradation of the RNA sample on the filter.

- At the end of filtration, remove the Sterivex from the syringe and remove as much of the residual water as possible by inserting the syringe plunger and passing air through the Sterivex.

- Proceed to sample preservation (Step 3)

2.2 **Filtration procedure with a vacuum pump :**

The set-up does not necessarily require bench space, but is easier to set up in a laboratory. Prepare the filtration material: vacuum pump, tubing, filter ramp adapted to the size of the Sterivex, gloves, Sterivex cartridge, caps and syringe.

- Wear gloves and remove the Sterivex from the blister pack (handle carefully, avoiding touching either end of the filter to avoid contamination).

- Insert the male Luer lock outlet tip of the Sterivex (Figure 2) into the clean silicone filter holder (Figure 3).

Figure 3 : Sterivex filtration system with vacuum pump

- Place the clean silicone filter holder onto the filtration ramp (Figure 3).

- Remove the 50ml syringe from the blister pack and remove the plunger from the syringe to use the syringe body as a funnel (leave the plunger in the blister pack to use at the end of the filtration process).

- Connect the body of the syringe to the female Luer lock inlet tip of the Sterivex (Figure 2 & 3).

- Gently homogenise the water sample.

- Slowly fill the syringe with water directly from the bottle as if it were a funnel (Figure 3).
- Switch on the pump and open the valves (the pressure must not exceed 500 mbar (50 kPa)).
- Fill the syringe body (funnel) to ensure that there is always water in it.
- Continue the filtration (using the same syringe and Sterivex) to filter a maximum volume of water and note the final volume of water filtered. Typical filtered volumes range from 0.5L to 5L: the volume of water filtered will depend on the microbial load and turbidity of the water sample.

Note: It is recommended that filtration does not exceed 30 minutes to limit the risk of degradation of the RNA sample on the filter.

- At the end of filtration, remove the Sterivex from the silicone filter holder.
- Remove as much of the residual water as possible by inserting the syringe plunger and passing air through the Sterivex.
- Proceed to sample preservation (step 3)

Sample preservation

3 As eRNA degrades very rapidly even at room temperature or at 4°C, the time between the end of the filtration and the préservation should be as short as possible. Freezing by either method should be performed immediately after filtration. Degradation can occur in just a few minutes, so do not wait for multiple samples.

Note: The use of liquid nitrogen or a freezer at -80°C is necessary, as some RNases are active at temperatures above -70°C.

3.1 **Preservation in liquid nitrogen**

Portable tanks of liquid nitrogen can be taken in the field to freeze samples immediately after collection and filtration.

Note: the use of liquid nitrogen requires special precautions. Wear suitable protective equipment and read the recommendations carefully.

- Place the outlet and the inlet caps on the Sterivex (Figure 2).
- Annotate the Sterivex with a marker pen
- Immerse Sterivex in liquid nitrogen. Sterivex can remain in the liquid nitrogen container for several hours if freezing is carried out in the field.
- Complete the field sheet.
- Once in the laboratory, place the labelled Sterivex in a clean bag/tube and place it in a -80°C freezer. Samples can be stored for months in a -80°C freezer prior to the eRNA extraction step.

3.2 Preservation in -80°C freezer

If liquid nitrogen cannot be used and a -80°C freezer is available, samples can be frozen directly in the freezer. However, as the samples must be frozen immediately after filtration, repeated opening and closing of the -80°C freezer may cause the temperature to rise. Therefore, this method should only be considered for a limited number of samples.

Note : Appropriate gloves should be worn when handling samples in a -80°C freezer.

- Place the outlet and the inlet caps on the Sterivex (Figure 2).
- Annotate the Sterivex with a marker pen or a pre-printed label
- Place the labelled Sterivex in a clean bag/tube and place it in a -80°C freezer. Samples can be stored for months in a -80°C freezer prior to the eRNA extraction step.
- Complete the field sheet.