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BEPREP STANDARD OPERATING PROCEDURE

Short title, ID:	BEPREP_SOP-1	**Category:**	Field SOP
Title:	Water sampling		
Version:	1.0	Created on:	2023-01-04
Status:	In use	Pages:	5
Related SOPs:			
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1. Abbreviations and definitions

SOP - standard operating procedure
CSA - case study area
PPE - Personal Protective Equipment
DRS - DNA-RNA Shield

2. Aim and scope

The aim of this SOP is to provide guidelines on how to concentrate DNA from natural freshwater habitats to sample viral and bacterial microbiota (for PCR, amplicon sequencing, shotgun sequencing, metagenomics), as well as to monitor the presence of vertebrate and invertebrate fauna (for barcoding or metabarcoding of species; sequences can also be used to accurately estimate genetic diversity).

3. Health and safety information and procedures

As contact with potential pathogens especially in urban or tropical water samples is possible, laboratory gloves should be worn at all times and changed between samples.

4. Materials and equipment

Item	**Purpose/Details**
Disposable laboratory gloves	PPE
5 L plastic bucket or canister	for collecting the water
FFP2 masks	to prevent contamination and improve fieldwork staff safety
100 ml plastic syringe	for colleting the water and pushing it through the filter unit
Sterivex-GP Filter unit	pore size 0.22 µm; Millipore cat. no. SVGPL10RC
Sterivex filter caps	to seal the filter; included in the associated Qiagen extraction kit
Combi-lock seal	To seal the filter if not using the Qiagen kit https://zarys.pl/koreczek-do-kaniul-luer-lock-koreczek-combi.html
Bleach (20% v/v)	Cleaning and sterilising the equipment between sampling points
Ethanol (70% v/v)	Cleaning and sterilising the equipment between sampling points

Sterile water	Cleaning and sterilising the equipment between sampling points
Paper towels	Cleaning
5 ml syringes	For filling the filter with DRS
DNA/RNA Shield	Zymo Research

Note: Bleach and ethanol are used to sterilize the plastic bucket/canister between sampling points and sites, therefore the required volume might vary depending on the number of sampling points and sites

5. Water sampling for bacterial microbiota, viromes and vertebrate DNA

To make this method as accessible and inexpensive as possible (and to avoid stirring up sediment), samples are taken **from the shore** of wetland or stream sites. If not possible, make sure that the water is taken upstream from where you are standing or with stirring up as little sediment as possible.

For amphibian monitoring, we suggest sampling water at peak amphibian activity, i.e., when eggs and larvae are or would normally be present. We suggest spatial replicates every 10-20 m perimeter.

1. All the equipment should be wiped or rinsed with bleach, ethanol and sterile water in that order between sampling points and sites.
2. Wearing disposable gloves (**which must be changed between sampling points and sites**) and a mask, collect about 5 litres of water (or less if there is very little water in the water body) from just under the surface of the water body in the sterilized plastic canister and swilled carefully to homogenize the water.

   Stirring up sediment should be carefully avoided. If the sample sites are very small or ephemeral puddles, water can be drawn up directly with the 100 ml syringe.
3. Draw up water from the canister with the 100 ml syringe, fit the filter on the syringe and push the water (about 30 ml) through until the filter clogs into a sterile 50 ml Falcon tube (**if you are studying viromes; otherwise, you do not need to save the filtrate**).
4. Write down the water volume that has been filtered.

Note: The amount of filtered water per filter unit will vary widely among sites, ranging between about 100 ml and more than one litre, depending on water turbidity.

5. Repeat steps 3 and 4 to obtain 2 clogged filters from each sampling point.
6. Dry each filter by pushing air through it using the same syringe.
7. Using the 5 ml syringe, carefully fill the filter with DNA/RNA Shield.
8. Cap the filter at both ends with the inlet and outlet caps and store at ambient temperature (or in a Styrofoam box with freezer packs if temperature in your vehicle is more than 20°C) or in a portable fridge until arrival in the laboratory.
9. Water samples in the 50 ml Falcon tubes (for viromes) should be kept cool (4 °C) and frozen (-20 °C) as soon as possible. **Remember that water expands, and that the Falcon tube should not be completely full.**
10. The number of sample points per CSA will likely vary, and will need to be agreed upon with the BEPREP team.

Figure 1. Phases of the sampling protocol. From left to right: collection and mixing of approximately 5 L of water (point 2), filtration (point 3), and detail of the syringe and Sterivex filter used.

6. Sample storage

In the field store the samples at room temperature or in a cooler in room temperature exceeds 20 °C.

In the laboratory filters filled with DNA/RNA shield can be stored at temperature below −20°C for an indefinite time, at ambient temperature (5°C- 30°C) for a maximum of 30 days, at 35°C- 40°C for a maximum of 7 days.

7. Sample transportation

Samples filled with DRS can be shipped at room temperature.

8. References

Zanovello L., Girardi M., Marchesini A., Galla G., Casari S., ...Hauffe H.C. 2023. A validated protocol for eDNA-based monitoring of within-species genetic diversity in a pond-breeding amphibian. *Sci Rep* **13**, 4346. https://doi.org/10.1038/s41598-023-31410-4

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