SLOVENSKI STANDARD
SIST EN 17805:2023

01-junij-2023

Kakovost vode - Vzorčenje, zbiranje in konzerviranje okoljske DNK iz vode

Water quality - Sampling, capture and preservation of environmental DNA from water

Wasserbeschaffenheit - Probenahme, Erfassung und Konservierung von Umwelt□DNA in Wasser

Qualité de l'eau - Échantillonnage, collecte et conservation de l'ADN environnemental prélevé dans l'eau

Ta slovenski standard je istoveten z: **EN 17805:2023**

ICS:

13.060.45	Preiskava vode na splošno	Examination of water in general
13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water

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English Version

Water quality - Sampling, capture and preservation of environmental DNA from water

Qualité de l'eau - Échantillonnage, collecte et conservation de l'ADN environnemental prélevé dans l'eau

Wasserbeschaffenheit - Probenahme, Erfassung und Konservierung von Umwelt-DNA in Wasser

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European foreword

This document (EN 17805:2023) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2023, and conflicting national standards shall be withdrawn at the latest by September 2023.

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Introduction

WARNING — Persons using this document should be familiar with water sampling protocols to assess biological diversity. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices.

Moreover, the need of notification, obtaining certificates or permits prior to sampling, depending on national or international laws and regulations such as the Nagoya Protocol on Access to Genetic Resources (https://www.cbd.int/abs/), needs to be considered.

The monitoring of organisms is key to the assessment of the status of aquatic ecosystems and is required by national and international legislation such as the European Union Water Framework Directive (2000/60/EC). A range of methods have been described how to monitor organisms in aquatic environments, leading to a wide range of European standards (e.g. EN 14011:2003, EN 14757:2015, EN 15460:2007). These approaches, however, necessitate the capture and/or collection of the organisms of interest, which can be a laborious and time-consuming process.

The possibility to detect the presence of organisms and/or quantify relative abundance (e.g. [6]) in aquatic environments via the analysis of environmental DNA (eDNA) provides a novel means to monitor biodiversity across a wide range of taxonomic groups, including microorganisms, plants and animals ([7][8][9]). This approach allows to examine organismic diversity without the need to directly isolate and capture organisms and it is expected to play a key role for future biomonitoring aiming at temporally and spatially highly resolved species inventories [10]. Albeit the power of the eDNA approach has been repeatedly reported [11], there is a great need for standardizing the application of eDNA-based assessment of aquatic biodiversity ([12], [13]). Note, however, that eDNA-based biomonitoring currently does not allow to obtain certain population parameters (e.g. individual size, sex) which can be obtained by traditional sampling techniques.

This document provides guidance how to sample and preserve eDNA from water samples, addressing the first and crucial step for any further downstream eDNA-based analyses of biodiversity. A specific technical report for the routine sampling of benthic diatoms from rivers and lakes adapted for metabarcoding analyses is CEN/TR 17245:2018.

1 Scope

This document specifies procedures for sampling, capture and preservation of environmental DNA (eDNA) in aquatic environments, stemming from organisms that are or have recently been present in a waterbody, have visited it or whose DNA has been introduced to the waterbody through some mechanism. This document also covers procedures for avoiding sample contamination and ensuring DNA quality, key properties of the filtering procedure and equipment and reporting standards.

This document does not include the collection of eDNA from biofilms, sediments or similar sample types and does not cover sampling designs.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— IEC Electropedia: available at https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
cross-contamination
unintended transfer of any source of and/or DNA from one sample to another sample

3.2
decontamination
procedure to remove any source and/or trace of DNA from material that might come into contact with the sample

3.3
enclosed filter
filtering device where the filter membrane is encapsulated and where the inflow and outflow can be closed for transport and storage

Note 1 to entry: The eDNA contained on the filter is typically extracted without removing the membrane from the filter capsule greatly reducing the risk of contamination of samples. See Figure A.1 C. in Annex A.

3.4
environmental DNA
eDNA
material stemming e.g. from dead or from living organisms and include single-stranded (ss) and double-stranded (ds) DNA fragments from nuclear and mitochondrial/plastid DNA of eukaryotes as well as plasmid DNA of prokaryotes

Note 1 to entry: Subsuming DNA from various sources such as unicellular or small multicellular organisms or tissue particles (e.g. shed cells, faeces) and gametes of multicellular organisms.

3.5
field equipment blank
sample obtained from processing target DNA-free water (e.g. distilled water) through all the equipment used and covering all procedures involved in the eDNA sampling process to allow checking that the equipment and procedures do not introduce DNA contamination

3.6
housed filter
systems in which a filter membrane is protected within a solid housing during the filtration process

Note 1 to entry: The filters are removed from the housing for eDNA extraction. The housing can be opened and the filter removed for preservation and later processing. See Figure A.1 B. in Annex A.

3.7
lysis buffer
buffer solution to preserve DNA present in the sample and to lyse/open cells as a first step of the DNA extraction

3.8
internal positive control
IPC
known fragment of synthetic or natural DNA containing an amplifiable and quantifiable sequence that will not naturally occur in the sample

Note 1 to entry: The IPC can be added to the sample or the preservation/lysis buffer at a known concentration to verify the efficiency of DNA preservation, DNA extraction, DNA amplification and DNA identification.

3.9
open filter
filtering device including filtration towers (laboratory) and filtration backpacks from which the filter membrane has to be removed by hand for further processing

Note 1 to entry: See Figure A.1 A. in Annex A.

3.10
pre-filter
filter membrane, mesh or hose strainer with a larger pore-size than the main filter membrane (for capturing the eDNA) through which water is passed first to remove larger particles of sediment, plant material or algae to increase the volume of water that can be filtered before saturation of the main filter

3.11
sample contamination
process by which exogenous DNA is unintentionally introduced to the sample during the sampling process

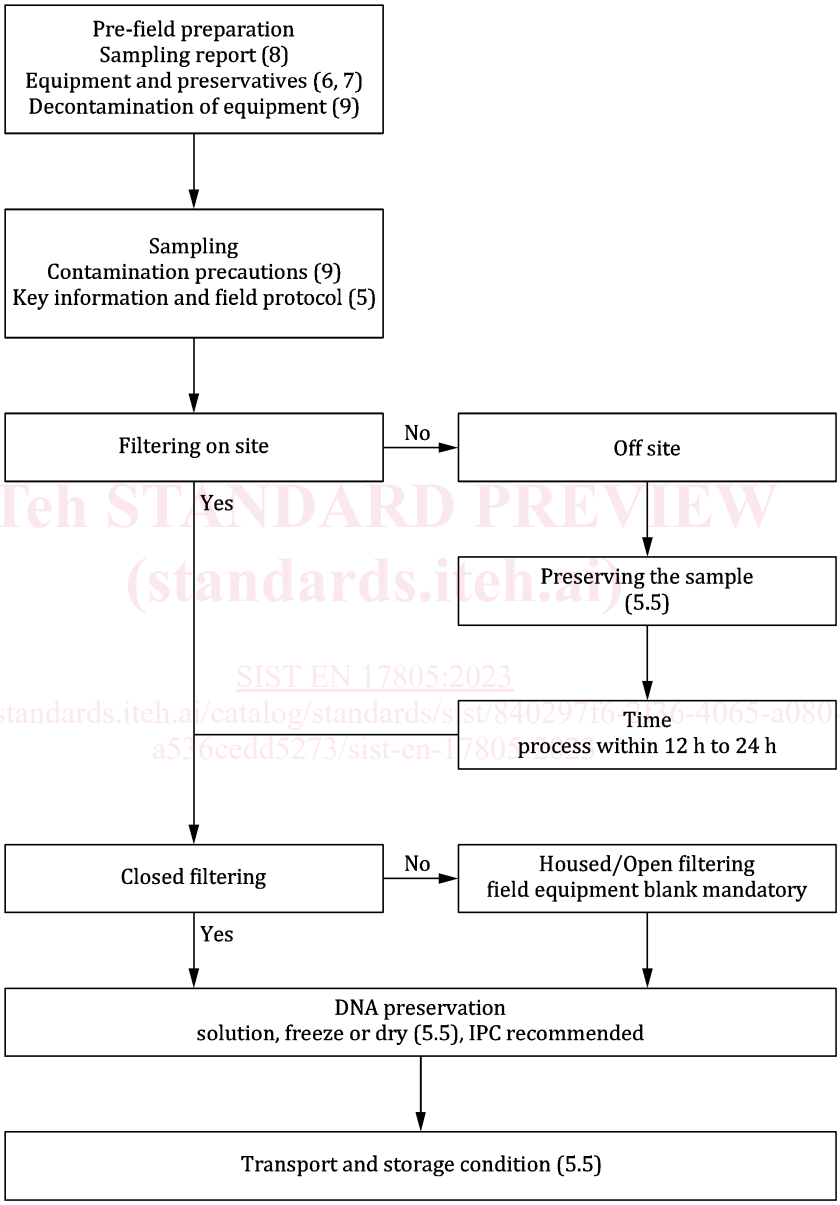
Note 1 to entry: DNA that is already present in the water before the eDNA sampling was undertaken is not considered as contamination.

3.12
target DNA
any source and/or trace of DNA from the surveyed species/taxa

4 Principle

A representative water sample from the surveyed water body is sampled according to an appropriate sampling design to capture and separate eDNA from the water sample. During the whole procedure (cross-)sample contamination with target DNA is avoided and eDNA integrity is guaranteed.

An overview on the key steps and considerations for the eDNA water sampling process is provided in Figure 1.



NOTE Numbers in parentheses refer to the respective clause/subclause.

Figure 1 — Key steps and considerations for the eDNA water sampling process

5 Procedure

5.1 General

Water shall be sampled to capture and separate eDNA via filtration or other processes. The probability of obtaining eDNA from the targeted organism(s) is positively correlated with:

— the number of samples per waterbody;

— the spatial representativeness of the samples;

— the volume of water filtered;

— the optimum sampling time point/season with regard to the organism(s) eDNA shedding rates, abundances, metabolic activity and locomotion.

5.2 Considerations prior to fieldwork

Depending on the different applications/goals of each eDNA survey, the most appropriate sampling conditions and design shall be assessed based on case-by-case evidence to obtain water samples representative of the water body and the organisms which shall be monitored. These might include hydrological, meteorological, seasonal/temporal and biological/ecological variation.

This is particularly important in lentic (non-flowing) water bodies since eDNA is often unevenly distributed when the water is not well mixed. Representative sampling can be achieved by merging subsamples collected at different points in the water body, or alternatively by continuous sampling systems that move across the water body while drawing up water. When surveying deep water bodies and targeting deep water dwelling organisms, it may be necessary to sample water from depth.

To maximize the probability of capturing target DNA, the following shall be considered when planning where and when to collect samples and subsamples:

1) Features of the water body, including its size, depth, flow and the distribution of microhabitats as well as inlets/outlets of the waterbody.

2) Biology of all target taxa, including habitat preferences and lifecycle. Detection probability for individual species can be increased by timing sampling to coincide with times of intense activity (e.g. spawning). Temporal variations in the amounts of released eDNA by the target species needs to be considered. It is also important to consider whether target taxa are likely to be present in the water body at the time of sampling, especially in the case of amphibious or migratory species.

5.3 Equipment preparation prior to fieldwork

Prior to fieldwork the collecting vessels and equipment need to be cleaned to avoid contamination (for detailed instructions, see Clause 9).

5.4 Sampling the eDNA from water

Various systems are used for sampling and filtering water. Some involve initially gathering water into a collecting vessel where it is mixed and then filtered subsequently; other systems filter the water directly as it is drawn up from the water body. When the water is not filtered directly in the water body, the filtration can be carried out on the shore or in the laboratory.

Water shall be sampled and/or filtered to capture tissue fragments, cells and DNA. This may be achieved manually with syringes or using a hand or powered pump. If a pump is used and water passes