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**Proficiency testing provider accredited by NAH under No NAH-8-0003/2023.**

# SURFACE WATER TESTING – Biological and toxicological tests

## WORK INSTRUCTIONS
## YEAR 2026 PROGRAMME
## PT ID: QDJV-2

| Parameter | Number of samples | Containers | Tartósítás, minta jellege |
|---|---|---|---|
| **chlorophyll-a** | 2 | plastic flask with 1.5 $dm^3$ sample | Surface water, preserved by cooling |
| **Total algal count** | 2 | plastic flask with 0.5 $dm^3$ sample | Surface water preserved by Lugol's iodine solution |
| **Daphnia immobilisation test** | 2 | plastic container with 20 $cm^3$ sample | Artificial test sample prepared from analytically pure (a.r.) reagents |

## 1. Determination of chlorophyll-a concentration:

**Sample ID: FSZ-HB-1, FSZ-HB-2**

**Analytical method:**

MSZ ISO 10260:1993 – Water quality. Measurement of biochemical parameters. Spectrophotometric determination of chlorophyll-a concentration.

Since this is a fresh (live) sample, the analysis should be performed within **24 hours**.

Until processing, samples must be stored at low temperature (refrigerated) and in the dark.

## 2. Determination of total algal count:

**Sample ID: FSZ-HB-3, FSZ-HB-4**

From the Lugol-preserved, non-concentrated, homogenized sample, a known volume is transferred into a counting chamber, and the sample is allowed to settle.

During homogenization, the sample should not be shaken too vigorously, as cell aggregates may disintegrate, especially in the case of certain cyanobacteria and other loosely associated algal communities (e.g. Synura species).

It is advisable to combine horizontal circular motion with gentle vertical up-and-down movement.

The settling rate is approximately 4 hours/cm, but the sample should be allowed to settle for at least 1 hour when using a 2 mL chamber.

Afterwards, the chamber is placed on the microscope stage and counting is performed using a 40× objective (at least 400× magnification).

Any dilution, as well as the counting field (strip width or chamber area: quarter, half, or full chamber), should be selected so that at least 400 individuals are counted in one analysis.

This ensures a maximum error of ±10%.

Larger organisms present in low abundance should be counted using a lower magnification objective (10×) by examining the entire chamber bottom.

The number of individuals can be clearly determined only for unicellular algae.

However, many algal species commonly occur as colonies or filaments consisting of tens to hundreds of cells. According to accepted algological convention, a single individual is defined as:

- a solitary cell,
- a colony,
- a coenobium,
- or a filament,

regardless of the number of cells forming it.

During the intercalibration study, all algal and cyanobacterial individuals/colonies larger than 3 µm in diameter must be counted.

Organisms crossing the counting frame are **excluded** on the left and top sides, **included** on the right and bottom sides.

If capacity allows, it is recommended to perform **multiple analyses from the same sample** and report the **average algal count**. This helps assess **within-laboratory variability** and improves result accuracy.

If possible, two biologists should examine the same sample (even multiple times), as this provides valuable data regarding **analytical accuracy and reproducibility**.

## 3. Daphnia test

**Sample IDs: FSZ-TOX-1, FSZ-TOX-2**

**Test standard:** MSZ EN ISO 6341:2013 – Water quality. Determination of the inhibition of mobility of *Daphnia magna* Straus (Cladocera, Crustacea). Acute toxicity test (ISO 6341:2012).

**Test organism:** *Daphnia magna Straus* 24-hour-old individuals

**Dilution water:** Synthetic dilution water (according to Section 6.3 of MSZ EN ISO 6341:2013), or water of controlled quality used for maintaining the *Daphnia* culture, or another suitable water.
Please note that if the test is not carried out in the same water used for maintaining the *Daphnia* culture, it is recommended to provide an approx. 2-week acclimatization period for the organisms to adapt to the alternative dilution water. This helps eliminate errors caused by stress due to changes between the culture medium and dilution water.

**Sample preparation:** From the solutions provided in vials, take 10 mL and dilute to a final volume of 2 L; this will serve as the stock solution for the tests.

From the stock solution, prepare a dilution series (1×, 2×, 4×, …) appropriate for determining 50% immobilisation (dilution factor, dilTE).

For each dilution, perform the test using 20 individuals of Daphnia that are not older than 24 hours. Ensure that freshly hatched Daphnia are not used, as they may not yet have had sufficient time to feed.

The number of mobile (non-immobilized) Daphnia is recorded after 24 and 48 hours in the probit template.

Before the experiment, a reference test with potassium dichromate must be performed using the given Daphnia culture, as required by the standard. The 24 h $EC_{50}$ value should also be recorded.

**Additional notes:** From the solutions provided in vials, take 10 mL and dilute to a final volume of 2 L; this will serve as the stock solution for the tests.

- Samples sent for toxicological testing may be stored in a refrigerator.
- For standardized evaluation, please use the attached probit template:
  In the “dilution” column, enter the actual dilution factor without “×”.

## 4. SUBMISSION OF RESULTS:

Participants are requested to report the measured values together with their **expanded measurement uncertainty** (coverage factor k = 2), expressed in the same unit as the measurement results.

Results must be submitted electronically via www.qualcoduna.hu electronic submission portal.

Results submission steps:

- Open the website and select **Login to electronic services**.
- Log in to access the participant interface.
- Select Recording and viewing measurement results.
- After submission, a confirmation will be generated (save/print it).

- Keep and verify the confirmation.
- Important: if no confirmation appears, submission was not successful.

**SUBMISSION DEADLINE: JULY 3, 2026 (FRIDAY)**

Results submitted after the deadline, values marked with "<" or ">", or results in different units will not be considered. (Reference: ISO 13528:2022. Statistical methods for use in proficiency testing by interlaboratory comparisons).

Reporting zero ("0") as a result will be considered a physically incorrect value and will be evaluated.

Budapest, 21st May, 2026.

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