



SLOVENSKI STANDARD
oSIST prEN ISO 5667-16:2016
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**Kakovost vode - Vzorčenje - 16. del: Navodilo za biološke preskuse vzorcev
(ISO/DIS 5667-16:2016)**

Water quality - Sampling - Part 16: Guidance on biotesting of samples (ISO/DIS 5667-16:2016)

Wasserbeschaffenheit - Probenahme - Teil 16: Anleitung zur Probenahme und Durchführung biologischer Testverfahren

Qualité de l'eau - Échantillonnage - Partie 16: Lignes directrices pour les essais biologiques des échantillons (ISO/DIS 5667-16:2016)

Ta slovenski standard je istoveten z: prEN ISO 5667-16

ICS:

13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water
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Water quality — Sampling —

Part 16: Guidance on biotesting of samples

Qualité de l'eau — Échantillonnage —

Partie 16: Lignes directrices pour les essais biologiques des échantillons

ICS: 13.060.45

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ISO/CEN PARALLEL PROCESSING

This draft has been developed within the International Organization for Standardization (ISO), and processed under the **ISO lead** mode of collaboration as defined in the Vienna Agreement.

This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel five month enquiry.

Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month approval vote in ISO and formal vote in CEN.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committees responsible for this document are ISO/TC 147, *Water quality*, Subcommittee SC 6, *Sampling (general methods)* and, under the Vienna Agreement, CEN/TC 230, *Water analysis*.

This second edition cancels and replaces the first edition (ISO 5667-16:1998), which has been technically revised.

ISO 5667 consists of the following parts, under the general title *Water quality — Sampling*:

- *Part 1: Guidance on the design of sampling programmes and sampling techniques*
- *Part 3: Preservation and handling of water samples*
- *Part 4: Guidance on sampling from lakes, natural and man-made*
- *Part 5: Guidance on sampling of drinking water from treatment works and piped distribution systems*
- *Part 6: Guidance on sampling of rivers and streams*
- *Part 7: Guidance on sampling of water and steam in boiler plants*
- *Part 8: Guidance on the sampling of wet deposition*
- *Part 9: Guidance on sampling from marine waters*

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- *Part 10: Guidance on sampling of waste waters*
- *Part 11: Guidance on sampling of groundwaters*
- *Part 12: Guidance on sampling of bottom sediments*
- *Part 13: Guidance on sampling of sludges*
- *Part 14: Guidance on quality assurance and quality control of environmental water sampling and handling*
- *Part 15: Guidance on the preservation and handling of sludge and sediment samples*
- *Part 16: Guidance on biotesting of samples*
- *Part 17: Guidance on sampling of bulk suspended solids*
- *Part 19: Guidance on sampling of marine sediments*
- *Part 20: Guidance on the use of sampling data for decision making - Compliance with thresholds and classification systems*
- *Part 21: Guidance on sampling of drinking water distributed by tankers or means other than distribution pipes*
- *Part 22: Guidance on design and installation of groundwater monitoring points*
- *Part 23: Guidance on passive sampling in surface waters*

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Introduction

Biological tests are suitable for determining the effect of environmental samples like treated communal and industrial waste water, fresh water, aqueous extracts (e.g. leachates, eluates) of solid material, pore water of sediments, whole sediments or chemical substances on test organisms under specific experimental conditions. These effects can be enhancing or inhibiting, and can be determined by the reaction of the organisms (e.g. death, growth, proliferation, morphological and physiological changes). Inhibiting effects are triggered by toxic water constituents or by other noxious influences.

The apparent toxicity measurable in the biological test is the result of the interaction between the constituents of an environmental sample or a toxic substance and the test organism. The protective potential of the biological system, for instance by metabolic detoxification and excretion, is an integral part of the biological test.

Apart from the direct toxic effect of one or more sample constituents, damaging biological effects can be exerted by the combined action of all noxious substances (e.g. by substances which are not toxic *per se* but affect the chemical or physical properties of the test batches by interfering with the test specific additives (e.g. nutrients, salts) and, consequently, the living conditions for the organisms. This applies for instance to oxygen-depleting substances, coloured substances or turbid matter which reduce light exposure.

Biological tests also include those tests which examine the effect of organisms on substances (e.g. microbial degradation studies).

The result of the biological test refer primarily to the organism used in the test and the conditions stipulated in the test procedure. A harmful effect stated by means of standardized tests can justify concern that aquatic organisms and biocoenosis might be endangered. The results, however, do not permit direct or extrapolative conclusions as to the occurrence of similar effects in the aquatic environment. This applies in particular to suborganismic tests, as important properties and physiological functions of intact organisms (e.g. protective integuments, repair mechanisms) are removed or deactivated.

In principle there is no organism which can be used to test all the effects on the biocoenosis or the ecosystem possible under the various constellations of abiotic and biotic conditions. Only a few ("model") species representing relevant ecological functions can be tested in practice.

Besides these fundamental and practical limitations in the selection of test organisms, the sample to be tested can also pose experimental problems on biotesting. Environmental samples (e.g. waste water, whole sediment) are complex mixtures and often contain sparingly soluble, volatile, unstable, coloured substances and/or suspended, sometimes colloidal, particles. The complexity and heterogeneity of materials give rise to a variety of experimental problems when performing biotests.

Special problems are related to the instability of the test material due to reactions and processes such as:

- physical (e.g. phase separation, sedimentation, volatilization);
- chemical (e.g. hydrolysis, photodegradation, precipitation); and/or
- biological (e.g. biodegradation, biotransformation, biological uptake in organisms).

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Other problems, especially if spectrometric measurements are applied, relate to turbidity and colour.

This part of ISO 5667 is one of a group of International Standards dealing with the sampling of waters and should be read in conjunction with the other parts of ISO 5667 and in particular with parts 1, 3 and 15.

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Water quality — Sampling — Part 16: Guidance on biotesting of samples

1 Scope

This part of ISO 5667 gives practical guidance on sampling, pre-treatment, performance and evaluation of environmental samples in the context of performing biological tests. Information is given on how to cope with the problems for biotesting arising from the sample and the suitability of the test design.

It is intended to convey practical experience concerning precautions to be taken by describing methods successfully proven to solve or to circumvent some of the experimental problems of biotesting of waters.

Reference has been made as far as possible to existing International Standards and guidelines. Information taken from published papers or oral communication is utilized as well.

Primarily dealt with are substance-related problems concerning sampling and pre-treatment of environmental samples (e.g. waste water samples) for the performance of biotests.

Special emphasis is laid on ecotoxicological testing with organisms (single-species biotests). Some features addressed in this general guidance apply as well to biotests using single-cell systems (*in vitro* biotest) and biodegradation studies as far as sampling and sample preparations are concerned. Testing of substances in the water solubility range is also addressed.

This part of ISO 5667 is applicable to biological tests for determining the effect of environmental samples like treated communal and industrial waste water, fresh water, aqueous extracts (e.g. leachates, eluates), pore water of sediments and whole sediments. This part of ISO 5667 is also applicable to chemical substances.

This part of ISO 5667 is not applicable to bacteriological examination of water. Appropriate methods are described in other International Standards.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

ISO 5667-10, *Water quality — Sampling — Part 10: Guidance on sampling of waste waters*

ISO 5667-15, *Water quality — Sampling — Part 15: Guidance on the preservation and handling of sludge and sediment samples*

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3 Terms and definitions

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

3.1

blank

mixture of water and nutrients without test organism

3.2

cell density

x

number of cells per unit volume of medium

[SOURCE: ISO 10253:2006^[1], 3.1]

Note 1 to entry: Cell density is expressed in cells per millilitre.

3.3

control

control medium (3.4), or control sediment (3.5), including organisms used in the test, without test sample

3.4

control medium

combination of water and additives (e.g. nutrients, salt) used in the test

3.5

control sediment

defined artificial or natural sediment used in the test

[SOURCE: ISO 10872:2010^[2], 3.7, modified]

3.6

dilution level

D

reciprocal value of the volume fraction of test sample in dilution water in which the test is conducted

EXAMPLE 250 ml of waste water in a total volume of 1 000 ml (volume fraction of 25 %) represents dilution level $D = 4$.

[SOURCE: ISO 15088:2007^[3], 3.2]

3.7

dilution water

water added to the test sample to prepare a series of defined dilutions

[SOURCE: ISO 20079:2005^[4], 3.7]

Note 1 to entry: The composition of the water is specified in the specific test.