



SLOVENSKI STANDARD

SIST ISO 21338:2011

01-junij-2011

Kakovost vode - Določevanje kinetike zaviralnih učinkov sedimenta, drugih trdnih in obarvanih vzorcev na emitirano svetlobo *Vibrio fischeri* (kinetic luminescent bacteria test)

Water quality - Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri* (kinetic luminescent bacteria test)

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Qualité de l'eau - Détermination cinétique des effets inhibiteurs des échantillons de sédiment, autres solides et des échantillons colorés sur la luminescence de *Vibrio fischeri* (essai cinétique de bactéries lumineuses)

Ta slovenski standard je istoveten z: ISO 21338:2010

ICS:

13.060.60	Preiskava fizikalnih lastnosti vode	Examination of physical properties of water
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en

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INTERNATIONAL STANDARD

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21338

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Water quality — Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri* (kinetic luminescent bacteria test)

*Qualité de l'eau — Détermination cinétique des effets inhibiteurs des échantillons de sédiment, autres solides et des échantillons colorés sur la luminescence de *Vibrio fischeri* (essai cinétique de bactéries luminescentes)*

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Reference number
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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 21338 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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Introduction

The method specified in this International Standard is a kinetic modification of the luminescent bacteria test specified in ISO 11348. The kinetic method overcomes the problems arising from intense colour and turbidity in the samples. There is no need for sedimentation or centrifugation of turbid samples, or for the correction of colour as described in ISO 11348.

This kinetic method uses luminometers capable of dispensing luminescent bacteria to the samples and measuring the luminescent intensity over a period of time. In the method, the bacterial suspension is dispensed and mixed with the sample in the measurement chamber of the luminometer. Several suitable instruments are commercially available, but only a few of them are capable of cooling the measurement chamber to $(15 \pm 1) ^\circ\text{C}$ as specified in ISO 11348. However, if the bacterial suspension and test samples are kept at $(15 \pm 1) ^\circ\text{C}$ in a thermo-block before the measurement and during the whole incubation, the actual temperature during the contact time is $(15 \pm 1) ^\circ\text{C}$.

The measurements specified in this International Standard can be carried out using freshly prepared bacteria, as well as freeze- or liquid-dried bacterial preparations. The various bacterial preparations can deliver different results, especially in the presence of heavy metals (see ISO 11348). The laboratories responsible for the results have the opportunity to select the most suitable bacterial preparation based on expert judgement and information about the samples to be tested.

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Water quality — Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri* (kinetic luminescent bacteria test)

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard 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 safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies the kinetic direct-contact method for determining the inhibitory effect of suspensions of sediment and other solid samples, and also for problematic turbid or coloured aqueous samples on the light emission of the marine bacterium *Vibrio fischeri* (NRRL B-11177).

This method is applicable to:

- a) sediment samples and water suspensions of sediments (fresh water, brackish, and seawater sediments);
- b) effluents (especially turbid and coloured);
- c) aqueous extracts (e.g. leachates, eluates, elutriates) of soil, solid waste, and other solid material (especially turbid and coloured).

2 Normative references

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

ISO 5667-16:1998, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

ISO 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method*

ISO 11348-1, *Water quality — Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) — Part 1: Method using freshly prepared bacteria*

ISO 11348-2, *Water quality — Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) — Part 2: Method using liquid-dried bacteria*

ISO 11348-3:2007, *Water quality — Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) — Part 3: Method using freeze-dried bacteria*

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3 Terms and definitions

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

3.1

contact time

duration of contact between one object or substance and another

NOTE In the test, the contact time is the time available to control or sample for contact with the test bacteria.

3.2

control sample

sample used in a laboratory in order to check or monitor the instrument or measurement performance or to monitor changes in a sample under investigation

3.3

correction factor

dimensionless multiplier to correct data for known influences affecting their values as measured

NOTE In the test, the correction factor, f_{kt} , serves to correct the initial luminescence intensity of the sample.

3.4

peak value

maximum signal recorded in response to a stimulus

NOTE In the test, the peak value is the maximum signal which is recorded immediately after all the bacteria are in contact with the sample.

3.5

reference sample

when the effect or behaviour of a substance is known from previous tests (reference substance) and when this substance is examined within the framework of a test series as test sample, this is called the reference sample

NOTE Adapted from ISO 5667-16:1998.

3.6

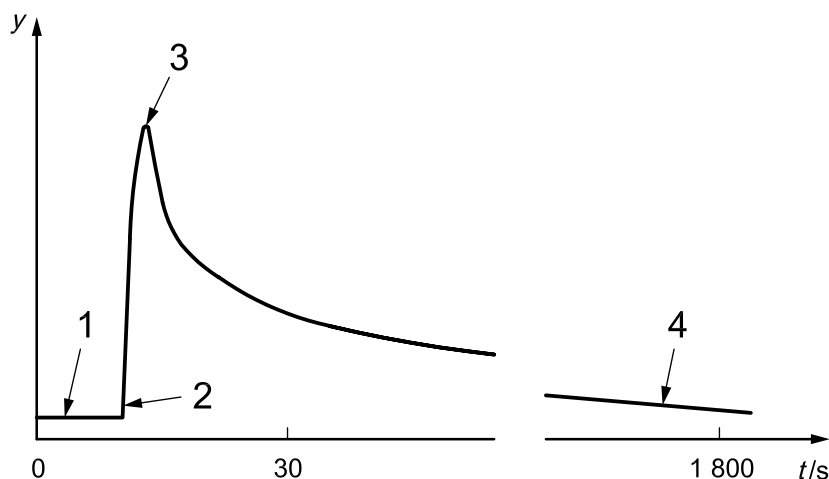
test sample

test sample is made from the sample by means of various preparatory steps specific to the sample and the test, e.g. by dissolving, homogenizing, sedimenting, filtering, neutralizing or aeration

[ISO 5667-16:1998]

4 Principle

The inhibition of light emission by cultures of *Vibrio fischeri* is measured kinetically by following the light emission of cultures from the very beginning of the assay. This is accomplished by dispensing the luminescent bacteria suspension into the sample in a cuvette or other suitable vessel (e.g. microtiter plate) already in the measuring position in the luminometer. The light emission is measured and recorded from the moment of dispensing of the bacterial suspension to the sample until the maximum value has been reached and not only at the maximum value of intensity (peak value) which usually occurs within 5 s of mixing, and after a contact time of 15 min and 30 min or optionally 5 min (Figure 1).



Key

- t time
- y relative light units
- 1 start measurement
- 2 inject bacteria
- 3 record peak value from 0 s to 5 s
- 4 mix the sample before recording signal at 30 min

Figure 1 — Principal schematic protocol for the kinetic luminescent bacteria test

Vibrio fischeri suspension is dispensed and mixed into the sample in the measurement chamber of the luminometer.

The test criterion is the decrease of the luminescence at each endpoint compared to the peak value, taking into account a correction factor, f_{kl} , which is measured from intensity changes of control samples during the exposure time. The inhibitory effect of the sample can be determined as the lowest ineffective dilution (LID) value, or as effective concentration (EC₂₀ or EC₅₀) values by means of dilution series (e.g. as described in Annex C). The LID value is the most concentrated test batch tested at which the inhibition of light emission is <20 %. For higher levels of inhibition, the dilution effect relationship can be determined graphically or by statistical analysis. The inhibition by a sample is expressed as the concentrations which results in 20 % and 50 % light reduction compared to the blank (EC₂₀ and EC₅₀). This value is interpolated within the dilution series.

No extra correction procedures for colour and turbidity are needed because these factors remain the same during the whole measurement. Inhibition at different contact times and different sample concentrations yields complete kinetic toxicological data about the sample (inhibition, expressed as a percentage vs. concentration vs. time) and enables assumptions to be made about the nature of the contaminants if compared with existing data (see Annex B).

5 Interferences

Volatile substances or substances which react with the dilution water or the test suspension, or alter their state during the test period, may affect the result or impair the reproducibility of the test results.

Since oxygen is required for the bioluminescence (Reference [18]), samples with high oxygen demand (or low oxygen concentration) may cause deficiency of oxygen and be inhibitory.

Readily biodegradable nutrients in the sample may cause a pollutant-independent reduction in bioluminescence (Reference [19]).