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Vattenundersökningar – Bestämning av vattenprovers hämmande effekt på emissionen av ljus hos *Vibrio fischeri* (Test med luminiscerande bakterier) – Del 3: Metod som använder frystorkade bakterier (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 (ISO 11348-3:2007)

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Europastandarden EN ISO 11348-3:2008 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN ISO 11348-3:2008.

Denna standard ersätter SS-EN ISO 11348-3, utgåva 1.

The European Standard EN ISO 11348-3:2008 has the status of a Swedish Standard. This document contains the official English version of EN ISO 11348-3:2008.

This standard supersedes the Swedish Standard SS-EN ISO 11348-3, edition 1.

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EUROPEAN STANDARD

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EUROPÄISCHE NORM

EN ISO 11348-3

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Supersedes EN ISO 11348-3:1998

English Version

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 (ISO 11348-3:2007)

Qualité de l'eau - Détermination de l'effet inhibiteur d'échantillons d'eau sur la luminescence de Vibrio fischeri (Essai de bactéries luminescentes) - Partie 3: Méthode utilisant des bactéries lyophilisées (ISO 11348-3:2007)

Wasserbeschaffenheit - Bestimmung der Hemmwirkung von Wasserproben auf die Lichtemission von Vibrio fischeri (Leuchtbakterientest) - Teil 3: Verfahren mit gefriergetrockneten Bakterien (ISO 11348-3:2007)

This European Standard was approved by CEN on 29 October 2008.

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Contents

Page

Foreword

The text of ISO 11348-3:2007 has been prepared by Technical Committee ISO/TC 147 “Water quality” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 11348-3:2008 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 May 2009, and conflicting national standards shall be withdrawn at the latest by May 2009.

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The text of ISO 11348-3:2007 has been approved by CEN as a EN ISO 11348-3:2008 without any modification.

Introduction

The measurements specified in ISO 11348 can be carried out using freshly prepared bacteria, as well as freeze-dried or liquid-dried bacterial preparations.

Standardized work carried out by DIN Normenausschuss Wasserwesen and ISO/TC 147/SC 5/WG 1 has shown that, in special cases, these different techniques may give different results, especially in the presence of heavy metals.

Such varying sensitivity is caused by differences in media composition used in the preparation of freeze-dried or liquid-dried bacteria. These protective media influence the bioavailability of toxicants and/or the light emission of luminescent bacteria. This means that the origin and type of preparation need to be taken into account when interpreting the results. This may be difficult sometimes, as freeze-dried and liquid-dried bacteria may be obtained from different suppliers. This, in turn, can mean that the composition is not known in detail and therefore cannot be interpreted by the user.

For this reason, in addition to toxicity measurements with liquid-dried bacteria (ISO 11348-2) and freshly prepared bacteria (ISO 11348-1), a procedure with freeze-dried bacteria is described in this part of ISO 11348, the performance of which can be interpreted by the user in every detail.

The laboratories responsible for the results have the opportunity to select the most suitable technique based on expert judgement and information about the water sample to be tested.

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

WARNING — Persons using this part of ISO 11348 should be familiar with normal laboratory practice. This 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 part of ISO 11348 be carried out by suitably trained staff.

1 Scope

ISO 11348 describes three methods for determining the inhibition of the luminescence emitted by the marine bacterium *Vibrio fischeri* (NRRL B-11177). This part of ISO 11348 specifies a method using freeze-dried bacteria.

This method is applicable to:

— waste water;

— aqueous extracts and leachates;

— fresh water (surface and ground water);

— sea and brackish water;

— eluates of sediment (freshwater, brackish and sea water);

— pore water;

— single substances, diluted in water.

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

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

3 Principle

The inhibition of light emission by cultures of *Vibrio fischeri* is determined by means of a batch test. This is accomplished by combining specified volumes of the test sample or the diluted sample with the luminescent bacteria suspension in a test tube.

The test criterion is the luminescence, measured after a contact time of 15 min or 30 min and optionally 5 min, taking into account a correction factor (f_{kt}), which is a measure of intensity changes of control samples during the exposure time. The inhibitory effect of the water sample can be determined as LID (see Annex B) or as EC_{20}- and/or EC_{50}-values by means of a dilution series. (EC is the effective concentration.)

4 Interferences

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

Losses of luminescence caused by light absorption or light scattering may occur in the case of strongly coloured or turbid waters. This interference can be compensated by a sample treatment for turbidity (7.2) or, for example, by using a double-chambered absorption correction test tube (see Annex A).

Since oxygen is required for the bioluminescence [6], samples with a high oxygen demand (and/or a low oxygen concentration) may cause a deficiency of oxygen and be inhibitory.

Readily biodegradable nutrients in the sample may cause a pollutant-independent reduction in bioluminescence [1].

Samples with a pH outside the range of pH = 6,0 and pH = 8,5 affect the luminescence of the bacteria [6], [7]. An adjustment of the sample is required when the toxic effect of pH is not wanted.

As the test organism *Vibrio fischeri* is a marine bacterium, testing salt-water samples with the standard procedure often leads to stimulation effects of bioluminescence, which may mask inhibition effects (see Annex D).

Salt concentrations in the initial sample exceeding 30 g/l NaCl, or contents of other compounds giving equal osmolarity may lead, together with the salt spiking required by the test, to hyperosmotic effects. The resulting salt concentration in the test samples should not exceed the osmolarity of a 35 g/l NaCl solution in order to avoid these effects.

5 Reagents and materials

Use chemicals of recognized analytical grade quality. Use distilled water or water of equivalent purity.

5.1 **Test bacteria**.

Use a strain of luminescence bacteria belonging to the species *Vibrio fischeri* NRRL B-11177. The bacterial suspensions used for toxicity measurements are prepared from commercially available freeze-dried reagents which can be stored in a freezer at −18 °C to −20 °C. The bacteria start glowing immediately after reconstitution and are ready to be used for the test.

5.2 **Sodium chloride solution**, as diluent.

Dissolve 20 g of sodium chloride (NaCl) in water and make up to 1 l with water.

5.3 **Sodium hydroxide solution**, e.g. c(NaOH) = 1 mol/l.

5.4 Hydrochloric acid, e.g. $c(HCl) = 1$ mol/l.

For the adjustment of the pH, it may be necessary to use acids or bases of lower or higher concentration.

5.5 Solution for freeze-dried bacteria.

20 g	Sodium chloride (NaCl)
2,035 g	Magnesium chloride hexahydrate ($MgCl_2 \cdot 6\ H_2O$)
0,30 g	Potassium chloride (KCl)

Dissolve in water and make up to 1 l with water. The solution may be stored in portions in a freezer at −18 °C to −20 °C.

5.6 Reference substances.

Prepare the following reference-substance stock solutions with sodium chloride solution (5.2) as diluent separately, without adjustment of the pH:

19,34 mg/l	Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7\ H_2O$)
6,8 mg/l	3,5-Dichlorophenol ($C_6H_4OCl_2$) (purity > 99 %)
105,8 mg/l	Potassium dichromate ($K_2Cr_2O_7$)

These concentrations are approximately twice the expected EC_{50}-values for the respective reference substances in this part of ISO 11348. The volumes required depend on the test set-up.

NOTE It is possible to use commercially available chemical preparations with defined concentrations of $ZnSO_4$ and $K_2Cr_2O_7$ (titrisol) for the preparation of the stock solutions of the reference substances.

6 Apparatus

6.1 Freezer, for the storage of preserved bacteria.

6.2 Incubator or refrigerator, to maintain the stock suspension (8.2) and, optionally, the “solution for freeze-dried bacteria” (5.5) (variant B) at a temperature of 4 °C ± 3 °C.

6.3 Thermostatically controlled thermo-block, to maintain the test samples at a temperature of 15 °C ± 1 °C. Within one test, the temperature deviation should be at most ± 0,3 °C.

6.4 Luminometer, measuring cell maintained at 15 °C ± 1 °C, equipped with suitable test tubes.

6.5 Test tubes, made of a chemically inert material, appropriate for the selected luminometer and having a capacity which facilitates the taking of a reading over the largest possible surface area and able to fit into the thermo-block (6.3).

6.6 pH-meter.

6.7 Chronometer.

6.8 Piston pipettes or **plastic syringes**, 10 µl, 500 µl and 1 000 µl.

6.9 Piston pipettes, with variable volume, 10 ml to 200 ml and 200 µl to 5 000 µl.

6.10 Conductometer.

6.11 Oxygen probe, in accordance with ISO 5814.