
**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**

*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



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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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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 11348-3 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 11348-3:1998), which has been technically revised.

ISO 11348 consists of the following parts, under the general title *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*
- *Part 2: Method using liquid-dried bacteria*
- *Part 3: Method using freeze-dried bacteria*

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.

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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*