INTERNATIONAL STANDARD

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Sterilization of health care products — Biological indicators —

Part 2: Biological indicators for ethylene oxide sterilization processes

Stérilisation des produits de santé — Indicateurs biologiques —
Partie 2: Indicateurs biologiques pour la stérilisation à l'oxyde d'éthylène

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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 11138-2 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

This second edition cancels and replaces the first edition (ISO 11138-2:1994), which has been technically revised.

ISO 11138 consists of the following parts, under the general title *Sterilization of health care products — Biological indicators*:

— *Part 1: General requirements*

— *Part 2: Biological indicators for ethylene oxide sterilization processes*

— *Part 3: Biological indicators for moist heat sterilization processes*

— *Part 4: Biological indicators for dry heat sterilization processes*

— *Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization processes*

Introduction

ISO 11138-1 specifies production, labelling, test methods and performance requirements for the manufacture of biological indicators including inoculated carriers and suspensions intended for use in validation and monitoring of sterilization processes. This part of ISO 11138 gives specific requirements for those biological indicators intended for use in ethylene oxide sterilization processes.

The intent of providing requirements in the ISO 11138 series of International Standards is to provide general requirements and requirements for test methods. This series of International Standards represents the current "state-of-the-art" according to the experts representing manufacturers, users and regulatory authorities involved in developing the standard. The intent is not to promote the use of biological indicators where such use is not advised, but rather to provide common requirements for the production of those biological indicators that are known to be in use today.

Standards exist providing requirements for the validation and control of ethylene oxide sterilization (see ISO 11135).

NOTE Some countries or regions may have published other standards covering requirements for sterilization or biological indicators.

Advice on selection, use and interpretation of results when using biological indicators can be found in ISO 14161.

Sterilization of health care products — Biological indicators —

Part 2:
Biological indicators for ethylene oxide sterilization processes

1 Scope

This part of ISO 11138 provides specific requirements for test organisms, suspensions, inoculated carriers, biological indicators and test methods intended for use in assessing the performance of sterilizers and sterilization processes employing ethylene oxide gas as the sterilizing agent, either as pure ethylene oxide gas or mixtures of this gas with diluent gases, at sterilizing temperatures within the range of 29 °C to 65 °C.

NOTE 1 Requirements for validation and control of ethylene oxide sterilization processes are provided by ISO 11135.

NOTE 2 National or regional regulations could provide requirements for work place safety.

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 11138-1:2006, *Sterilization of health care products — Biological indicators — Part 1: General requirements*

ISO 18472, *Sterilization of health care products — Biological and chemical indicators — Test equipment*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 11138-1 apply.

4 General requirements

The requirements of ISO 11138-1 apply.

5 Test organism

5.1 The test organisms shall be spores of *Bacillus atrophaeus, Bacillus subtilis* or other strains of microorganisms of demonstrated equivalent performance as required by this part of ISO 11138.

NOTE 1 Some strains of *Bacillus subtilis* have been reclassified as *Bacillus atrophaeus*.

NOTE 2 *Bacillus atrophaeus* ATCC 9372, NCTC 10073, NCIMB 8058, DSM 2277, NRRL B-4418 and CIP 77.18 have been found to be suitable.

5.2 If a test organism other than *Bacillus atrophaeus* is used, the suitability of the resistance of that test organism shall be determined.

6 Suspension

The requirements of ISO 11138-1 apply.

7 Carrier and primary packaging

7.1 The suitability of the carrier and primary packaging materials for biological indicators for use in ethylene oxide sterilization processes shall be demonstrated in accordance with the requirements of ISO 11138-1:2006, 5.2 and Annex B.

7.2 The exposure conditions for establishing compliance shall be:

a) minimum exposure temperature: $\geqslant$ 55 °C;

b) sterilizing agent: ethylene oxide gas at a concentration not less than 800 mg/l at $\geqslant$ 70 % RH;

c) maximum exposure temperature: as stated by the manufacturer;

d) exposure time: $\geqslant$ 6h.

NOTE These conditions have been selected to represent a realistic challenge to the carrier while remaining within the practical limits of an ethylene oxide sterilization process.

8 Inoculated carriers and biological indicators

The requirements of ISO 11138-1 apply.

9 Population and resistance

9.1 The manufacturer shall state the resistance characteristics in accordance with ISO 11138-1:2006, 6.4.

9.2 The viable count shall be stated with increments $\leqslant 0{,}1 \times 10^n$ per unit (e.g. per ml of suspension, per inoculated carrier or per biological indicator).

9.3 For inoculated carriers and biological indicators, the viable count shall be $\geqslant 1{,}0 \times 10^6$.

9.4 The resistance shall be expressed as the D value in minutes at 54 °C and/or 30 °C. The D value of each batch/lot of biological indicators or inoculated carriers shall be stated in minutes to one decimal place at 54 °C or 30 °C, or at both temperatures.

9.5 Suspensions, inoculated carriers or biological indicators containing *Bacillus atrophaeus* spores shall have a D value of not less than 2,5 min at 54 °C and/or not less than 12,5 min at 30 °C, when tested according to the conditions in Annex A. Other microorganisms shall have D values supporting the application.

9.6 The resistance of a biological indicator may also be indicated by the term F_{BIO} value (see ISO 11138-1:2006, 3.7).

The resistance characteristics specified in this part of ISO 11138 and any other part of ISO 11138 apply to the specific test conditions stated in the other parts.

9.7 D values are determined according to methods given in Annexes C and D of ISO 11138-1:2006.

9.8 Determination of D value and survival-kill response characteristics require the use of a resistometer applying the reference resistometer process parameters (see Annex A).

9.9 The survival-kill window can be calculated using the formulae in ISO 11138-1:2006, Annex E.

NOTE This information may be of value to the user when comparing different batches from the same manufacturer.

EXAMPLE

Using the formulae in ISO 11138-1:2006, Annex E with the minimum population and minimum D value requirements specified in this part of ISO 11138, the survival-kill response characteristics are:

— at 54 °C: survival time not less than 10 min and kill time $\leqslant$ 25 min;

— at 30 °C: survival time not less than 50 min and kill time $\leqslant$ 125 min.

Annex A
(normative)

Method for determination of resistance to ethylene oxide sterilization

A.1 General

This method requires the use of a test apparatus referred to as a resistometer in this part of ISO 11138. The specifications of the resistometer process parameters for ethylene oxide sterilization processes are provided in ISO 18472.

Specific requirements related to the test method are provided in A.2.

A.2 Method

A.2.1 Load the samples on to suitable sample holders.

A.2.2 Preheat the resistometer chamber to the selected testing conditions (30 °C or 54 °C).

A.2.3 Place the loaded sample holders in the chamber, close the chamber and initiate the process cycle.

A.2.4 Carry out the following sequence of operations:

— Step 1: evacuate the chamber to a vacuum set point of 10 kPa ± 0,5 kPa.

— Step 2: admit sufficient water vapour to raise the relative humidity in the chamber to 60 % ± 10 %. Maintain these conditions for a period of 30 min ± 1 min. The samples should be allowed to warm to above the dew point prior to injection of water vapour to avoid the potential for condensation.

— Step 3: admit ethylene oxide to the chamber to obtain a concentration of 600 mg/l ± 30 mg/l within 60 s. For the 0 min exposure time, no ethylene oxide shall be admitted.

— Step 4: maintain these conditions for the required exposure time ± 5 s.

— Step 5: at the end of the exposure period, evacuate the chamber to 10 kPa or less within 60 s and then admit filtered air, or an inert gas (such as nitrogen) to ambient pressure.

— Step 6: repeat step 5 four additional times.

— Step 7: at the end of the above process, remove the samples from the chamber and transfer the samples to the growth medium and incubate (see ISO 11138-1:2006, Clause 7).

A.2.5 The transfer period should be documented and the same time period should be used for all tests.

A.3 Determination of resistance

Resistance characteristics shall be determined according to methods given in Annexes C, D and E of ISO 11138-1:2006.

Bibliography

[1] ISO 11135:1994, *Medical devices — Validation and routine control of ethylene oxide sterilization*

[2] ISO 14161:2000, *Sterilization of health care products — Biological indicators — Guidance for the selection, use and interpretation of results*