INTERNATIONAL STANDARD

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Sterilization of health care products — Biological indicators —

Part 2:
Biological indicators for ethylene oxide sterilization

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

Reference number
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Contents

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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.

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.

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

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

— *Part 1: General*

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

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

Annex A forms an integral part of this part of ISO 11138.

Introduction

ISO 11138-1 specifies general production, labelling and performance requirements for the manufacture of biological indicators intended for use as monitors of sterilization cycles, and this part of ISO 11138 gives specific requirements for ethylene oxide sterilization cycles. The procedures and methods described should be carried out by suitably trained personnel.

Biological indicators are not intended for use in any process other than that specified by the manufacturer on the labelling. The use of an inappropriate biological indicator can give misleading results.

Biological indicators should always be used in combination with physical and/or chemical monitoring in demonstrating the efficacy of a sterilizing process. When a physico-chemical variable of a sterilizing process is outside its specified limits, a sterilization cycle should always be regarded as unsatisfactory, irrespective of the results obtained from biological indicators.

The performance of a biological indicator can be affected by the conditions of storage prior to use, the methods of use or the techniques employed after exposure to the process. For these reasons, the recommendations of the manufacturer for storage and use should be followed and biological indicators should be transferred to the specified recovery conditions as soon as possible after exposure to the process. Biological indicators should not be used beyond any expiry date stated by the manufacturer.

Biological indicators are used to test the effectiveness of sterilization processes and equipment. These studies should be conducted by suitably trained personnel.

Sterilization of health care products — Biological indicators —

Part 2:
Biological indicators for ethylene oxide sterilization

1 Scope

This part of ISO 11138 provides specific requirements for test organisms and biological indicators intended for use in assessing the performance of sterilizers employing pure ethylene oxide gas or admixtures of the gas with diluent gases at sterilizing temperatures within the range of 20 °C to 65 °C.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 11138. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this part of ISO 11138 are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 11138-1:1994, *Sterilization of health care products — Biological indicators — Part 1: General.*

3 Definitions

For the purposes of this part of ISO 11138, the definitions given in ISO 11138-1 apply.

4 General

The requirements of ISO 11138-1 shall apply, except as modified in subsequent clauses of this part of ISO 11138.

5 Test organisms

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

NOTE 1 *Bacillus subtilis* NCTC 10073 and CIP 7718, and *B. subtilis* ATCC 9372 have been found to be suitable.

6 Suspensions

Replicate determinations of the viable test organism count on the same batch of suspension shall be within ± 35 % of the nominal population.

7 Carrier and primary packaging

For specific requirements for the carrier and primary packaging, see ISO 11138-1:1994, subclause 4.4.

The test conditions used to validate the acceptability of the carrier and primary packaging materials shall be:

Temperature: not less than 55 °C

Relative humidity: not less than 70 %

Gas concentration: not less than 800 mg/l

Exposure time: not less than 6 h

NOTE 2 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 Biological indicators

8.1 The number of recoverable test organisms on each biological indicator shall be controlled during manufacture to be either within ± 50 % of the nominal population stated by the manufacturer or within the minimum and maximum populations stated by the manufacturer.

8.2 Retrospective determination of the count shall be made by performing a viable test organism count under the manufacturer's stated culture conditions on a suspension of test organisms obtained by physical removal of the test organisms from the carrier through ultrasonication, shaking with glass beads, or other appropriate, validated methods. Counts obtained shall be regarded as acceptable if they are within − 50 % and + 300 % of the nominal population stated by the manufacturer or the midpoint between the minimum and maximum populations stated by the manufacturer.

8.3 For inoculated carriers and biological indicators intended for use in routine monitoring, the nominal population shall be not less than 1×10^6 stated in increments not greater than $0{,}1 \times 10^6$.

NOTE 3 Inoculated carriers and biological indicators intended for other purposes, e.g. qualification, validation or other specific tests, could require other nominal populations.

9 Resistance

9.1 The manufacturer shall state the D value of each batch of biological indicators or inoculated carriers to an accuracy of ± 0,5 min.

9.2 Determination of the resistance characteristics of each batch of biological indicators shall be performed according to annex A.

9.3 The D values obtained by either the survivor curve method and/or by the fraction negative analysis using the MPN procedure (see ISO 11138-1:1994, annexes B and C respectively) for test organism populations on the biological indicator shall be not less than 12,5 min when exposed to 600 mg/l ± 30 mg/l ethylene oxide at (60 ± 10) % relative humidity and 30 °C ± 1 °C, and/or 2,5 min if the test conditions are the same except temperature is controlled to 54 °C ± 1 °C.

10 Test methods

Test methods given in this part of ISO 11138 are reference methods. When alternative method(s) are used these shall be defined and validated, and have proven correlation with the reference method(s).

Annex A
(normative)

Method for determination of resistance to ethylene oxide sterilization

A.1 Ethylene oxide biological indicator resistometer

A.1.1 The equipment shall be capable of maintaining the conditions given in table A.1 within the limits given for exposure periods between 1 min and 120 min to an accuracy of ± 10 s. In addition, equipment shall be capable of exposures of greater than 6 h.

Table A.1 — Conditions for resistance studies of biological indicators

Ethylene oxide	600 mg/l ± 30 mg/l
Temperature	30 °C ± 1 °C or 54 °C ± 1 °C
Relative humidity	(60 ± 10) %

A.1.2 The equipment shall be provided with means to evacuate the reaction chamber to less than 10 kPa (100 mbar) to permit adequate air removal prior to admission of the sterilant and to exhaust the sterilant at the end of the exposure period. Air admitted at the end of the cycle shall be filtered through a filter having the ability to remove not less than 99,9 % of 0,5 µm particles.

A.1.3 The time to achieve the required gas concentration from commencement of gas admission shall not exceed 60 s and the time to exhaust the gas to 10 kPa (100 mbar) at the end of the exposure period shall not exceed 60 s.

A.1.4 The chamber and door shall be provided with means to maintain the temperature of the inner surface of the chamber at the required operating temperature.

A.1.5 The supply of ethylene oxide gas to the chamber shall be filtered and preheated to ensure that neither liquid ethylene oxide nor particles of polymer are admitted to the chamber.

A.1.6 The equipment shall be capable of automatic operation and shall be provided with a system for recording temperature, pressure and humidity within the chamber which is independent of the control function and such that the limits of error on the recording equipment do not exceed 50 % of the tolerance allowed for each control variable. For example, the chamber temperature is required to be controlled within ± 1 K and thus the maximum allowable error limit on the temperature recorder is ± 0,5 K.

A.2 Operation of resistometer

A.2.1 Load the carriers, inoculated carriers or biological indicators onto a suitable sample holder.

A.2.2 Preheat the resistometer chamber to the desired temperature (30 °C ± 1 °C or 54 °C ± 1 °C).

A.2.3 Place the loaded sample holder in the chamber, close the chamber and leave for the time required to allow the temperature to stabilize.

A.2.4 The following sequence of operations shall be carried out under automatic control.

a) Evacuate the chamber to 10 kPa ± 0,4 kPa (100 mbar ± 4 mbar).

b) Admit sufficient water vapour to raise the relative humidity in the chamber to (60 ± 10) %. Maintain these conditions for a period of 28 min to 30 min.

c) Admit ethylene oxide to the chamber to obtain a concentration of 600 mg/l ± 30 mg/l within 60 s.

NOTE 4 For the 0 min exposure time, no ethylene oxide should be admitted.

d) Maintain these conditions for the required exposure time.

e) At the end of the exposure period, evacuate the chamber to 10 kPa ± 0,4 kPa (100 mbar ± 4 mbar) within 60 s and then admit filtered air,

or an inert gas (such as nitrogen) to ambient pressure.

f) Repeat stage e) four additional times.

A.2.5 At the end of the cycle remove the holder and samples from the chamber.

A.3 Determination of resistance

Resistance is determined according to the procedures given in ISO 11138-1:1994, clause 5 (and the related annexes).

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