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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

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

This third edition cancels and replaces the second edition (ISO 11138-2:2006), which has been technically revised.

A list of all parts of ISO 11138 can be found on the ISO website.

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 document gives specific requirements for those biological indicators intended for use in ethylene oxide sterilization processes.

The ISO 11138 series 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 and ISO 14937).

NOTE It is possible that some countries or regions 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 document specifies 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 and ISO 14937.

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

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 11138-1:2017, *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.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp/

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 document.

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[1)].

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 11138-1:2017, 5.2 and Annex B.

7.2 The exposure conditions to determine compliance shall be

a) minimum exposure temperature: greater than or equal to 55 °C,

b) sterilizing agent: ethylene oxide gas at a concentration not less than 800 mg/l in air at greater than or equal to 70 % RH,

c) maximum exposure temperature: as stated by the biological indicator manufacturer, and

d) exposure time: greater than or equal to 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 11138-1:2017, 6.4.

9.2 The viable count shall be stated with increments less than or equal to $0{,}1 \times 10^n$ 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 greater than or equal to $1{,}0 \times 10^6$.

9.4 The resistance shall be expressed as the *D* value in minutes, at 54 °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.

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 when tested according to the conditions in Annex A using test gas mixtures (see Annex B). Other microorganisms shall have *D* values supporting the application.

1) These are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

9.6 Suspensions, inoculated carriers or biological indicators containing *Bacillus atrophaeus* spores shall have a *D* value of not less than 2,0 min at 54 °C when tested according to the conditions in Annex A using a test gas consisting of 100 % EO (see Annex B). Other microorganisms shall have *D* values supporting the application.

9.7 The resistance characteristics specified in this document and any other parts of ISO 11138 shall be defined using the specific critical variables associated with the referenced sterilization process.

9.8 *D* values are determined according to methods given in ISO 11138-1:2017, Annex C and Annex D.

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

9.10 The survival-kill window can be calculated using the formulae in ISO 11138-1:2017, Annex E.

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

EXAMPLE Using the formulae in ISO 11138-1:2017, Annex E with the minimum population and minimum *D* value requirements specified in this document, the survival-kill response characteristics are:

— at 54 °C, 2,5 *D* value; survival time greater than or equal to 10 min and kill time less than or equal to 25 min;

— at 54 °C, 2,0 *D* value; survival time greater than or equal to 8 min and kill time less than or equal to 20 min.

Annex A
(normative)

Method for determination of resistance to ethylene oxide sterilization

A.1 Principle

This method requires the use of a test apparatus referred to as a resistometer in this document. 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 Procedure

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

A.2.2 Preheat the resistometer chamber to the test condition of 54 °C.

A.2.3 Place the loaded sample holders in the chamber, close the chamber and initiate the test 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 gas 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 gas shall be admitted.

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

— Step 5: At the end of the exposure time, 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 or more if necessary to reduce operator exposure to any sterilant remaining in the chamber.

— Step 7: At the end of the above test cycle, remove the samples from the chamber and transfer the samples to the growth medium and incubate (see ISO 11138-1:2017, Clause 7).

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

A.3 Determination of resistance

Resistance characteristics shall be determined according to methods given in ISO 11138-1:2017, Annexes C, D and E.

Annex B
(informative)

Rationale for the inclusion of a second minimum *D* value specification as a result of changes to the test gas used to evaluate resistance and deletion of the requirement for a minimum *D* value at 30 °C

B.1 Inclusion of a second minimum *D* value

This document requires *D* value testing of ethylene oxide (EO) biological indicators (BIs) in an ISO 18472 compliant resistometer using ethylene oxide gas at a nominal concentration of 600 mg/l. Historically, this was often accomplished by BI manufacturers using test gas mixtures. As of 31 December 2014, the U.S. EPA Clean Air Act prohibited the sale and use of HCFC-based (hydrochlorofluorocarbon) products in the United States, requiring U.S.-based BI manufacturers to change the *D* value test gas from HCFC/EO to 100 % EO.

Round robin *D* value testing at 54 °C completed by three U.S.-based biological indicator manufacturers on four different biological indicator products demonstrated that *D* values obtained using 100 % EO were consistently lower than *D* values obtained in the HCFC/EO mixture, even though the nominal EO concentration for both sets of tests was 600 mg/l. The reported differences between *D* values were as high as 40 %. This study has now been published in *Pharmaceutical Technology* (2014)[4].

ISO 11138-2:2006 has been amended by changing the 54 °C *D* value requirement to add an option of a 2,0 min *D* value specification for tests performed in 100 % EO, based on data reported in the peer reviewed publication. Providing an option for BI manufacturers to maintain compliance with the standard without changing the actual BI resistance was preferred over driving manufacturers to increase BI resistance to meet the standard. This scenario would create more risk of positive BIs for end users whose sterilization processes were validated against the lower resistance BIs.

B.2 Deletion of the requirement for a *D* value at 30 °C

Due to missing data to support the requirement for a 12,5 minute minimum *D* value at 30°C, and the absence of evidence for the actual use of this test method, it has been deleted from this document.

Bibliography

[1] ISO 11135, *Sterilization of health-care products — Ethylene oxide — Requirements for the development, validation and routine control of a sterilization process for medical devices*

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

[3] ISO 14937, *Sterilization of health care products — General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices*

[4] Krushefski G. "Effects of 100% Ethylene Oxide Test Gas on the Resistance of Ethylene Oxide Biological Indicators," Pharmaceutical Technology 38 (12) 2014

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