INTERNATIONAL STANDARD

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Sterilization of health care products — Biological indicators —

Part 3:
Biological indicators for moist heat sterilization

Stérilisation des produits de santé — Indicateurs biologiques —
Partie 3: Indicateurs biologiques pour stérilisation à la chaleur humide

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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 11138-3 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*

Annexes A and B form an integral part of this part of ISO 11138.

Introduction

This International Standard gives general production, labelling and performance requirements for the manufacture of biological indicators intended for use as monitors of moist heat sterilization cycles. The procedures and methods described are to 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 physicochemical 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 3:
Biological indicators for moist heat 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 moist heat as the sterilant at sterilizing temperatures in excess of 100 °C.

See the Introduction for use of biological indicators.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 11138. At the time of publication, the editions indicated were 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 editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 11134:1994, *Sterilization of health care products — Requirements for validation and routine control — Industrial moist heat sterilization.*

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 and the following definition apply.

3.1 z-value: For a thermal sterilization process, the change in exposure temperature which corresponds to a 10-fold change in D-value.

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 organism shall be spores of *Bacillus stearothermophilus* or other strains or organisms of demonstrated equivalent performance as required by this standard.

NOTE 1 *Bacillus stearothermophilus* NCTC 10003, DSM 494, *B. stearothermophilus* ATCC 12980, DSM 22 and *B. stearothermophilus* CIP 52.81, DSM 5934, ATCC 7953, NCTC 10007 have been found to be suitable.

6 Suspensions

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

7 Carriers 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 establish suitability shall be as follows:

— **Temperature**: not less than the manufacturer's stated maximum exposure temperature + 5 °C. When not stated, a temperature of 145 °C shall be used.

— **Exposure time**: not less than the manufacturer's stated maximum exposure time. When not stated, an exposure time of 30 min shall be used.

NOTE 2 These conditions have been selected to represent a realistic challenge to the carrier while remaining within the practical limits of a steam 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 stated value.

8.3 For inoculated carriers or biological indicators intended for use in routine monitoring, the nominal number of test organisms shall be not less than 1×10^5 and shall be stated in increments not greater than $0{,}1 \times 10^5$.

For self-contained biological indicator systems, the nominal number of test organisms may be less than 1×10^5 for routine monitoring, provided the requirements of 9.3 are met.

NOTE 3 Inoculated carriers and/or biological indicators intended for other purposes, for example qualification, validation and other specific tests, could require other nominal populations: a nominal population of 1×10^6 could be required for some routine monitoring applications.

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 in accordance with annex A.

9.3 The D-values obtained by either the survivor curve method or by quantal or fraction negative analysis using the MPN procedure (see clause 5, and annexes B, C and D of ISO 11138-1) for test organism populations on the biological indicator shall be not less than 1,5 min when exposed to moist heat at 121 °C ± 1 °C. The population log times the D-value shall not be less than 10 min.

9.4 The D-values of the test organisms on the inoculated carrier shall be determined at not less than two other temperatures in the range 110 °C to 130 °C by either of the two methods given. These data shall be used to calculate the z-value, which shall be not less than 6 °C and which shall be stated in increments not greater than 0,1 ° C. The z-value shall be calculated according to annex B.

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, validated and have proven correlation with the reference method(s).

Annex A
(normative)

Method for determination of resistance to moist heat sterilization

A.1 Steam biological indicator resistometer

A.1.1 The equipment shall be capable of maintaining the following conditions within the limits given for exposure periods between 5 s and 180 min to an accuracy of ± 1 s:

— **Temperature:** (110 °C to 145 °C) ± 0,5 °C

— **Pressure:** (140 kPa to 413 kPa) ± 2,5 kPa

— **Vacuum:** (4 kPa to 100 kPa) ± 0,5 kPa or, for equipment used to test biological indicators intended for use only with cycles incorporating a forced-air removal stage, not less than 10 kPa.

A.1.2 The equipment shall be provided with means to evacuate the reaction chamber to less than 5 kPa within 5 min to permit adequate air removal prior to admission of steam. Steam flushing and/or repeated alternate steam admission followed by evacuation shall not be used to effect air removal.

A.1.3 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.1.4 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 chamber shall be supplied with moist heat from a source external to the chamber. The steam supply shall meet the requirements of ISO 11134.

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

A.1.7 The time for the temperature rise within the resistometer chamber shall not exceed 10 s.

A.1.8 At the end of the exposure period, the temperature in the resistometer chamber shall be reduced to 100 °C or less in a period not exceeding 10 s and the chamber shall return to ambient pressure in not more than 5 s.

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.

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 Carry out the following sequence of operation under automatic control.

a) Evacuate the chamber to 4,5 kPa ± 0,5 kPa (or 10 kPa for forced-air removal cycles) within 5 min.

b) Admit steam to the chamber to obtain the required temperature and pressure.

NOTE 4 For the 0-min exposure time, no steam should be admitted.

c) Maintain these conditions for the required exposure time.

d) At the end of the exposure period, evacuate the chamber to 10 kPa ± 3,5 kPa and then admit filtered air, or an inert gas (such as nitrogen) to ambient pressure within 5 s.

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 clause 5 (and the related annexes of ISO 11138-1).

Annex B
(normative)

Calculation of z-value

Using the procedures and data specified in 9.3, calculate the z-value, in degrees Celsius, using the following formula:

$$z = \frac{T_2 - T_1}{\log_{10} D_1 - \log_{10} D_2}$$

where D_1 and D_2 are the D-values obtained at temperatures T_1 and T_2, respectively (see 9.4).