INTERNATIONAL STANDARD

ISO 11138-3

Second edition
2006-07-01

Sterilization of health care products — Biological indicators —

Part 3: Biological indicators for moist heat sterilization processes

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

Reference number
ISO 11138-3:2006(E)

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Published in Switzerland

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 11138-3 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

This second edition cancels and replaces the first edition (ISO 11138-3:1995), 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 moist heat 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 to provide common requirements for the production of those biological indicators known to be in use today.

Standards exist providing requirements for the validation and control of moist heat sterilization (see ISO 17665).

NOTE Some countries or regions may have published 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 3: Biological indicators for moist heat 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 sterilization processes employing moist heat as the sterilizing agent.

Moist heat as the sterilizing agent is defined in this part of ISO 11138 as dry saturated steam. While air-steam mixtures may be used in moist heat sterilization processes, the methods and performance requirements of this part of ISO 11138 might not be applicable for biological indicators used in such processes.

NOTE 1 Requirements for validation and control of moist heat sterilization processes are provided by ISO 17665.

NOTE 2 National or regional regulations may 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 — Biolgical 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 *Geobacillus stearothermophilus* or other strains of microorganism of demonstrated equivalent performance as required by this part of ISO 11138.

NOTE 1 *Bacillus stearothermophilus* has been reclassified as *Geobacillus stearothermophilus*.

NOTE 2 *Geobacillus stearothermophilus* ATCC 7953 (NCTC 10007, DSM 22 and CIP 52.81), ATCC 12980 (equivalent to NRRL B-4419), have been found to be suitable.

5.2 If a test organism other than *Geobacillus stearothermophilus* or *Bacillus subtilis* ATCC 35021 (5230) is used, the suitability of the resistance of that test organism shall be determined.

NOTE For processes at less than 121 °C, microorganisms such as *Bacillus subtilis* ATCC 35021 (5230) could be used, particularly in sterilization of heat-sensitive liquids.

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 moist heat 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: ⩾ 5 °C above the manufacturer's stated maximum temperature;

b) sterilizing agent: dry saturated steam; if the biological indicator is intended for use in a moist heat process not using dry saturated steam, e.g. an air/steam mixture, the appropriate air steam mixture should be used and noted as an exception to this part of ISO 11138;

c) maximum exposure temperature: as stated by the manufacturer; if not stated by the manufacturer, a temperature of 140 °C shall be used;

d) exposure time: ⩾ 30 min.

NOTE These conditions have been selected to represent a realistic challenge to the carrier while remaining within the practical limits of a moist heat 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 $\geqslant 1{,}0 \times 10^5$.

9.4 The resistance shall be expressed as the D value in minutes at 121 °C. The D value of each batch/lot of biological indicators or inoculated carriers shall be stated in minutes to one decimal place at 121 °C.

9.5 Suspensions, inoculated carriers or biological indicators containing *Geobacillus stearothermophilus* spores shall have a D_{121} value of $\geqslant$ 1,5 min when tested according to the conditions given in Annex A. Other microorganisms shall have D values supporting the application. The z value of the test organisms in the suspension, on the inoculated carrier or in the biological indicator shall be determined at not less than three temperatures, in the range of 110 °C to 130 °C. These data shall be used to calculate the z value, which shall be $\geqslant$ 6 °C (see Annex B).

9.6 The resistance of a biological indicator may also be indicated by the term F_{BIO} value (see 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 standards.

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 requires the use of a resistometer applying the 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 121 °C: survival time $\geqslant$ 4,5 min and kill time $\leqslant$ 13,5 min.

Annex A
(normative)

Method for determination of resistance to moist heat 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 moist heat 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 test samples on to suitable sample holders.

A.2.2 Pre-heat the resistometer chamber to the required operating temperature, e.g. 121 °C ± 0,5 °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 set point of 4,5 kPa ± 1 kPa within 2 min.

— Step 2: Admit steam to the chamber to obtain the required temperature and pressure within 10 s. For the 0 minute exposure time, no steam shall be admitted.

— Step 3: Maintain these conditions for the required exposure time.

— Step 4: At the end of the exposure period, evacuate the chamber to 10 kPa or less within 1 min. The time taken to achieve a temperature of less than 100 °C shall be less than 5 s. Then admit filtered air to ambient pressure.

— Step 5: At the end of the above process, remove the samples from the chamber, and cool down rapidly. Transfer the samples to the growth medium and incubate (see 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.

Annex B
(normative)

Calculation of z value and correlation coefficient, r^2

B.1 Using all the data obtained from either Annex C or D of ISO 11138-1:2006, plot the $\log_{10}$ of the D value against exposure temperature in degrees Celsius. The z value is equal to the negative reciprocal of the slope of the best-fit rectilinear curve as determined by regression analysis.

NOTE See 9.5 for requirements regarding calculation of z value and correlation coefficient, r^2.

B.2 The slope of the best-fit rectilinear curve is calculated using the following formula:

$$m = \frac{(nG)-(AB)}{(nC)-(A^2)}$$

where

m is the slope of the best-fit rectilinear curve;

n is the number of D value/temperature pairs;

$G = \sum\left[t(\log_{10} y)\right]$;

$A = \sum(t)$;

$B = \sum(\log_{10} y)$;

$C = \sum(t^2)$.

The data required for the calculation are given in Table B.1.

Table B.1 — Examples of data collected for regression analysis

D value (min) = y	Exposure temperature (°C) = t	$\log_{10} y$	t^2	$t(\log_{10} y)$	$(\log_{10} y)^2$
y_1	t_1	$\log_{10} y_1$	$(t_1)^2$	$t_1(\log_{10} y_1)$	$(\log_{10} y_1)^2$
y_2	t_2	$\log_{10} y_2$	$(t_2)^2$	$t_2(\log_{10} y_2)$	$(\log_{10} y_2)^2$
y_3	t_3	$\log_{10} y_3$	$(t_3)^2$	$t_3(\log_{10} y_3)$	$(\log_{10} y_3)^2$
y_n	t_n	$\log_{10} y_n$	$(t_n)^2$	$t_n(\log_{10} y_n)$	$(\log_{10} y_n)^2$
	$A = \sum_{i=1}^{i=n} t_i$	$B = \sum_{i=1}^{i=n} \log_{10} y_i$	$C = \sum_{i=1}^{i=n} (t_i)^2$	$G = \sum_{i=1}^{i=n} \left[t_i (\log_{10} y_i)\right]$	$E = \sum_{i=1}^{i=n} (\log_{10} y_i)^2$
Assigned variable	A	B	C	G	E