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Sterilization of health care products — Biological indicators —

Part 4:
Biological indicators for dry heat sterilization processes

Stérilisation des produits de santé — Indicateurs biologiques —

Partie 4: Indicateurs biologiques pour la stérilisation à la chaleur sèche

Reference number
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Foreword

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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 11138-4 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 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 sterilization processes. This part of ISO 11138 gives specific requirements for those biological indicators intended for use in dry 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 that are known to be in use today.

Standards exist providing general requirements for the validation and control of general sterilization processeses (see ISO 14937)[1].

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.

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1) Although ISO/TC 198 has agreed to develop a standard applicable to dry heat processes, it was not available for reference at the time this document was prepared.

Sterilization of health care products — Biological indicators —

Part 4:
Biological indicators for dry 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 dry heat as the sterilizing agent at sterilizing temperatures within the range of 120 °C to 180 °C.

NOTE 1   Requirements for validation and control of dry heat sterilization processes are provided by ISO 14937.

NOTE 2   Requirements for work place safety may be provided by national or regional regulations.

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 *Bacillus atrophaeus* 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* CIP 77.18, NCIMB 8058, DSM 675, NRRL B-4418, and ATCC 9372 or *Bacillus subtilis,* DSM 13019 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.

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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 dry 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: $\geqslant$ 5 °C above the manufacturer's stated maximum temperature;

b) sterilizing agent: dry heat in ambient air;

c) maximum exposure temperature: as stated by the manufacturer; if not stated by the manufacturer, the maximum temperature shall be $\geqslant$ 180 °C;

d) exposure time: $\geqslant$ 30 min.

NOTE   These conditions have been selected to represent a realistic challenge to the carrier while remaining within the practical limits of a dry 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 according to 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 160 °C. The D value of each batch/lot of biological indicators or inoculated carriers shall be stated in minutes to one decimal place at 160 °C.

9.5 Suspensions, inoculated carriers or biological indicators containing *Bacillus atrophaeus* spores shall have a D_{160} value of not less than 2,5 min when tested according to the conditions 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 150 °C to 180 °C. These data shall be used to calculate the z value, which shall $\geqslant$ 20 °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 require the use of a resistometer applying the resistometer process parameters (see Annex A).

NOTE   The values stated above would fit a dry heat sterilizer with forced air distribution, running a cycle of 160 °C with a holding time of 2 h.

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 $\geqslant$ 10 min and kill time $\leqslant$ 25 min;

— at 30 °C: survival time $\geqslant$ 50 min and kill time $\leqslant$ 125 min.

Annex A
(normative)

Method for determination of resistance to dry 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 dry 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 samples on to suitable sample holders.

A.2.2 Preheat the resistometer chamber to the required operating temperature, e.g. 160 °C ± 1 °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: Maintain the above conditions for the required holding time ± 5 s.

— Step 2: At the end of the exposure period, remove the test samples from the chamber, and cool down rapidly. 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 the methods given in Annexes C, D and E of ISO 11138-1:2006.

Annex B
(normative)

Calculation of z value

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

B.3   Table B.2 shows example calculations for the slope of the best-fit rectilinear curve.

Table B.2 — Examples of calculations for slope

D value (min)= y	Exposure temperature (°C) = t	$\log_{10} y$	t^2	$t(\log_{10} y)$	$(\log_{10} y)^2$
$y_1 = 4{,}2$	$t_1 = 150$	$\log_{10} y_1 = 0{,}623\ 2$	$(t_1)^2 = 225\ 00$	$t_1(\log_{10} y_1) = 93{,}480\ 0$	$(\log_{10} y_1)^2 = 0{,}388\ 4$
$y_2 = 2{,}1$	$t_2 = 160$	$\log_{10} y_2 = 0{,}322\ 2$	$(t_2)^2 = 256\ 00$	$t_2(\log_{10} y_2) = 51{,}552\ 0$	$(\log_{10} y_2)^2 = 0{,}103\ 8$
$y_3 = 1{,}2$	$t_3 = 170$	$\log_{10} y_3 = 0{,}079\ 2$	$(t_3)^2 = 289\ 00$	$t_3(\log_{10} y_3) = 13{,}464\ 0$	$(\log_{10} y_3)^2 = 0{,}006\ 3$
	$A = \displaystyle\sum_{i=1}^{i=3} t_i$	$B = \displaystyle\sum_{i=1}^{i=3} \log_{10} y_i$	$C = \displaystyle\sum_{i=1}^{i=3} (t_i)^2$	$G = \displaystyle\sum_{i=1}^{i=3} \left[t_i (\log_{10} y_i) \right]$	$E = \displaystyle\sum_{i=1}^{i=5} (\log_{10} y_i)^2$
Assigned variable	$A = 480$	$B = 1{,}024\ 6$	$C = 77\ 000$	$G = 158{,}496\ 0$	$E = 0{,}498\ 5$

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

$$m = \frac{\left[(3)(158{,}496\ 0) \right] - \left[(480)(1{,}024\ 6) \right]}{\left[(3)(77\ 000) \right] - \left(480^2 \right)}$$

$$m = \frac{(475{,}488\ 0) - (491{,}808\ 0)}{(231\ 000) - (230\ 400)}$$

$$m = \frac{-16{,}320\ 0}{600}$$

$$m = -0{,}027\ 2$$

B.4   The z value is equal to the negative reciprocal of the slope obtained and is calculated using the following formula:

$$z \text{ value} = -1\left(\frac{1}{m} \right)$$

using the above calculated slope, the resulting z value is:

$$z = -1\left(\frac{1}{-0{,}027\ 2} \right) = 36{,}764\ 7 \ °C \text{ rounded to one decimal point}$$

$$z = 36{,}8 \ °C$$

B.5 The correlation coefficient, r^2, for the linearity of the z value curve is calculated using the following formula:

$$r^2 = \frac{\left\{(G) - \left[(A)(B/n)\right]\right\}^2}{\left[(C) - \left(A^2/n\right)\right]\left[(E) - \left(B^2/n\right)\right]}$$

where all variables are as defined in B.2 and $E = \sum\left(\log_{10} y\right)^2$.

B.6 Example calculations for the correlation coefficient for the linearity of the z value curve

Using the values from Table B.2,

$$r^2 = \frac{\left[(158{,}496\,0) - \left[(480)(1{,}024\,6/3)\right]\right]^2}{\left[(77\,000) - \left(480^2/3\right)\right]\left[(0{,}498\,5) - \left(1{,}024\,6^2/3\right)\right]}$$

$$r^2 = \frac{\left\{(158{,}496\,0) - \left[(163{,}936\,0)\right]\right\}^2}{\left[(77\,000) - (76\,800)\right]\left[(0{,}498\,5) - (0{,}349\,9)\right]}$$

$$r^2 = \frac{(-5{,}440\,0)^2}{(200)(0{,}148\,6)}$$

$$r^2 = \frac{29{,}593\,6}{29{,}720\,0}$$

$$r^2 = 0{,}995\,7$$