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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

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Foreword

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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 second edition cancels and replaces the first edition (ISO 11138-4:2006), which has been technically revised.

A list of all the 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 sterilization processes. This document gives specific requirements for those biological indicators intended for use in dry heat 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 to provide common requirements for the production of those biological indicators that are known to be in use today.

A standard exists providing general requirements for the validation and control of dry heat sterilization processeses (see ISO 20857).

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 4: Biological indicators for dry heat 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 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 20857.

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

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* 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* CIP 77.18, NCIMB 8058, DSM 675, NRRL B-4418 and ATCC 9372 or *Bacillus subtilis,* DSM 13019 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 dry heat 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 the following:

a) minimum exposure temperature: greater than or equal to 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 exposure temperature shall be greater than or equal to 180 °C;

d) exposure time: greater than or equal to 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 11138-1:2017, 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 minute 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 2,0 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

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.

three temperatures, in the range of 150 °C to 180 °C. These data shall be used to calculate the *z* value, which shall be greater than or equal to 20 °C (see Annex B).

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

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

9.8 Determination of *D* value and survival-kill response characteristics require the use of a resistometer applying the reference 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 should 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 160 °C: 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 dry heat 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 dry heat 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.

NOTE Preconditioning of the samples according to the manufacturer's recommendations have been found to provide more consistent results.

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 test 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 11138-1:2017, 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 the methods given in ISO 11138-1:2017, Annexes C, D and E.

Annex B
(normative)

Calculation of *z* value

B.1 Using all the data obtained from either ISO 11138-1:2017, Annex C or D, 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 coefficient of determination, r^2.

B.2 The slope of the best-fit rectilinear curve is calculated using Formula (B.1):

$$m = \frac{\left(nG\right) - \left(AB\right)}{\left(nC\right) - \left(A^2\right)} \tag{B.1}$$

where

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

n is the number of *D* value/temperature pairs;

t is the exposure time;

γ is the minimum *D* value;

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

$A = \sum\left(t\right)$;

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

$C = \sum\left(t^2\right)$;

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

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

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

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

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

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

B.4 The z value is equal to the negative reciprocal of the slope obtained and is calculated using Formula (B.2):

$$z \text{ value} = -1\left(\frac{1}{m}\right) \tag{B.2}$$

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

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

z = 36,8 °C

B.5 The coefficient of determination, r^2, for the linearity of the z value curve is calculated using Formula (B.3):

$$r^2 = \frac{\left\{\left(G\right) - \left[\left(A\right)\left(B/n\right)\right]\right\}^2}{\left[\left(C\right) - \left(A^2/n\right)\right]\left[\left(E\right) - \left(B^2/n\right)\right]} \tag{B.3}$$

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

B.6 Example calculations for the coefficient of determination for the linearity, r^2, of the z value curve are given below.

NOTE Calculations reflect rounding.

Using the values from Table B.2,

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

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

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

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

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

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