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Sterilization of health care products — Biological indicators —

Part 1: General

Stérilisation des produits sanitaires — Indicateurs biologiques — Partie 1: Généralités

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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 11138-1 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, B, C, D, E and F form an integral part of this part of ISO 11138.

Introduction

This part of ISO 11138 specifies (see clause 1) general production, labelling and performance requirements for the manufacture of biological indicators intended for use as monitors of sterilization cycles. The procedures and methods described should 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 physico-chemical 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 1: General

1 Scope

This part of ISO 11138 specifies general production, labelling and performance requirements for the manufacture of biological indicators and suspensions intended for use in the validation and monitoring of sterilization cycles.

NOTE 1 Subsequent parts of ISO 11138 specify the particular requirements for biological indicators for defined sterilization processes.

This part of ISO 11138 does not contain requirements for product directly inoculated with test organisms, or recovery procedures for such inoculated product. Nor does it specify requirements for biological indicators that use more than one strain or species of microorganism on a carrier.

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 8601:1988, *Data elements and interchange formats — Information interchange — Representation of dates and times.*

ISO 9002:1994, *Quality systems — Model for quality assurance in production, installation and servicing.*

ISO 11138-2:1994, *Sterilization of health care products — Biological indicators — Part 2: Biological indicators for ethylene oxide sterilization.*

ISO 11138-3:—[1], *Sterilization of health care products — Biological indicators — Part 3: Biological indicators for moist heat sterilization.*

3 Definitions

For the purposes of all parts of ISO 11138, the following definitions apply.

3.1 biological indicator; BI: Inoculated carrier contained within its primary pack ready for use and providing a defined resistance to the specified sterilization process.

3.2 carrier: Supporting material on which test organisms are deposited.

3.3 primary pack: System which protects the inoculated carrier from damage and contamination without preventing penetration of the sterilizing agent(s).

1) To be published.

3.4 secondary pack: Container system in which biological indicators are packed for transport and storage.

3.5 inoculated carrier: Carrier on which a defined number of test organisms have been deposited.

3.6 test organism: Microorganism used for the manufacture of inoculated carriers.

3.7 viable test organism count: Number of viable test organisms in a unit volume of a suspension or on an inoculated carrier, estimated by growth of discrete colonies under the stated culture conditions.

3.8 inactivation: Loss of the ability of the test organisms to germinate, outgrow and/or multiply under the specified culture conditions.

3.9 culture conditions: Manufacturer's stated combination of conditions including the growth medium with the period and temperature of incubation, used to promote germination, outgrowth and/or multiplication of the test organism.

3.10 recognized culture collection: International depository authority under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent and Regulation.

3.11 D value; Decimal reduction value: Exposure time or absorbed radiation dose required to secure inactivation of 90 % of a population of test organisms under stated conditions.

3.12 survivor curve: Graphical representation of inactivation against increasing exposure to stated conditions.

3.13 process challenge device: Object which simulates the worst case of conditions as they are given for the sterilizing agent(s) in the items of the goods to be sterilized.

NOTES

2 The process challenge device is so constituted that a biological indicator can be arranged in the place most difficult for the sterilizing agent(s) to reach.

3 The design of the process challenge device depends on the kind of goods to be sterilized and the sterilization procedure. The biological indicator should not interfere with the function of the test body.

4 In some process challenge devices an inoculated carrier may be used instead of a biological indicator.

3.14 colony-forming unit (CFU): Visible growth of microorganisms arising from a single cell or multiple cells.

3.15 self-contained biological indicator: Biological indicator presented in such a way that the primary pack, intended for incubation, contains the growth medium required for recovery.

3.16 survival-kill window: Extent of exposure to a sterilization process under defined conditions when there is a transition from all biological indicators showing growth (survival exposure) to all biological indicators showing no growth (kill exposure).

3.17 nominal population: Stated number of microorganisms.

NOTE 5 The actual number of microorganisms will differ from the nominal population of microorganism as a result of the accuracy of the inoculation and recovery methods.

3.18 resistometer: Equipment designed to create defined combinations of the physico-chemical variables of a sterilization process within defined limits.

4 Production, performance and labelling requirements

4.1 Manufacturing controls and quality systems

4.1.1 All operations required by this part of ISO 11138 shall be controlled according to a quality system complying with the requirements of ISO 9002.

4.1.2 Traceability of manufacturing components shall be maintained.

Manufacturing components should include all materials and components incorporated in or coming into direct contact with the test organism suspension, the inoculated carrier or the biological indicator.

4.1.3 The finished product supplied by the manufacturer (suspension, inoculated carrier or biological indicators) shall have no organisms, other than the test organism, present in sufficient numbers to impair the utility of the product. This shall be validated, controlled, monitored and recorded, during production.

4.2 Test organisms

4.2.1 Test organisms shall be of a strain suitable for handling without special containment facilities.

4.2.2 Test organisms shall be of a defined strain, lodged with a recognized culture collection, and shall be unambiguously identified by reference to the culture collection number.

4.2.3 When the strain of the test organism to be used is not lodged with a recognized culture collection, the manufacturer shall be responsible for lodging that particular strain with a recognized culture collection.

4.2.4 The originating inoculum for each batch of test organism suspension shall be

a) traceable to the reference culture lodged with the recognized culture collection; and

b) verified as to its identity and purity.

The method(s) used for the maintenance of cultures of the test organism should be designed and maintained to ensure that the cultures are protected from contamination and induced changes in their inherent properties.

Verification tests are specific for each strain of test organism and should be documented and validated by the manufacturer.

4.3 Test organism suspensions

4.3.1 The culture medium and incubation conditions used for preparation of the test organism suspension shall be defined by the manufacturer. These conditions shall consistently produce test organism suspensions that meet the performance requirements of this part of ISO 11138 and the particular performance requirements provided in ISO 11138-2 and ISO 11138-3, as appropriate.

4.3.2 The method of harvesting and subsequent treatment shall ensure that the suspension to be used in the inoculation of carriers is free from residues of the culture medium which could adversely influence the performance of the inoculated carrier or biological indicator.

This shall not be required where the manufacturer has demonstrated that residues of the culture medium do not adversely influence the performance of the inoculated carrier or biological indicator.

4.3.3 Manufacturers of test organism suspensions and/or biological indicators shall maintain adequate records in order to allow traceability of biological indicators and test organism suspension back to the culture obtained from the culture collection.

4.3.4 If a test organism suspension is distributed for use in the preparation of inoculated carriers or the preparation of inoculated product, then each container of test organism suspension shall be accompanied by the following information:

a) the name of the test organism;

b) the name or abbreviation of the culture collection from which the test organism has been obtained and the reference number of the species;

c) the nominal volume of suspension, in millilitres (or in grams, if not a suspension);

d) a unique code by which the manufacturing history can be traced;

e) the viable count in test organisms per millilitre;

f) the recommended storage conditions;

g) the expiry date or shelf life, expressed in accordance with ISO 8601 (i.e.YY-MM-DD);

h) the manufacturer's name, trademark, address or other means of identification; and

i) disposal instructions.

4.3.5 When requested by the purchaser, the manufacturer shall supply details of the resistance and performance characteristics of the suspension. These data shall be determined by a method agreed upon by the purchaser and manufacturer.

4.3.6 The conditions for storage of suspensions of test organisms and their expiry date shall be defined by the manufacturer. These conditions shall be monitored during storage. These conditions shall maintain the test organism suspensions so that they continue to meet the performance requirements of this part of ISO 11138 and the particular performance requirements provided in ISO 11138-2 and ISO 11138-3, as appropriate.

4.3.7 The viable test organism count of the suspension shall be determined.

Where the user requires information on the growth index of the test organism, this should be determined by expressing the viable test organism count as a percentage of the total count determined microscopically.

4.3.8 The manufacturer shall ensure that transport to a third party is carried out under controlled conditions compatible with the storage conditions specified for the suspension of test organisms (see 4.3.6).

4.4 Carrier, primary packaging and design

4.4.1 Carriers and primary packaging shall not contain any contamination (physical, chemical or microbial) that would adversely affect the performance of the biological indicator.

4.4.2 The carrier and primary packaging shall not be degraded by the sterilization process with which it is intended to be used in such a way that the performance characteristics of the inoculated carrier are adversely affected.

The carrier should withstand transport in the primary and secondary pack and handling at the point of use without breakage.

The design of the carrier and/or primary pack should be such that

a) it will minimize the loss of the original inoculum of test organisms during transport and handling, and during shelf life;

b) it is appropriate for use as part of a process challenge device.

4.4.3 Compliance with 4.4.2 shall be tested by observation of carrier and primary packaging exposed to extreme ranges and rates of change of chemical and physical variables of the sterilization process.

NOTE 6 These limits are given in the relevant subsequent parts of ISO 11138.

4.4.4 During and after the sterilization process, the carrier and primary package shall neither retain nor release any substance to such an extent that, on transfer to the growth medium, under the culture conditions, there will be inhibition of the growth of low numbers of surviving test organisms.

Tests for compliance with this requirement shall be performed in accordance with annex F.

4.4.5 The manufacturer shall provide the purchaser with a statement of the maximum and the minimum values of each dimension of the carrier on request.

4.5 Inoculated carriers

4.5.1 In the preparation of a batch of inoculated carriers, only one strain of a species of test organism shall be used.

4.5.2 Inoculated carriers shall be prepared by inoculating carriers with test organism suspension, followed by drying under controlled conditions.

4.5.3 The conditions under which inoculation is carried out shall be specified, validated and controlled to ensure that the inoculated carrier remains free from microorganisms, other than the test organism, which may affect adversely the performance of the product as specified in ISO 11138-2 and ISO 11138-3, as appropriate.

4.5.4 The same nominal population of test organisms shall be deposited on each inoculated carrier used in the manufacture of a batch of biological indicators.

4.5.5 The conditions for storage of inoculated carriers and their expiry date shall be defined by the manufacturer. These conditions shall be monitored during storage. These conditions shall maintain consistently the performance requirements of this part of ISO 11138 and the particular performance requirements for inoculated carriers in ISO 11138-2 and ISO 11138-3, as appropriate.

4.5.6 Where the inoculated carriers are packaged for conversion into biological indicators, they shall be packed in a manner that does not affect the nominal population or performance of individual inoculated carriers.

4.5.7 Each batch of inoculated carriers shall be accompanied by the following information:

a) "inoculated carriers";

b) the name of the test organism;

c) directions for use, especially data about the medium and conditions to be used for recovery of test organisms after exposure to the sterilization process;

d) the name of the culture collection from which the test organism has been obtained and the reference number of the species;

e) the number of test organisms per inoculated carrier;

f) the batch number or unique code by which the manufacturing history can be traced;

g) data about the resistance characteristics of the inoculated carriers to the sterilization process for which they are suitable, including the test conditions and methods used to determine these characteristics;

h) the number of inoculated carriers in the secondary pack;

i) the recommended storage conditions;

j) the expiry date of the inoculated carriers, expressed in accordance with ISO 8601 (i.e. YY-MM-DD);

k) the manufacturer's name, trademark, address or other means of identification;

l) the sterilization process for which the inoculated carriers are suitable; and

m) disposal instructions.

4.6 Biological indicators

4.6.1 Biological indicators shall be prepared by packaging individual inoculated carriers in a primary pack.

4.6.2 The primary packaging shall be designed, constructed and validated to ensure that the biological indicator presented in the primary packaging meets the performance requirements of ISO 11138-2 and ISO 11138-3, as appropriate.

4.6.3 The primary packaging shall be designed, constructed and valided to ensure that, when stored and transported in accordance with the manufacturer's instructions, the biological indicator and inoculated carrier are protected from both contamination and loss of the inoculum from the carrier.

4.6.4 The conditions under which primary packaging is carried out shall be specified, validated and controlled to ensure that the inoculated carrier remains free from microorganisms, other than the test organism, that may affect adversely the performance of the product as specified in ISO 11138-2 and ISO 11138-3, as appropriate.

4.6.5 The primary pack shall be validated for its intended use.

Appropriate International Standards or national standards should be used.

4.6.6 Each biological indicator primary package shall be labelled with the following information:

a) the name of the test organism;

b) the batch number of the biological indicator;

c) the expiry date of the biological indicator, expressed in accordance with ISO 8601 (i.e. YY-MM-DD);

d) an indication of the sterilization process for which the biological indicator is suitable; and

e) the manufacturer's name, trademark, address or other means of identification.

4.6.7 Biological indicators shall be packed in a secondary pack for transport and storage.

4.6.8 The secondary pack shall be labelled with the following information:

a) "biological indicators";

b) the information specified in 4.6.6;

c) the name of the culture collection from which the test organism has been obtained and the reference number of the species;

d) the number of test organisms on each biological indicator as determined for the batch of inoculated carriers;

e) the number of biological indicators in the secondary pack;

f) the recommended storage conditions;

g) the resistance of the test organism on the inoculated carrier in its primary pack, including the test conditions and methods used to determine these characteristics;

h) directions for use, especially data about the medium and conditions to be used for recovery of test organisms after exposure to the sterilization process; and

i) disposal instructions.

4.6.9 Each secondary pack shall be supplied with a copy of a certificate for that batch of biological indicators which shall include the following information:

a) information specified in 4.6.8;

b) data about the resistance characteristics of the indicators to the sterilization process for which the inoculated carriers are suitable for monitoring; and

c) a reference to this part of ISO 11138 and any other relevant International Standards.

4.6.10 Each secondary pack shall be supplied with written instructions for users for the handling and recovery of the biological indicators and the directions shall state the following instructions.

a) Biological indicators shall be stored under the conditions specified by the manufacturer.

b) Biological indicators of a batch shall not be used beyond the expiry date stated by the manufacturer.

c) After exposure to the sterilizing procedure to be tested, the biological indicators shall be examined for recoverable test organisms within a time period specified by the manufacturer.

d) When examining biological indicators for recoverable test organisms, the methods and conditions prescribed by the manufacturer shall be applied, or, if alternative methods are used, those methods shall be validated.

4.7 Self-contained biological indicators

4.7.1 Self-contained biological indicators shall comply with all the requirements of this part of ISO 11138.

4.7.2 The self-contained biological indicator system should be sufficiently robust to withstand transport in the secondary pack as well as handling at the point of use without breakage.

The design of the self-contained biological indicator system should be such that

a) it will minimize the loss of the original inoculum of test organisms during transport and handling; and

b) it is appropriate for use as part of a process challenge device.

4.7.3 During or after the sterilization process, the materials of which the self-contained biological indicator system is made shall neither retain nor release any substance to such an extent that will inhibit the growth of low numbers of surviving test organisms under culture conditions (see annex F).

5 Determination of resistance

5.1 Resistance testing requirements

5.1.1 The resistance of each batch of biological indicators shall be tested to demonstrate conformance with the performance requirements specified in ISO 11138-2 and ISO 11138-3, as appropriate.

5.1.2 Resistance testing (see 5.4 and 5.5) shall include determination of the number of recoverable test organisms and determination of the resistance characteristics by a combination of two or more of the following methods:

a) determination of the D value through the construction of a survivor curve;

b) determination of the D value through fraction negative analysis;

c) calculation of the survivor-kill window using the determined D value, and verification of the survival-kill response characteristics.

5.1.3 The values obtained by these tests shall be within the ranges specified in ISO 11138-2 and ISO 11138-3, as appropriate. At least two of these values shall be stated on the labelling of the secondary pack (see 4.6.8) and the certificate accompanying each batch of inoculated carriers (see 4.5.7).

NOTE 7 Relevant subsequent parts of ISO 11138 may require additional determinations (e.g. a *z*-value for moist heat sterilization biological indicators in ISO 11138-3).

5.2 Calculation of survival-kill window

The survival-kill window shall be calculated by using one of the D-values determined in annexes B and D along with the following formulae:

Survival time/dose = not less than the D value X ($\log_{10}$ labelled viable test organism count per carrier − 2)

Kill time/dose = not more than the D value X ($\log_{10}$ labelled viable test organism count per carrier + 4)

5.3 Determination of number of recoverable test organisms

The number of recoverable test organisms shall be determined in accordance with annex A.

5.4 D value determination

5.4.1 The data used in the calculation of a D value for the biological indicators shall be determined in accordance with annex B (i.e. construction of a survivor curve using direct enumeration) and/or annex C [fraction negative analysis or Most Probable Number (MPN) method].

5.4.2 The D value shall be calculated in accordance with annexes B and/or D.

5.4.3 Other methods of analysing fraction negative data may be used, but equivalence with the reference method shall be demonstrated.

5.5 Survival-kill response determination

The survival-kill response characteristics shall be determined and verified in accordance with annex E.