INTERNATIONAL STANDARD

ISO 11138-1

Second edition
2006-07-01

Sterilization of health care products — Biological indicators —

Part 1: General requirements

Stérilisation des produits de santé — Indicateurs biologiques — Partie 1: Exigences générales

Reference number
ISO 11138-1:2006(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

Foreword ... iv

Introduction ... v

1 Scope ... 1
1.1 General ... 1
1.2 Exclusions ... 1

2 Normative references ... 1

3 Terms and definitions ... 2

4 General manufacturing requirements ... 4
4.1 Manufacturing controls ... 4
4.2 Test organism ... 5
4.3 Information supplied by manufacturer (labelling) ... 6
4.4 Storage and transport ... 6

5 Specific manufacturing requirements ... 7
5.1 Suspensions ... 7
5.2 Carrier, primary and secondary packaging ... 7
5.3 Inoculated carrier ... 8
5.4 Biological indicators ... 8
5.5 Self-contained biological indicators ... 8

6 Determination of resistance ... 8
6.1 General resistance requirements ... 8
6.2 Test organism ... 9
6.3 Population of test organisms ... 9
6.4 Resistance characteristics ... 9
6.5 Test conditions ... 10

7 Culture conditions ... 10
7.1 Incubator ... 10
7.2 Growth medium ... 10
7.3 Incubation ... 10

Annex A (normative) **Determination of viable count** ... 11

Annex B (normative) **Determination of growth inhibition by carriers and primary packaging materials exposed to sterilization processes** ... 13

Annex C (normative) ***D*** **value determination by survivor curve method** ... 15

Annex D (normative) ***D*** **value determination by fraction negative method** ... 19

Annex E (normative) **Survival-kill response characteristics** ... 35

Annex F (informative) **Relationship between components of biological indicators** ... 36

Bibliography ... 37

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 11138-1 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

This second edition cancels and replaces the first edition (ISO 11138-1:1994), 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

This part of ISO 11138 specifies general requirements for 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. Subsequent parts of ISO 11138 provide additional specific requirements for biological indicators for defined sterilization processes.

A graphic description of a biological indicator and its components is presented in Annex F. The presentation includes the two types of biological indicator which are covered by ISO 11138. This shows that inoculated carriers can be presented directly to the sterilizing agent without prior packaging, or included in a primary package that permits access by the sterilizing agent.

The resistance characteristics depend on the type of test organism, its numbers, the method of preparation and the effects of the primary package. Advice on selection, use and interpretation of results of biological indicators can be found in ISO 14161[7].

For any individual sterilization process, including those covered in subsequent parts of ISO 11138, the resistance of the biological indicator will also depend on its microenvironment during testing. In theory, this could lead to an infinite variation in the preparation of biological indicators. Moreover, a sterilization process could be manipulated in infinite variety to suit each possible set of conditions to which products could be exposed. It has therefore been routine practice to manufacture biological indicators that, when exposed to a set of conditions in a defined sterilization process, provide resistance characteristics expressed as D values and, where relevant, z values. Such values are set out in the subsequent parts of ISO 11138.

ISO 11138, parts 1 to 5 represent the current “state-of-the-art” according to the experts representing manufacturers, users and regulatory authorities involved in developing this International Standard.

Biological indicators for specific sterilization processes not covered by reference test conditions in subsequent parts of ISO 11138 should comply with the general requirements in this part, including the resistance testing procedures. Such biological indicators might not be well enough described, or might be used for novel sterilization processes, or might be represented by isolated bioburden microorganisms. If microorganisms other than risk group 1 (WHO,1993[27]) are included in these biological indicators, the appropriate containment and safety levels must be met.

Standards exist providing requirements for the validation and control of sterilization processes (see Bibliography).

NOTE Some countries or regions might have published other standards covering requirements for sterilization or biological indicators (see Bibliography).

Sterilization of health care products — Biological indicators —

Part 1: General requirements

1 Scope

1.1 General

1.1.1 This part of ISO 11138 provides general requirements for production, labelling, test methods and performance characteristics of biological indicators, including inoculated carriers and suspensions, and their components, to be used in the validation and routine monitoring of sterilization processes.

1.1.2 This part of ISO 11138 specifies basic and common requirements that are applicable to all subsequent parts of ISO 11138. Requirements for biological indicators for particular specified processes are provided in the subsequent parts of ISO 11138. If no specific subsequent part is provided, this part applies.

NOTE National or regional regulations may apply.

1.2 Exclusions

This part of ISO 11138 does not apply to microbiological test systems for processes that rely on physical removal of microorganisms, e.g. filtration processes or processes that combine physical and/or mechanical removal with microbiological inactivation, such as use of washer disinfectors or flushing and steaming of pipelines. This part of ISO 11138, however, could contain elements relevant to such microbiological test systems.

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

ISO 11135:1994, *Medical devices — Validation and routine control of ethylene oxide sterilization*

ISO 11137-1, *Sterilization of health care products — Radiation — Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices*

ISO 11137-2, *Sterilization of health care products — Radiation — Part 2: Establishing the sterilization dose*

ISO 11137-3, *Sterilization of health care products — Radiation — Part 3: Guidance on dosimetric aspects*

ISO 11607-1, *Packaging for terminally sterilized medical devices — Part 1: Requirements for materials, sterile barrier systems and packaging systems*

ISO 11607-2, *Packaging for terminally sterilized medical devices — Part 2: Validation requirements for forming, sealing and assembly processes*

ISO 11737-1, *Sterilization of medical devices — Microbiological methods — Part 1: Determination of a population of microorganisms on products*

ISO 13485, *Medical devices — Quality management systems — Requirements for regulatory purposes*

ISO 15223, *Symbols to be used with medical device labels, labelling and information to be supplied*

ISO 17665-1, *Sterilization of health care products — Moist heat — Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices*

ISO 18472, *Sterilization of health care products — Biological and chemical indicators — Test equipment*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
biological indicator
test system containing viable microorganisms providing a defined resistance to a specified sterilization process

[ISO/TS 11139, definition 2.3]

3.2
carrier
supporting material on or in which test microorganisms are deposited

3.3
colony forming unit
CFU
individual visible units of growth of microorganisms arising from a single cell or multiple cells

3.4
culture collection number
unique identification of the test organism allocated by a scientifically recognised service culture collection

3.5
culture conditions
combination of growth media and manner of incubation used to promote germination, growth and/or multiplication of microorganisms

NOTE The manner of incubation may include the temperature, time and any other conditions specified for incubation.

[ISO/TS 11139, definition 2.10]

3.6
D **value**
D_{10} **value**
time or dose required to achieve inactivation of 90 % of a population of the test microorganism under stated dose conditions

[ISO/TS 11139, definition 2.11]

3.7
F_{BIO} **value**
product of the logarithm of the population and the D value where the F_{BIO} value is an expression of the resistance of the biological indicator

3.8
inactivation
loss of ability of microorganisms to grow and/or multiply

[ISO/TS 11139, definition 2.21]

3.9
inactivation curve
graphical representation of inactivation of test organism against increasing exposure to the sterilizing agent at stated conditions

3.10
inoculated carrier
supporting material on or in which a defined number of viable test organisms have been deposited

NOTE See Annex F.

3.11
nominal population
manufacturer's stated number of viable microorganisms

NOTE This is generally expressed in $\log_{10}$ function (e.g. 10^6).

3.12
packaging system
combination of the sterile barrier system and protective packaging

[ISO/TS 11139, definition 2.28]

3.13
primary package
element of the packaging system which maintains the integrity of the product

NOTE The packaging system protects the inoculated carrier from damage and contamination without preventing penetration of the sterilizing agent.

3.14
process challenge device
PCD
item designed to constitute a defined resistance to a sterilization process and used to assess performance of the process

[ISO/TS 11139, definition 2.33]

3.15
resistometer
test equipment designed to create defined reference combinations of the physical and/or chemical variables of a sterilization process

3.16
secondary package
container in which biological indicators are packed for transport and storage

3.17
self-contained biological indicator
biological indicator presented in such a way that the primary package, intended for incubation, contains the incubation medium required for recovery of the test organism

3.18
survival-kill window
extent of exposure to a sterilization process under defined conditions where there is a transition from all biological indicators showing growth (survival time) to all biological indicators showing no growth (kill time)

3.19
suspension
viable test organisms suspended in a fluid

NOTE Suspension can be a biological indicator if ready to use in a sealed glass ampoule, or may be an intermediate component used to produce an inoculated carrier or biological indicator.

3.20
viable count
actual number of recoverable colony-forming units or other appropriate units

NOTE See Annex A.

3.21
***z* value**
change in exposure temperature of a thermal sterilization process, which corresponds to a tenfold change in D value

NOTE See ISO 11138-3 and ISO 11138-4.

4 General manufacturing requirements

4.1 Manufacturing controls

4.1.1 Quality systems

The manufacturer shall establish, document and maintain a formal quality system (e.g. ISO 13485, GMPs or other national or regional requirements) to cover all operations required by this part of ISO 11138. In particular, the manufacturer shall take precautions at all stages of production to minimize contamination that would adversely affect the performance of the biological indicator.

4.1.2 Traceability

4.1.2.1 Traceability of manufacturing components shall be maintained.

4.1.2.2 Manufacturing components shall include all materials incorporated in, or coming into direct contact with, the test organism suspension, the inoculated carrier or its primary package.

4.1.3 End product requirements

The finished product shall comply with the requirements set out in this part of ISO 11138, see:

a) manufacturing (Clause 5);

b) labelling (4.3);

c) resistance characteristics (6.4);

d) storage and transport (4.4).

NOTE 1 Advice on methods for the use of biological indicators is provided in ISO 14161.

NOTE 2 National and/or regional requirements might exist, for example in the various national or regional pharmacopoeias.

4.1.4 Personnel

The procedures and methods in this part of ISO 11138 shall be carried out by suitably trained and experienced laboratory personnel (see 4.1.1).

4.2 Test organism

4.2.1 Strain

4.2.1.1 Test organisms shall be of a defined strain, available through a recognised culture collection, and shall be identified by appropriate test methods.

4.2.1.2 The test organism should be a strain that is:

a) suitable for handling without special containment facilities, does not need specific containment procedures for handling and does not have specific transport or mailing requirements (e.g. Risk Group 1, WHO 1993);

b) sufficiently stable to maintain its resistance characteristics for the duration of the stated shelf-life when transported and stored in accordance with label directions.

NOTE Traditionally, the test organisms of biological indicators have been bacterial spores, usually derived from *Bacillus* or *Geobacillus* species.

4.2.1.3 Test organisms other than bacterial spores may be used if they have been shown to provide appropriate resistance to the sterilization process.

4.2.2 Originating inoculum for suspension

4.2.2.1 The initial inoculum for each batch of test organism suspension shall be:

a) traceable to the reference culture and available through a recognized culture collection;

b) verified as to its identity and purity.

4.2.2.2 The methods used for maintaining test organism cultures shall be designed to protect them from contamination and to minimize any induced changes in the inherent properties of the test organisms.

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

4.2.3 Test organism count

4.2.3.1 The viable test organism count of the suspension shall be determined in accordance with Annex A.

4.2.3.2 If the user requires information on the growth index of the test organism, this shall be provided by expressing the viable test organism count as a percentage of the total direct microscopic count.

4.3 Information supplied by manufacturer (labelling)

4.3.1 The following information shall be provided on the label of each individual unit of suspension, inoculated carrier packaging and biological indicator:

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

— the name of the test organism;

— an indication of the sterilization process for which the suspension, inoculated carriers or biological indicators are suitable;

— the expiry date, expressed according to ISO 8601, e.g. YYYY-MM-DD;

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

Internationally recognized symbols may be used where appropriate (see 4.1.3 and ISO 15223).

4.3.2 The information given in Table 1 shall be provided within the secondary packaging of each batch of product.

4.3.3 Requirements for labelling may be achieved by the use of appropriate symbols (ISO 15223).

Table 1 — Information to be provided by the manufacturer

Information requirement	Suspension	Inoculated carrier	Biological indicator
The name or abbreviation of the culture collection from which the test organism has been obtained and the reference number of the strain	Required	Required	Required
The nominal volume of suspension, in ml	Required	—	—
The process for which the product is suitable for use, the resistance and the procedure and carrier used to determine the resistance [a]	Required	Required	Required
The specified storage conditions	Required	Required	Required
Disposal instructions	Required	Required	Required
Directions for use, especially data about the medium, incubation and other conditions to be used for recovery of test organisms after exposure to the sterilization process	Required	Required	Required
The number of test organisms per ml (suspension), or per unit (inoculated carrier or biological indicator) [a]	Required	Required	Required
The number of product units in the secondary pack	—	Required	Required
A reference to this part of ISO 11138	Required	Required	Required

[a] Test methodology used to determine resistance and population should be supplied by the manufacturer upon request.

4.4 Storage and transport

4.4.1 Storage and transport conditions for the test organism suspension shall be maintained such that the test organism suspension complies with the requirements of this part of ISO 11138 and, where relevant, a subsequent part of ISO 11138.

4.4.2 If inoculated carriers are packaged, they shall be packaged in a way that does not affect the nominal population or performance of individual inoculated carriers.

4.4.3 Storage and transport conditions for inoculated carriers shall be maintained such that the inoculated carriers comply with the requirements of this part of ISO 11138 and where relevant, a subsequent part of ISO 11138.

4.4.4 Individually-packaged biological indicators shall be placed in a secondary package for transport and storage. Packaging for transport and storage shall ensure that biological indicators comply with this part of ISO 11138 and, where relevant, a subsequent part of ISO 11138.

5 Specific manufacturing requirements

5.1 Suspensions

5.1.1 Culture medium and incubation conditions shall consistently produce test organism suspensions that meet the performance requirements of this part of ISO 11138 and any relevant subsequent part of ISO 11138.

5.1.2 The suspending medium for the test organism suspension shall not adversely affect the stability of the test organism and shall be compatible with the procedures and materials employed in the manufacture of inoculated carriers and biological indicators.

5.1.3 The method of harvesting and subsequent treatment of suspensions to be used in the inoculation of carriers should ensure that residues do not adversely influence the performance of the inoculated carrier or biological indicator.

5.2 Carrier, primary and secondary packaging

5.2.1 The materials of the carrier and the primary and secondary packaging shall not contain any contamination (physical, chemical or microbial) that would adversely affect the performance of the biological indicator.

5.2.2 The carrier, the primary and secondary packaging, and the specified storage conditions shall be designed so that the performance characteristics of the biological indicator meet the requirements of this part of ISO 11138 throughout the stated shelf life of the product. The manufacturer shall provide the purchaser with a statement of the maximum and minimum values of each dimension of the carrier on request.

5.2.3 During and after the sterilization process, the carrier and the primary packaging shall not retain or release any substance to such an extent that, on transfer to the incubation medium, under the culture conditions, the growth of low numbers of surviving test organisms will be inhibited. Testing shall comply with Annex B.

5.2.4 The carrier, the primary packaging, and the secondary packaging shall withstand planned transport and handling at the point of use, without breakage.

5.2.5 Raw materials used for the carrier and the primary packaging shall withstand exposure to the sterilization process for which they are intended in such a way that the performance characteristics of the inoculated carrier or biological indicator are maintained. Compliance shall be tested by observation of the carrier and the primary packaging exposed to the extreme ranges and rates of change of the chemical and physical variables of the sterilization process.

NOTE Reference sterilization conditions can be found in subsequent parts of ISO 11138.

5.2.6 Sterilization conditions likely to be used should be investigated by the manufacturer of biological indicators and applied for testing of the applicability of the biological indicator.