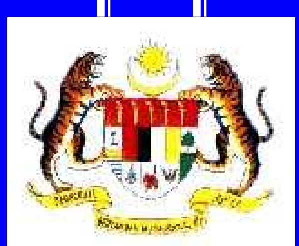

MALAYSIAN STANDARD

MS ISO 11138-1:2010

STERILIZATION OF HEALTH CARE PRODUCTS - BIOLOGICAL INDICATORS - PART 1: GENERAL REQUIREMENTS (ISO 11138-1:2006, IDT)

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ICS: 11.080.01

Descriptors: medical device, sterilization, biological indicators, general requirements

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Committee representation

The Industry Standards Committee on Medical Devices and Facilities for Healthcare (ISC R) under whose authority this Malaysian Standard was adopted, comprises representatives from the following organisations:

Association of Malaysia Medical Industries
Atomic Energy Licensing Board
Biomedical Engineering Association Malaysia
Department of Standards Malaysia
Federation of Malaysian Manufacturers
Institute for Medical Research
Malaysia Medical Device Association
Malaysian Association of Standards Users
Malaysian Medical Association
Malaysian Nuclear Agency
Malaysian Organisation of Pharmaceutical Industries
Malaysian Rubber Board
Malaysian Rubber Export Promotion Council
Ministry of Health Malaysia
Ministry of Health Malaysia (Medical Device Bureau)
Pharmaceutical Association of Malaysia
Radiation Physics, Biophysics and Medical Physics Sub-Group of Institute of Physics Malaysia
SIRIM Berhad (Secretariat)
SIRIM QAS International Sdn Bhd
Universiti Kebangsaan Malaysia
Universiti Teknologi Malaysia

The Technical Committee on Quality Management and Corresponding General Aspects for Medical Devices which recommended the adoption of the ISO Standard as Malaysian Standard consists of representatives from the following organisations:

Association of Private Hospitals of Malaysia
Biomedical Engineering Association Malaysia
Federation of Malaysian Manufacturers
Institute of Quality Malaysia
Malaysia Medical Device Association
Malaysian Medical Association
Malaysian Nuclear Agency
Malaysian Rubber Export Promotion Council
Malaysian Rubber Glove Manufacturers' Association
Ministry of Health Malaysia
SIRIM Berhad (Secretariat)
SIRIM QAS International Sdn Bhd
SterilGamma (M) Sdn Bhd

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NATIONAL FOREWORD

The adoption of the ISO Standard as a Malaysian Standard was recommended by the Technical Committee on Quality Management and Corresponding General Aspects for Medical Devices under the authority of the Industry Standards Committee on Medical Devices and Facilities for Healthcare.

This Malaysian Standard is identical with ISO 11138-1:2006, *Sterilization of health care products - Biological indicators - Part 1: General requirements*, published by the International Organization for Standardization (ISO). However, for the purposes of this Malaysian Standard, the following apply:

a) in the source text, "this International Standard" should read "this Malaysian Standard";

b) the comma which is used as a decimal sign (if any), to read as a point; and

c) reference to International Standards should be replaced by corresponding Malaysian Standards as follows:

Referenced International Standards	Corresponding Malaysian Standards
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*	MS 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*	MS 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*	MS 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*	MS 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*	MS ISO 11607-2, *Packaging for terminally sterilized medical devices - Part 2: Validation requirements for forming, sealing and assembly processes*
ISO 13485, *Medical devices - Quality management systems - Requirements for regulatory purposes*	MS 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*	MS ISO 15223, *Symbols to be used with medical device labels, labelling and information to be supplied*

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NATIONAL FOREWORD *(continued)*

Referenced International Standards	Corresponding Malaysian Standards
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*	MS 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*

Compliance with a Malaysian Standard does not of itself confer immunity from legal obligations.

NOTE. IDT on the front cover indicates an identical standard i.e. a standard where the technical content, structure, and wording (or is an identical translation) of a Malaysian Standard is exactly the same as in an International Standard or is identical in technical content and structure although it may contain the minimal editorial changes specified in clause 4.2 of ISO/IEC Guide 21-1.

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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.

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*

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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).

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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*

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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]

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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

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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);

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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.

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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.

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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.

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5.3 Inoculated carrier

5.3.1 The inoculated carrier shall be made of materials that will withstand exposure to the sterilization process without distortion, melting, corrosion or other failure that would impair the use of the inoculated carrier.

5.3.2 Only one strain of test organism shall be used in a batch of inoculated carriers, unless the manufacturer has demonstrated that the use of multiple strains does not significantly affect test organism performance in the specified sterilization process.

5.3.3 Prior to inoculation, the carrier should be sterilized in accordance with ISO 17665-1, ISO 11135, ISO 11137 parts 1 to 3 or other relevant sterilization methods. If sterilization is not practicable, acceptable bioburden limits of the carrier prior to inoculation may be established in accordance with ISO 11737-1 (see Annex B).

5.3.4 Carriers shall be inoculated so as to maintain a consistent microbial population (see 6.3).

5.4 Biological indicators

5.4.1 Individually packaged biological indicators shall be prepared by placing individual inoculated carriers in a primary pack.

5.4.2 The primary packaging shall be validated for its intended use (see Annex B).

5.4.3 Appropriate international or national standards for packaging should be used (see ISO 11607-1 and ISO 11607-2).

5.5 Self-contained biological indicators

The performance of self-contained biological indicators shall be validated, including the ability of the culture medium to promote growth of the test organisms after being subjected to the sterilization process.

6 Determination of resistance

6.1 General resistance requirements

6.1.1 The resistance of each lot/batch of biological indicators shall be tested to demonstrate conformance with the performance requirements specified in this part of ISO 11138 and any relevant subsequent part of ISO 11138.

6.1.2 Resistance characteristics of biological indicators intended for sterilization processes not specified in any subsequent part of ISO 11138 shall be defined using the elements of this clause in which test conditions for the subject processes are described.

6.1.3 It is recognised that the validation and monitoring of some sterilization processes may use biological indicators that do not meet the minimum population and/or resistance criteria specified in ISO 11138. These biological indicators are acceptable provided that:

a) all other requirements of ISO 11138 (including the method of test for population and resistance) are met;

b) the product information includes a clear statement of the population and resistance;

c) the product label carries a clear warning that the population and/or resistance (as appropriate) is below the value specified in the relevant part of ISO 11138.

6.1.4 Resistance testing shall include determination of the viable count and determination of the resistance characteristics (see 6.3 and 6.4).

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6.1.5 The resistance of a biological indicator may be indicated by the term F_{BIO} value (see 3.7).

6.2 Test organism

The test organism shall be specified.

6.3 Population of test organisms

6.3.1 The viable count shall be determined (see Annex A).

6.3.2 The viable count shall be between 50 % and 300 % of the manufacturer's stated value when it is determined by the manufacturer or a third party during the stated shelf life using the method specified by the manufacturer.

6.4 Resistance characteristics

6.4.1 Resistance characteristics shall be determined by a combination of at least two of the following methods:

d) determination of the D value through the construction of a survivor curve (see Annex C);

e) determination of the D value through a fraction negative method (see Annex D);

f) verification of the survival/kill response characteristics (see Annex E).

6.4.2 The values obtained by these methods shall be within the ranges specified in the relevant subsequent parts of ISO 11138. At least two of these values shall be included in the labelling of the biological indicators (see 4.3).

6.4.3 The D value shall be within $\pm$ 20 % of the manufacturer's stated value when determined by the manufacturer during the stated shelf life using the method specified by the manufacturer.

Ideally, the survivor curve is linear over the full range of inactivation. In practice, deviations from this ideal occur, but linearity must be maintained within acceptable limits. Construction of a survivor curve by enumeration establishes the resistance for surviving populations greater than approximately 5×10^1, whereas the fraction negative method establishes a statistically based calculation of surviving test organisms below that level. Good correlation of the D values obtained by the two methods can therefore be used to establish that there are no serious deviations from a linear survivor curve.

Subsequent parts of ISO 11138 may require additional determinations (e.g. z value for biological indicators for moist heat sterilization [ISO 11138-3] or dry heat sterilization [ISO 11138-4]).

The resistance characteristics specified in this part of ISO 11138 and any subsequent part of ISO 11138 apply to the specific test conditions stated in those parts.

6.4.4 The survivor curve, when plotted as a semi-logarithmic curve of the $\log_{10}$ of the viable test organism count against time, shall be linear with a correlation coefficient of at least 0,8 (see Annex C).

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6.5 Test conditions

Resistance characteristics shall be determined using specified testing conditions. See Table 2.

Table 2 — Minimum test samples according to method

Test method per ISO 11138-1	Minimum number of test samples	Minimum number of exposure conditions	Minimum total number of test samples
Initial count of viable test organism [a]	4	—	4
Annex C Survivor curve method	4	5	20
Annex D Fraction negative method	20	5 [b]	100 [b]
Annex E survival-kill window	50	2	100
Minimum total number depending on choice of combination of methods:			124 or 204

NOTE Common test conditions for specific sterilisation methods have developed over time and are presented in subsequent parts of ISO 11138.

[a] The viable count of the unprocessed inoculated carrier or biological indicator.

[b] The extra set of testing conditions at the exposure subsequent to t_6 (see Table D.1) is not used in the calculations, but is a condition for accepting the test results as valid.

7 Culture conditions

7.1 Incubator

7.1.1 The incubator shall be set to provide, and monitored to confirm, the specified culture conditions.

7.1.2 In addition to routine monitoring of temperature, the temperature distribution within the incubator should be validated.

7.2 Growth medium

7.2.1 The growth medium shall be specified and demonstrated to support the growth of an inoculum of less than 100 test organisms.

7.2.2 Labelling shall include information on culture conditions after exposure to the sterilization process (see 4.3).

7.2.3 The growth medium shall be validated to ensure that it can neutralize any sterilizing agent residuals that might influence the viability of the test organism (see 5.2.3).

7.3 Incubation

7.3.1 The incubation time and temperature shall be validated.

7.3.2 Manufacturers shall provide instructions for incubation (see Table 1). An incubation period is commonly recognized to be 7 days for established sterilization processes, such as moist heat and ethylene oxide, using well characterized test organisms, such as *Geobacillus stearothermophilus* and *Bacillus atrophaeus*, respectively. Where sufficient data are not available to support a reference incubation period of 7 days for a novel sterilization method, at least 14 days shall be used as the reference incubation period on which to base the validation.

NOTE National or regional requirements for incubation period validation may also exist.

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Annex A
(normative)

Determination of viable count

A.1 General

A.1.1 Enumeration techniques are used to determine the number of viable test organisms in suspension on inoculated carriers, or from packaged biological indicators, by counting of distinct colony forming units (CFUs). The method is used when the expected number of recoverable test organisms is above 5×10^1 CFUs.

A.1.2 The relevant products shall be examined for recoverable test organisms in accordance with A.2 to A.4. This method applies to both processed and unprocessed test samples and can be used for the determination of initial viable count (unprocessed samples) as well as for D value determination using the survivor curve method (processed samples).

A.1.3 Alternative methods of enumeration with demonstrated equivalence to direct plating techniques may be used.

A.2 Minimum number of test samples

A minimum of four test samples from each lot/batch or exposure shall be used.

A.3 Sample preparation and culture methods

A.3.1 The test samples shall be placed in an appropriate volume of suspending medium. The test organisms shall be eluted from the test samples by a validated procedure (e.g. maceration with glass beads, grinding and/or blending in a homogenizer and/or blender, vortexing, ultrasonication or other appropriate procedure). (See ISO 11737-1.)

A.3.2 The concentration of microorganisms in the suspensions shall be adjusted by dilution, if necessary, in appropriate sterile dilution fluid. Numbers of CFUs should be within a specified range for the method used whenever possible.

For cultures poured into molten agar or spread on solidified agar in regular size Petri dishes, numbers of CFUs between 30 and 300 are considered to be the most accurate.

A.3.3 An appropriate method for the enumeration of viable organisms shall be used.

Appropriate methods may include membrane filtration techniques, direct spreading on semi-solid agar growth medium or mixing with molten tempered agar growth medium (see ISO 11737-1).

A.3.4 The biological indicator manufacturer shall identify or make available a suitable medium for recovery of test organisms and/or complete data and instructions for the preparation of such a medium.

A.4 Incubation and enumeration

A.4.1 The plated samples or the membrane filters shall be incubated at temperatures and times specified by the manufacturer.

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In general, the incubation periods and temperatures are 55 °C to 60 °C for not less than 48 h for thermophilic microorganisms and 30 °C to 37 °C for not less than 48 h for mesophilic microorganisms.

NOTE Desiccation of the growth medium can adversely affect growth at elevated incubation temperatures.

A.4.2 After the appropriate incubation period, the numbers of colony forming units on the plates or membrane filters shall be counted, and the mean number of recoverable test organisms per appropriate unit shall be calculated.

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Annex B
(normative)

Determination of growth inhibition by carriers and primary packaging materials exposed to sterilization processes

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B.1 General

This method is used to determine the suitability of the carrier and primary packaging materials for the intended sterilization process by identifying possible inhibitory effects of these materials on growth of the test organisms after sterilization. The physical properties of these materials shall have already been tested for suitability. Test methods are given in subsequent parts of ISO 11138. Specifications for resistometers are given in ISO 18472.

B.2 Materials

B.2.1 A suspension of test organisms of the same strain shall be prepared in the same manner as the organisms to be used for inoculation of carriers. The suspension shall be of known population, as determined by viable count, to permit dispensing of test samples with a population of less than 100 viable organisms.

B.2.2 The incubator shall be set to provide, and monitored to confirm, the temperature specified in the culture conditions.

B.2.3 The growth medium shall be as specified in the culture conditions.

B.2.4 Test samples shall be uninoculated carriers or primary packaging materials prepared in accordance with B.3.

B.3 Method

B.3.1 Prepare nine containers of growth medium and equilibrate to the incubation temperature specified in the culture conditions. Use the same volume of growth medium as typically used for the suspension, inoculated carrier or biological indicator.

B.3.2 Take a representative sample of twelve uninoculated carriers, and divide the carriers into six groups of two. They shall be packaged in the material used in the manufacture of the biological indicators.

B.3.3 Take three groups of the carriers from the sample taken in B.3.2, each containing two carriers, and then expose them to the sterilization process.

B.3.4 Set the operational conditions of the resistometer to the values specified in the relevant subsequent parts of this part of ISO 11138, as appropriate.

B.3.5 At the end of the process, unwrap the carriers and aseptically transfer them to the growth medium without subjecting them to intermediate treatment. Place the contents of one group of two carriers into each of the three containers of growth medium previously equilibrated to the incubation temperature (see B.3.1). Record the time taken to complete the transfer.

B.3.6 Incubate the growth medium containing the carrier samples at the specified temperature for 2 h ± 10 min to allow any inhibitory substances to desorb from the carriers. Remove the growth medium from the incubator and inoculate each container with a volume of the test organism suspension calculated to

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contain less than 100 test organisms. Return the inoculated medium to the incubator. Incubate for the validated time period specified for recovery of biological indicators under normal conditions of use.

B.3.7 Control is performed by transferring the three remaining groups of two carriers, not exposed to the process, to the three remaining containers of incubation medium. Incubate these containers for 2 h ± 10 min, then inoculate each container with less than 100 test organisms, and incubate them for the specified incubation period in the same manner as described in B.3.6.

B.3.8 Microorganism identification may be performed if the use of non-sterile carriers is suspected of influencing the test results.

B.3.9 For growth medium controls, incubate three containers of growth medium, without carriers, for 2 h ± 10 min. Then inoculate each container with less than 100 test organisms and incubate for the specified incubation period in the same manner as described in B.3.6.

B.3.10 At the end of the specified incubation period, remove all nine containers from the incubator and examine for viable microorganisms in accordance with the manufacturer's specified methods for normal conditions of use.

B.3.11 Report results as "growth" or "no growth" of the test organism.

B.4 Interpretation of results

B.4.1 If "no growth" occurs in one or more of the growth medium controls, the test procedure shall not be regarded as valid.

NOTE "No growth" in the growth medium controls can be indicative of failure to control the population of the test organism inoculum or of inappropriate recovery conditions (i.e. growth medium, incubation time, incubation temperature etc.).

B.4.2 If "no growth" occurs in one or more of the carrier controls, the carrier shall not be regarded as suitable for the manufacture of inoculated carriers or biological indicators.

NOTE "No growth" in the carrier control where growth occurred in the growth medium could indicate that the carrier material is inhibitory to the growth of the test organism.

B.4.3 If "no growth" occurs in one or more of the three tests of carriers exposed to the sterilization process, the carrier material shall not be regarded as suitable for the manufacture of inoculated carriers or biological indicators.

NOTE "No growth" can be caused by either high levels of absorption of sterilant or by degradative changes in the carrier material during the sterilization process.

B.5 Determination of growth inhibition by primary packaging materials

B.5.1 Samples of the primary packaging material shall be tested in a similar way as the carrier material (i.e. following the steps given in this clause, using the primary packaging materials as the test samples).

B.5.2 The test shall be carried out using samples of primary packaging material equivalent to twice the area normally in contact with the inoculated carrier, or for self-contained biological indicators, equivalent to the area normally in contact with the recovery medium. The test samples shall be immersed in the growth medium.

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Annex C
(normative)

D value determination by survivor curve method

C.1 General

This method establishes the number of surviving test organisms by direct counting of colony forming units (CFUs). This method is also referred to as the “direct enumeration method”. See also Annex A.

NOTE The method has a practical lower limit of approximately 5×10^1 CFUs.

C.2 Materials

C.2.1 Test samples representing spore suspensions, inoculated carriers or packaged biological indicators shall be included in the materials.

NOTE Test methods are given in subsequent parts of ISO 11138. Specifications for resistometers are given in ISO 18472.

C.2.2 The incubator shall be set to provide, and monitored to confirm, the temperature specified in the culture conditions.

C.2.3 The growth medium as specified in the culture conditions shall be included in the materials.

C.3 Procedure

C.3.1 Test samples shall be subjected to defined exposure conditions. The range of exposures shall be stated. See Table 2.

C.3.2 A minimum of five exposures shall be used and shall include:

a) one exposure in which the sample is not subjected to the sterilant (e.g. 0 time exposure);

NOTE The sterilant may be absent or replaced by an inert gas or medium.

b) at least one exposure in which the viable population is reduced to 0,01 % of the original inoculum (4 $\log_{10}$ reduction);

c) a minimum of three exposures covering the intervals between exposure a) and exposure b) above.

C.3.3 Not less than four test samples shall be used for each exposure in each determination. The same number of replicates shall be used for each exposure.

C.3.4 If the sterilizing agent leaves a residue in or on the test samples, this shall be neutralized as rapidly as possible so as not to interfere with the test results. If a neutralization procedure is required, it shall be validated.

C.3.5 Within 2 h of each exposure, the test samples shall be treated to remove the test organisms from the carrier and a viable count assay performed (see Annex A) using the specified culture conditions and methods stated by the manufacturer for recovery under normal conditions for use.

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C.3.6 The suspensions shall be adjusted in appropriate sterile dilution fluid. For cultures poured into molten agar or spread on solidified agar in regular size Petri dishes, numbers of colony forming units between 30 and 300 are considered to be statistically valid.

C.3.7 Using all the data obtained, plot the $\log_{10}$ of the surviving population against exposure time in minutes or dose level and determine the best-fit rectilinear curve by regression analysis using the method of least squares. Survivor data points within 0,5 logarithms of the initial population shall not be included in the regression analysis. Calculate the negative reciprocal of the slope of the line obtained, which is equal to the D value in minutes at the stated exposure conditions.

a) The slope of the best-fit rectilinear curve is calculated using the following formula:

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

where

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

n is the number of data points;

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

$A = \sum(t)$;

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

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

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

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

Recovered population [a] = y	**Exposure duration** (minutes) = t	$\log_{10} y$	t^2	$t(\log_{10} y)$	$(\log_{10} y)^2$
y_1	t_1 = 0,0	$\log_{10} y_1$	$(t_1^2) = 0$	$t_1(\log_{10} y_1) = 0$	$(\log_{10} y_1)^2$
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_4	t_4	$\log_{10} y_4$	(t_4^2)	$t_4(\log_{10} y_4)$	$(\log_{10} y_4)^2$
y_5	t_5	$\log_{10} y_5$	(t_5^2)	$t_5(\log_{10} y_5)$	$(\log_{10} y_5)^2$
	$A = \sum_{i=1}^{i=5} t_i$	$B = \sum_{i=1}^{i=5} \log_{10} y_i$	$C = \sum_{i=1}^{i=5}\left(t_i^2\right)$	$G = \sum_{i=1}^{i=5}\left[t_i\left(\log_{10} y_i\right)\right]$	$E = \sum_{i=1}^{i=5}\left(\log_{10} y_i\right)^2$
Assigned Variable	A	B	C	G	E

[a] As per C.3.7, data points within 0,5 logarithms of y_1 shall not be included in the regression analysis

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b) Example calculations for the slope of the best-fit rectilinear curve are found in Table C.2 and below.

Table C.2 — Examples of calculations for slope

Recovered population [a] = y	Exposure duration (minutes) = t	$\log_{10} y$	t^2	$t(\log_{10} y)$	$(\log_{10} y)^2$
y_1 = 2,5 X 10^6	t_1 = 0,0	$\log_{10} y_1$ = 6,397 9	(t_1^2) = 0	$t_1(\log_{10} y_1)$ = 0	$(\log_{10} y_1)^2$ = 40,933 1
y_2 = 3,4 X 10^5	t_2 = 2,0	$\log_{10} y_2$ = 5,531 5	(t_2^2) = 4	$t_2(\log_{10} y_2)$ = 11,063 0	$(\log_{10} y_2)^2$ = 30,597 5
y_3 = 3,1 X 10^4	t_3 = 4,0	$\log_{10} y_3$ = 4,491 4	(t_3^2) = 16	$t_3(\log_{10} y_3)$ = 17,965 6	$(\log_{10} y_3)^2$ = 20,172 7
y_4 = 1,7 X 10^3	t_4 = 6,0	$\log_{10} y_4$ = 3,230 4	(t_4^2) = 36	$t_4(\log_{10} y_4)$ = 19,382 4	$(\log_{10} y_4)^2$ = 10,435 5
y_5 = 1,9 X 10^2	t_5 = 8,0	$\log_{10} y_5$ = 2,278 8	(t_5^2) = 64	$t_5(\log_{10} y_5)$ = 18,230 4	$(\log_{10} y_5)^2$ = 5,192 9
	$A = \sum_{i=1}^{i=5} t_i$	$B = \sum_{i=1}^{i=5} \log_{10} y_i$	$C = \sum_{i=1}^{i=5} (t_i)^2$	$G = \sum_{i=1}^{i=5} \left[t_i (\log_{10} y_i) \right]$	$E = \sum_{i=1}^{i=5} (\log_{10} y_i)^2$
Assigned Variable	A = 20	B = 21,930 0	C = 120	G = 66,641 4	E = 107,331 7

[a] As per C.3.7 Data points within 0,5 logarithms of y_1 shall not be included in the regression analysis.

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

$$m = \frac{\left[(5)(66{,}641\ 4)\right] - \left[(20)(21{,}930\ 0)\right]}{\left[(5)(120)\right] - \left(20^2\right)}$$

$$m = \frac{(333{,}207\ 0) - (438{,}600\ 0)}{(600) - (400)}$$

$$m = \frac{-105{,}393\ 0}{200}$$

$$m = -0{,}527\ 0$$

c) The D value is equal to the negative reciprocal of the slope obtained and is calculated using the following formula:

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

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

$$D = -1\left(\frac{1}{-0{,}527\ 0}\right) = 1{,}897\ 5 \text{ min (rounded to one decimal place } D = 1{,}9 \text{ min)}$$

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C.3.8 The value obtained for the correlation coefficient for the linearity of the survivor curve shall be not less than 0,8.

a) The correlation coefficient for the linearity of the survivor 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 C.3.7 a) and $E = \sum\left(\log_{10} y\right)^2$

b) Example calculations for the correlation coefficient for the linearity of the survivor curve

Using the values from Table C.2:

$$r^2 = \frac{\left\{(66{,}641\,4) - \left[(20)(21{,}930\,0/5)\right]\right\}^2}{\left[(120) - \left(20^2/5\right)\right]\left[(107{,}331\,7) - \left(21{,}930\,0^2/5\right)\right]}$$

$$r^2 = \frac{\left\{(66{,}641\,4) - \left[(87{,}720\,0)\right]\right\}^2}{\left[(120) - (80)\right]\left[(107{,}331\,7) - (96{,}185\,0)\right]}$$

$$r^2 = \frac{\left[(-21{,}078\,6)\right]^2}{\left[(40)\right]\left[(11{,}146\,7)\right]}$$

$$r^2 = \frac{444{,}307\,4}{445{,}868\,0}$$

$$r^2 = 0{,}996\,5$$

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Annex D
(normative)

D value determination by fraction negative method

D.1 General

D.1.1 This method establishes the number of surviving test organisms by indirect calculation based on the recoverable number of microorganisms as determined by visual observation of growth in fluid growth medium. The method referred to as “fraction negative analysis” is a method in which a fraction of the test samples shows no growth (the fraction negative range) and the calculation is based on the results obtained with this data. A “total kill analysis” is also a fraction negative method in which all the test samples show no growth and the calculation is based on the results obtained with this requirement. This method is used when the recoverable number of test organisms is less than 5 × 10 ° CFUs/unit of measure.

D.1.2 The Holcomb-Spearman-Karber procedure (see D.3.1) and Limited-Holcomb-Spearman-Karber procedure (see D.3.2) require successive exposures which span the fraction negative range.

NOTE Other methods may be applicable, particularly when the survival-kill window is known. One such alternative method is provided by the Stumbo-Murphy-Cochran procedure (see D.3.3).

D.1.3 Test samples shall be subjected to defined exposure conditions with all process variables, except time, remaining within defined windows (steady-state). Where the process variables are considered to be acceptably narrow, time is expressed as “t”. Where the control of process variables is too wide to be considered constant, methods of integration may be used to calculate equivalent time “U”. Both terms are found in the literature.

D.1.4 The number of samples exposed, n, in each exposure and the intervals between sequential exposures, d, both affect the reliability of the test.

D.2 Materials

D.2.1 Test samples shall be representative of spore suspensions, inoculated carriers or packaged biological indicators.

D.2.2 The relevant resistometer shall be used.

NOTE Test methods are given in subsequent parts of ISO 11138. Specifications for resistometers are given in the resistometer standard (ISO 18472).

D.2.3 The incubator shall be set to provide, and monitored to confirm, the temperature specified in the culture conditions.

D.2.4 The growth medium shall be as specified in the culture conditions.

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D.3 Methods

D.3.1 Holcomb-Spearman-Karber procedure (HSKP)

D.3.1.1 Introduction

D.3.1.1.1 Test samples shall be subjected to graded exposures to the defined exposure conditions with all process variables, except time, remaining constant. The total number of test samples shall be not less than 100. The minimum number of 20 replicates should be used for each exposure.

D.3.1.1.2 A minimum of five exposure conditions should be used including at least one set of samples in which all test samples show growth, two sets of samples in which a fraction of the test samples show growth, and two sets of test samples, from sequential exposures, in which no growth is observed.

NOTE Details of requirements for specific resistometer process parameters are provided in the subsequent parts of ISO 11138.

D.3.1.1.3 When the sterilizing agent leaves a residue in or on the test samples, this shall be neutralized as rapidly as possible so as not to interfere with the test results. If a neutralization procedure is required, it shall be validated.

D.3.1.1.4 Samples shall be cultured after exposure according to the manufacturer's specified method.

D.3.1.1.5 Each inoculated carrier is transferred aseptically to a test tube containing an adequate volume of the specified growth medium. The volume of medium shall be the same for each replicate. If the growth medium is included by the manufacturer as an integral part of the biological indicator, the manufacturer's culturing instructions shall be followed. The manufacturer of the biological indicators shall identify or make available a suitable recovery medium and/or the complete data for preparing one (see also 4.3).

D.3.1.1.6 The test samples shall be incubated following the manufacturer's specified methods. The cultures shall be examined after the manufacturer's recommended incubation period or validated incubation time period (see 7.3). Growth of the test organism can be indicated by turbidity of the broth medium, growth on the surface of the broth, or sediment at the bottom of the tube, depending upon the characteristics of the test organism. If the growth medium is an integral part of the biological indicator, e.g. self-contained biological indicators, growth or no growth of the test organism shall be interpreted according to the manufacturer's instructions.

Growth of the test organism in self-contained biological indicators may be indicated by a pH colour change.

D.3.1.1.7 The results are recorded as the ratio of inoculated carriers with non-recoverable test organisms to the total number of inoculated carriers tested at each sub-lethal exposure.

D.3.1.2 Calculations using the HSKP

D.3.1.2.1 The calculations are based on a minimum of five exposure conditions and shall include at least:

— one set of samples in which all tested samples show growth;

— two sets of samples in which a fraction of the samples shows growth;

— two sets of samples, from sequential exposures, in which no growth is observed (see Table D.1).

NOTE HSKP is similar to the Limited Holcomb-Spearman-Karber procedure (see D.3.2), except that it uses a generic formula which is not limited to the same number of replicates at each exposure condition or constant time intervals between exposures.

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D.3.1.2.2 The average D value is calculated using the following formula:

$$D = \frac{U_{HSK}}{\log_{10} N_0 + 0{,}250\ 7}$$

where

$$U_{HSK} = \sum_{i=1}^{k-1} U_i \ ;$$

N_0 is the average viable count per indicator determined by the total viable count method (see Annex A).

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

Table D.1 — Examples of data collected for HSKP

Time of exposure to sterilizing agent t	Number of test samples exposed n	Number of test samples showing no growth r
t_1 (U_1)	n_1	r_1 (r = 0) [a]
t_2	n_2	r_2
t_3	n_3	r_3
t_4	n_4	r_4
t_5 (U_{k-1})	n_5	r_5
t_6 (U_k)	n_6	r_6 ($r = n_6$)
t_7	n_7	r_7 ($r = n_7$) [a]

NOTE t_1 is defined as the longest exposure time to the sterilizing agent in the exposure set where all test samples show growth. Exposure times t_2 through t_5 are increasing exposure times in the fraction negative area. Exposure times t_6 and t_7 are two sequential exposure times at which all samples show no growth.

[a] The test is valid if there are no negative units, i.e. no negative test samples (r = 0), with all units showing growth at the exposure preceding t_1, and all negative test samples ($r = n_7$), i.e. none showing growth at the exposure subsequent to t_6.

D.3.1.2.3 For times of exposure to sterilizing agent, t_1 to t_6, the factors χ and γ are calculated as shown:

$$\chi_i = \frac{t_i + t(i+1)}{2}$$

$$\gamma_i = \frac{r_i + 1}{n_i + 1} - \frac{ri}{ni}$$

where

r_i = the number of test samples showing no growth at an exposure time t_i;

n_i = the number exposed at exposure time t_i.

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At t_1, all test samples show growth and so $\gamma_i = \frac{r_i + 1}{n_i + 1}$.

From the calculated values of χ_i and γ_i above, the value U_i can be calculated for each exposure time, t_i, as follows:

$$U_i = \chi_i \gamma_i$$

D.3.1.2.4 The mean time to sterility, U_{HSK}, from any of the test samples can then be calculated as the sum of U_i for each exposure time t_1 to t_6:

$$U_{HSK} = \sum_{i=1}^{i=6} U_i$$

D.3.1.2.5 Where the interval between exposure times, d, is constant and the same number of test samples, n, is used at each exposure time, the mean to sterility, U_{HSK}, can be calculated from the equation

$$U_{HSK} = U_K - \frac{d}{2} - \frac{d}{n} \sum_{i=1}^{i=6} r_i$$

D.3.1.2.6 The mean D value, $\overline{D}$, can be calculated using the following formula:

$$\overline{D} = \frac{U_{HSK}}{\log_{10} N_0 + 0{,}250\,7}$$

NOTE log (Euler's constant) = log (0,577 2) = – 0,250 7.

where N_0 is the initial viable count of test organisms per test sample (see Annex A).

D.3.1.2.7 The 95 % confidence interval for $\overline{D}$ (p = 0,05) D_{calc}, is calculated using the following formula:

$$D_{calc} = \overline{D} \pm 2\sqrt{V}$$

D.3.1.2.8 The variance, V, is calculated using the following formula:

$$V = a\left(\frac{2{,}302\,6}{\ln N_0 + 0{,}577\,2}\right)^2$$

D.3.1.2.9 The "a" for the variance is calculated using the following formula:

$$a = 0{,}25 \sum_{i=2}^{i=6} \left[t_{(i+1)} - t_{(i-1)}\right]^2 \left[r_i \frac{(n_i - r_i)}{n_i^2 (n_i - 1)}\right]$$

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D.3.1.3 Example calculation of the Holcomb-Spearman-Karber procedure (HSKP)

Table D.2 — Examples of data with non-constant time intervals and non-constant number of samples

Exposure time to sterilizing agent min t	Number of test samples exposed n	Number of test samples showing no growth r_i
$t_1 = 10$	n_1 20	r_1 0
$t_2 = 18$	n_2 19	r_2 4
$t_3 = 28$	n_3 21	r_3 8
$t_4 = 40$	n_4 20	r_4 12
$t_5 = 50$	n_5 20	r_5 16
$t_6 = 60$	n_6 20	r_6 20
$t_7 = 70$	n_7 20	r_7 20

D.3.1.3.1 Calculate χ_i and γ_i (for each exposure):

$$\chi_i = \frac{t_i + t_{(i+1)}}{2}$$

$$\chi_1 = \frac{t_1 + t_{(i+1)}}{2}$$

$$\chi_1 = \frac{10 + 18}{2} = 14$$

$$\chi_2 = \frac{18 + 28}{2} = 23$$

$$\chi_3 = \frac{28 + 40}{2} = 34$$

$$\chi_4 = \frac{40 + 50}{2} = 45$$

$$\chi_5 = \frac{50 + 60}{2} = 55$$

$$\chi_6 = \frac{60 + 70}{2} = 65$$

$$\gamma_i = \frac{r_{i+1}}{n_{i+1}} - \frac{r_i}{n_i}$$

$$\gamma_1 = \frac{r_{1+1}}{n_{1+1}} - \frac{r_1}{n_1}$$

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$$\gamma_1 = \frac{4}{19} - \frac{0}{20} = 0{,}21$$

$$\gamma_2 = \frac{8}{21} - \frac{4}{19} = 0{,}17$$

$$\gamma_3 = \frac{12}{20} - \frac{8}{21} = 0{,}22$$

$$\gamma_4 = \frac{16}{20} - \frac{12}{20} = 0{,}2$$

$$\gamma_5 = \frac{20}{20} - \frac{16}{20} = 0{,}2$$

$$\gamma_6 = \frac{20}{20} - \frac{20}{20} = 0$$

NOTE For the calculations of γ_4 and γ_5, both γs = 0,2. This happens because of the number of test samples showing no growth increase at a constant rate in this example.

D.3.1.3.2 Calculate U_i for each exposure time, t_i:

$$U_i = \chi_i \gamma_i$$

$$U_1 = \chi_1 \gamma_1 = 14 \times 0{,}21 = 2{,}94$$

$$U_2 = 23 \times 0{,}17 = 3{,}91$$

$$U_3 = 34 \times 0{,}22 = 7{,}48$$

$$U_4 = 45 \times 0{,}2 = 9{,}0$$

$$U_5 = 55 \times 0{,}2 = 11{,}0$$

$$U_6 = 65 \times 0 = 0$$

D.3.1.3.3 The mean time to sterility, U_{HSK}, is calculated using the following formula:

$$U_{HSK} = \sum_{i=1}^{i=6} \mu_i$$

$$U_{HSK} = \mu_1 + \mu_2 + \mu_3 + \mu_4 + \mu_5 + \mu_6$$

$$U_{HSK} = 2{,}94 + 3{,}91 + 7{,}48 + 9{,}0 + 11{,}0 + 0 = 34{,}33$$

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D.3.1.3.4 The mean D value, $\overline{D}$, is calculated using the following formula:

$$\overline{D} = \frac{U_{\text{HSK}}}{\log_{10} N_0 + 0{,}250\ 7}$$

where

N_0 = initial population of 1×10^5;

$$\overline{D} = \frac{34{,}33}{5{,}000 + 0{,}250\ 7} = 6{,}54 \text{ min.}$$

D.3.1.3.5 The 95 % confidence interval for $\overline{D}$ (p = 0,05) D_{calc} is calculated using the following formula:

$$D_{\text{calc}} = \overline{D} \pm 2\sqrt{V}$$

D.3.1.3.6 The variance, V, is calculated using the following formula:

$$V = a\left(\frac{2{,}302\ 6}{\ln N_0 + 0{,}577\ 2}\right)^2$$

D.3.1.3.7 The "a" in the variance formula for each t_i and summing all results is calculated using the following formulae:

$$a = 0{,}25 \sum_{i=2}^{i=6} \left\{\left[t_{(i+1)} - t_{(i-1)}\right]^2 \left[r_i\ \frac{n_i - r_i}{n_i^2(n_i - 1)}\right]\right\}$$

$$a = 0{,}25\left\{\left(t_{1+1} - t_{1-1}\right)^2\left[r_1\frac{n_1 - r_1}{n_1^2(n_1-1)}\right] + \left(t_{2+1} - t_{2-1}\right)^2\left[r_2\ \frac{n_2 - r_2}{n_2^2(n_2-1)}\right] + \left(t_{3+1} - t_{3-1}\right)^2\left[r_3\ \frac{n_3 - r_3}{n_3^2(n_3-1)}\right]\right\}$$

$$+\left(t_{4+1} - t_{4-1}\right)^2\left[r_4\ \frac{n_4 - r_4}{n_4^2(n_4-1)}\right] + \left(t_{5+1} - t_{5-1}\right)^2\left[r_5\ \frac{n_5 - r_5}{n_5^2(n_5-1)}\right] + \left(t_{6+1} - t_{6-1}\right)^2\left[r_6\ \frac{n_6 - r_6}{n_6^2(n_6-1)}\right]$$

$$a = 0{,}25 \times [(28 - 10)^2 \times 4\left(\frac{19-4}{361 \times 18}\right) = 2{,}991\ 7 +$$

$$(40 - 18)^2 \times 8\left(\frac{21-8}{441 \times 20}\right) = 5{,}707\ 0 +$$

$$(50 - 28)^2 \times 12\left(\frac{20-12}{400 \times 19}\right) = 6{,}113\ 7 +$$

$$(60 - 40)^2 \times 16\left(\frac{20-16}{400 \times 19}\right) = 3{,}368\ 4 +$$

$$(70 - 50)^2 \times 20\left(\frac{20-20}{400 \times 19}\right) = 0{,}000\ 0]$$

$$a = 0{,}25\ [2{,}991\ 7 + 5{,}707\ 0 + 6{,}113\ 7 + 3{,}368\ 4 + 0{,}000\ 0] = 0{,}25 \times 18{,}180\ 8$$

$$a = 0{,}25 \times 18{,}180\ 8 = 4{,}545\ 2$$

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D.3.1.3.8 The variance, V, is calculated using the following formula now that "a" is calculated:

$$V = a\left(\frac{2{,}302\ 6}{\ln N_0 + 0{,}577\ 2}\right)^2$$

where

$N_0 = 1 \times 10^5$;

$$V = 4{,}545\ 2\left[\frac{2{,}302\ 6}{\ln(1\times 10^5) + 0{,}577\ 2}\right]^2$$

$$= 4{,}545\ 2\left(\frac{2{,}302\ 6}{11{,}513 + 0{,}577\ 2}\right)^2$$

$$= 4{,}545\ 2 \times (0{,}190\ 45)^2$$

$$= 4{,}545\ 2 \times 0{,}036\ 27$$

$$V = 0{,}164\ 9.$$

D.3.1.3.9 The 95 % confidence interval for $\overline{D}$ (p = 0,05) D_{calc} is calculated using the following formula:

$$D_{\text{calc}} = \overline{D} \pm 2\sqrt{V}$$

D.3.1.3.10 Lower confidence limit:

$$D_{\text{calc}} = \overline{D} - 2\sqrt{V}$$

$$= 6{,}54 - 2\ \sqrt{0{,}164\ 9}$$

$$= 6{,}54 - (2 \text{ x } 0{,}4061) = 5{,}73$$

D.3.1.3.11 Upper confidence limit:

$$D_{\text{calc}} = \overline{D} + 2\sqrt{V}$$

$$= 6{,}54 + 2\sqrt{0{,}164\ 9}$$

$$= 6{,}54 + (2 \times 0{,}406\ 1) = 7{,}35$$

D.3.2 Limited Holcomb-Spearman-Karber procedure (LHSKP)

D.3.2.1 Calculations using LHSKP

D.3.2.1.1 The calculations for LHSKP are based on a minimum of five exposure conditions and shall include at least:

- one set of samples in which all tested samples show growth;
- two sets of samples in which a fraction of the samples shows growth;
- two sets of samples in which no growth is observed (see Table D.3).

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D.3.2.1.2 LHSK procedure is similar to HSKP (see D.3.1), except that it uses a formula which requires the same number of replicates at each exposure condition and constant time intervals between exposures.

Table D.3 — Examples of data collected for LHSKP with constant time intervals and constant number of samples

Exposure time to sterilizing agent min t	Number of test samples exposed n	Number of test samples showing no growth r_i
t_1 (U_1)	n_1	r_1 (r = 0)
t_2	n_2	r_2
t_3	n_3	r_3
t_4	n_4	r_4
t_5 (U_{k-1})	n_5	r_5
t_6 (U_k)	n_6	r_6 (r = n)
t_7	n_7	r_7 (r = n) [a]

[a] The test is valid if there are no negative units, i.e. no negative test samples (r = 0), with all units showing growth at the exposure preceding U_1, and all negative replicates (r = n), i.e. no replicate showing growth at the exposure subsequent to U_k.

D.3.2.1.3 The mean time to sterility, U_{HSK}, is calculated using the following formula:

$$U_{HSK} = U_k - \frac{d}{2} - \frac{d}{n} \sum_{i=1}^{k-1} r_i$$

where

U_{HSK} is the mean time to sterility;

U_k is the first exposure to show no growth of the replicates;

d is the time or dose interval between exposures (being identical);

n is the number of replicates at each exposure (identical number at each exposure, e.g. 20);

N_0 is the average viable number count per indicator determined by the total viable count method (see Annex A);

$\sum_{i=1}^{k-1} r_i$ is the sum of the negatives between U_2 and U_{k-1} inclusive.

D.3.2.1.4 The mean D value, $\overline{D}$, can be calculated using the following formula:

$$\overline{D} = \frac{U_{HSK}}{\log_{10} N_0 + 0{,}250\,7}$$

NOTE When following the method above, the LHSK procedure makes it possible to calculate the variance, V, the standard deviation (SD) and the 95 % confidence interval (the upper and lower confidence limits).

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D.3.2.1.5 The variance, V, is calculated using the following formula:

$$V = \frac{d^2}{n^2(n-1)} \times \sum_{i=1}^{k-1} ri\,(n - r_i)$$

D.3.2.1.6 The standard deviation (SD) is calculated using the following formula:

$$\text{SD} = \sqrt{V}$$

D.3.2.1.7 The 95 % confidence interval for $\overline{D}$ (p = 0,05) D_{calc}, is calculated using the following formula:

$$D_{\text{calc}} = \overline{D} \pm 2\text{SD limits}$$

D.3.2.1.8 D lower confidence limit

$$= \frac{U_{\text{HSK}} - 2\text{SD}}{\log_{10} N_0 + 0{,}250\,7}$$

D.3.2.1.9 D upper confidence limit

$$= \frac{U_{\text{HSK}} + 2\text{SD}}{\log_{10} N_0 + 0{,}250\,7}$$

D.3.2.2 Example calculations of the Limited Holcomb-Spearman-Karber procedure (LHSKP)

Table D.4 — Examples of data with constant time intervals and constant number of samples

Exposure time to sterilizing agent min t	Number of test samples exposed n	Number of test samples showing no growth r_i
t_1 = 20 (U_1)	n_1 = 20	r_1 = 0 (r = 0)
t_2 = 22	n_2 = 20	r_2 = 1
t_3 = 24	n_3 = 20	r_3 = 7
t_4 = 26	n_4 = 20	r_4 = 15
t_5 = 28 (U_{k-1})	n_5 = 20	r_5 = 19
t_6 = 30 (U_k)	n_6 = 20	r_6 = 20 (r = n) [a]
t_7 = 32	n_7 = 20	r_7 = 20 (r = n)

[a] The test is valid if there are no negative units, i.e. no negative replicates (r = 0), at the exposure preceding U_1, and all negative replicates, i.e. all replicates showing growth, (r = n) at the exposure subsequent to U_k.

D.3.2.2.1 The D value is calculated using the following formula:

$$\overline{D} = \frac{U_{\text{HSK}}}{\log_{10} N_0 + 0{,}250\,7}$$

where $N_0 = 1 \times 10^6$

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D.3.2.2.2 The mean exposure time U_{HSK} required to obtain no growth (sterility) is calculated using the following formula:

$$U_{HSK} = U_k - \frac{d}{2} - \frac{d}{n} \sum_{i=1}^{k-1} r_i$$

where

U_k = 30

d = 2

n = 20

$N_0 = 1 \times 10^6$

$$U_{HSK} = 30 - \frac{2}{2} - \frac{2}{20} \times (0 + 0 + 1 + 7 + 15 + 19) = 24,8$$

$$\overline{D} = \frac{24,8}{6,000 + 0,2507} = 3,97 \text{ min (rounded to one decimal place } D = 4,0 \text{ min)}$$

D.3.2.2.3 The variance, V, is calculated using the following formula:

$$V = \frac{d^2}{n^2(n-1)} \times \sum_{i=1}^{k-1} r_i\,(n - r_i) = \frac{2^2}{(20)^2(20-1)} \times [(1 \times 19) + (7 \times 13) + (15 \times 5) + (19 \times 1)] = 0,107\,4$$

D.3.2.2.4 The standard deviation (SD) is calculated using the following formula:

$$\text{SD} = \sqrt{V}$$

$$\text{SD} = \sqrt{0,1074} = 0,327\,7$$

D.3.2.2.5 The 95 % confidence intervals for $\overline{D}$ (p = 0,05) D_{calc} is calculated using the following formula:

$$D_{calc} = \overline{D} \pm 2\text{SD}$$

$$D \text{ lower confidence limit} = \frac{U_{HSK} - 2\text{SD}}{\log_{10} N_0 + 0,2507}$$

$$= \frac{24,8 - (2 \times 0,3227)}{6,000 + 0,2507} = \frac{24,144}{6,2507} = 3,86 \text{ min.}$$

where

$N_0 = 1 \times 10^6$;

$$D \text{ upper confidence limit} = \frac{U_{HSK} + 2\text{SD}}{\log_{10} N_0 + 0,2507} = \frac{24,8 + (2 \times 0,3227)}{6,000 + 0,2507} = \frac{25,455}{6,2507} = 4,07 \text{ min.}$$

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D.3.3 The Stumbo-Murphy-Cochran-Procedure (SMCP)

D.3.3.1 Introduction

D.3.3.1.1 Other methods of analysing fraction negative data may be used when equivalence with the methods of D.3.1 and D.3.2 is demonstrated.

D.3.3.1.2 SMCP, a most probable number (MPN) method, can be practical to use when the response characteristics are predictable.

D.3.3.1.3 The formula for SMCP requires one result in the fraction negative range consisting of time, t, the number of units negative for growth, r, the number of replicates, n, at one exposure time within the fraction negative range and the initial number of microorganisms per replicate, N_0.

D.3.3.1.4 To obtain valid data using SMCP, the D value should be calculated as the average of at least three runs in the fraction negative range in order to confirm reproducibility.

D.3.3.1.5 The same materials apply as those in D.2.

D.3.3.1.6 For a confidence interval of 95 %, not less than 50 replicates at each exposure condition shall be used and the condition $r/n < 0{,}9$ shall be met in order to establish test criteria equivalent to D.3.1 and D.3.2. Test samples shall be subjected to a defined exposure condition within the survival/kill window of the batch/lot.

D.3.3.2 Calculations using the Stumbo-Murphy-Cochran-Procedure

D.3.3.2.1 The D value is calculated using the following formula:

$$D = \frac{t}{\log_{10} A - \log_{10} B}$$

where

t is the exposure time

$\log_{10} A$ = $\log_{10}$ of initial population, N_0, per replicate;

$\log_{10} B$ = $\log_{10}$ of population after exposure time, t.

D.3.3.2.2 This formula can be restated for fraction negative data sets:

$$D = \frac{t}{\log_{10} N_0 - \log_{10}\left(\ln \frac{n}{r}\right)}$$

or

$$D = \frac{t}{\log_{10} N_0 - \log_{10} N_{\mu_i}}$$

where $\log_{10} B = \log_{10} (\ln n/r)$ or $\log_{10} [2{,}303 \log_{10} (n/r)]$

and where

N_{μ_i} is the natural log of the quotient of the number of replicates per test divided by the number of negative samples;

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n is the number of replicates per exposure time;

r is the number of units sterile or showing no growth.

D.3.3.2.3 The 95 % confidence interval for $\overline{D}$ (p = 0,05) D_{calc} is calculated using the following formula:

$$D_{calc} = \frac{t}{\log_{10} N_0 - \log_{10}\left(\ln\frac{(1)}{a}\right)}$$

where $a = \frac{r}{n} \pm 1{,}96\sqrt{\frac{r}{n} \times \frac{1-r/n}{n}}$

D.3.3.2.4 The formula above can only be used if $n \times \frac{r}{n} \times \frac{n-r}{n}$ is $\geqslant$ 0,9.

D.3.3.3 Example calculations of SMCP

Table D.5 — Calculations of D value using only one data set in the fractional negative zone

Exposure time min t	Number of test samples exposed n	Number of test samples showing no growth r
t = 24	n = 100	r = 37

D.3.3.3.1 The D value is calculated using the following formula:

$$D = \frac{t}{\log_{10} N_0 - \log_{10}\left(\ln\frac{n}{r}\right)}$$

where

t is the exposure time;

N_0 is the initial viable count per test organism per sample = 1×10^6;

$\log_{10} A$ = $\log_{10}$ of initial population, N_0, per sample;

$\log_{10} B$ = $\log_{10}$ of population after exposure time, t,

or

= $\log_{10}$ (ln n/r) or $\log_{10}$ [2,303 $\log_{10}$ (n/r)];

n is the number of replicates per exposure time;

r is the number of units sterile or showing no growth.

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$$D = \frac{24}{6{,}000 - \log_{10}\left(\ln 2{,}702\ 7\right)}$$

$$D = \frac{24}{6{,}000 - \log_{10}\left(0{,}994\ 3\right)}$$

$$D = \frac{24}{6{,}000 - \left(-0{,}002\ 5\right)}$$

$$D = \frac{24}{6{,}002\ 5}$$ = 4,00 min (rounded to one decimal place D = 4,0 min)

D.3.3.3.2 The 95 % confidence interval for $\overline{D}$ (p = 0,05) D_{calc} is calculated using the following formula.

If $n \times \frac{r}{n} \times \frac{n-r}{n} \geqslant$ to 0,9, then the 95 % confidence interval can be calculated using the following equations:

$$D \text{ lower confidence limit} = \frac{t}{\log N_0 - \log_{10}(\ln 1/a)}$$

where

$$a = \frac{r}{n} + 1{,}96\sqrt{\frac{r}{n} \times \frac{1-\frac{r}{n}}{n}}$$

$$D_{calc} = \frac{24}{6{,}000 - \log_{10}(\ln 1/a)}$$

where

$$a = \frac{37}{100} + 1{,}96\sqrt{\frac{37}{100} \times \frac{1-37/100}{100}}$$

$$= 0{,}37 + 1{,}96\sqrt{0{,}37 \times \frac{0{,}63}{100}}$$

$$= 0{,}37 + 1{,}96\sqrt{0{,}37 \times 0{,}006\ 3}$$

$$= 0{,}37 + 1{,}96\sqrt{0{,}002\ 331}$$

$$= 0{,}37 + 1{,}96 \times 0{,}048\ 28$$

$$a = 0{,}465$$

$$D_{calc} = \frac{24}{6{,}000 - \log_{10}\left(\ln \frac{1}{0{,}465}\right)}$$

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$$= \frac{24}{6{,}000 - \log_{10}(0{,}765\ 7)}$$

$$= \frac{24}{6{,}000 - (-0{,}115\ 9)}$$

$$= \frac{24}{6{,}000 + 0{,}115\ 9}$$

$$D_{\text{calc}} = \frac{24}{6{,}115\ 9} = 3{,}92$$

$$D \text{ upper confidence limit} = \frac{t}{\log_{10} N_0 - \log_{10}(\ln 1/a)}$$

where

$$a = \frac{r}{n} - 1{,}96\sqrt{\frac{r}{n} \times \frac{1 - \frac{r}{n}}{n}}$$

$$D_{\text{calc}} = \frac{24}{6{,}000 - \log_{10}(\ln 1/a)}$$

where

$$a = \frac{37}{100} - 1{,}96\sqrt{\frac{37}{100} \times \frac{1 - 37/100}{100}}$$

$$= 0{,}37 - 1{,}96\sqrt{0{,}37 \times \frac{0{,}63}{100}}$$

$$= 0{,}37 - 1{,}96\sqrt{0{,}37 \times 0{,}006\,3}$$

$$= 0{,}37 - 1{,}96\sqrt{0{,}002\ 331}$$

$$= 0{,}37 - 1{,}96 \times 0{,}048\ 28$$

$$a = 0{,}37 - 0{,}095 = 0{,}275$$

$$D_{\text{calc}} = \frac{24}{6{,}000 - \log_{10}\left(\ln \frac{1}{0{,}275}\right)}$$

$$= \frac{24}{6{,}000 - \log_{10}(1{,}291)}$$

$$= \frac{24}{6{,}000 - 0{,}111}$$

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$$D_{calc} = \frac{24}{5,889} = 4,08$$

$$= \frac{24}{6,000 - \log_{10}\left(\ln\frac{1}{0,275}\right)}$$

$$= \frac{24}{6,000 - \log_{10}(1,291)}$$

$$= \frac{24}{6,000 - 0,111}$$

$$D_{calc} = \frac{24}{5,889} = 4,08$$

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Annex E
(normative)

Survival-kill response characteristics

E.1 General

Monitoring the survival-kill response characteristics of a lot/batch of biological indicators provides an additional means of ensuring the consistent performance of units within a given lot/batch.

E.2 Materials

E.2.1 Test samples shall be representative of spore suspensions, inoculated carriers or packaged biological indicators.

E.2.2 The relevant resistometer shall be used.

NOTE Test methods are given in subsequent parts of ISO 11138. Specifications for resistometers are given in the resistometer standard (see ISO 18472).

E.2.3 The incubator shall be set to provide, and monitored to confirm, the temperature specified in the culture conditions.

E.2.4 The growth medium shall be as specified in the culture conditions.

E.3 Method

E.3.1 Not less than 50 replicates shall be used to confirm both the survival time and the kill time (see Table 2). The D value calculated by survivor curve method (see Annex C) or a fraction negative method (see Annex D) shall be used for the stipulation of the survival-kill response characteristics.

E.3.2 Samples shall be cultured after exposure according to the manufacturer's specified method.

E.3.3 The survival performance is the labelled exposure time that results in surviving test organisms for each biological indicator. The kill performance is the labelled exposure time that results in kill of all test organisms of each biological indicator.

E.3.4 Survival-kill performance characteristics shall be determined in a resistometer using the relevant resistometer process parameters.

NOTE Reference conditions for specific sterilization processes are provided in subsequent parts of ISO 11138.

E.3.5 Relevant values for survival time and kill time can be obtained by using the following equations:

survival time = not less than ($\log_{10}$ nominal population $-$ 2) $\times$ D value;

kill time = not more than ($\log_{10}$ nominal population $+$ 4) $\times$ D value.

E.3.6 The number of units run per exposure will depend on both the capacity and the operating characteristics of the resistometer being used. It may be necessary to run several exposures at both the survival and kill times in order to test the total number of units required.

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Annex F
(informative)

Relationship between components of biological indicators

Table F.1 — Relationship between components of biological indicators

Graphic illustrations	Components	Terminology
⇩	microorganisms	test organisms
⇩	microorganisms suspended in fluid [a]	test organism suspensions [b]
⇩	microorganisms inoculated on surfaces [c]	inoculated carrier [b]
⇩	inoculated carrier in primary package	individually packaged biological indicator
	growth medium with processed inoculated carrier	testing of growth properties of processed inoculated carrier
Sterilizarion process unprocessed testing of growth properties of inoculated carrier	ready to use system with combination of inoculated carrier and growth medium	self contained biological indicator

NOTE The illustrations reflect the common physical configurations of components in a graphical presentation. Test organism suspensions in fluids have the suspending medium as the carrier material, not a solid material (see 3.2).

[a] The fluid employed may vary depending on whether the microorganisms are to be held for storage purposes or employed for testing purposes.

[b] May be defined as a biological indicator if used to monitor a sterilization process.

[c] In some instances the surface may be product for testing purposes.

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