BRITISH STANDARD

BS EN
866-1 : 1997

Biological systems for testing sterilizers and sterilization processes

Part 1. General requirements

The European Standard EN 866-1 : 1997 has the status of a British Standard

ICS 11.080

Committees responsible for this British Standard

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ABHI Special Interest Section (Sterilizers and Disinfectors)
Association of British Healthcare Industries
BLWA Ltd. (the Association of the Laboratory Supply Industry)
Department of Health (Medical Devices Agency)
European Sterilization Packaging Association (UK)
Medical Sterile Products Association
Public Health Laboratory Service
Royal College of Pathologists
Royal Pharmaceutical Society of Great Britain

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National foreword

This Part of BS EN 866 has been prepared by Subcommittee LBI/35/3 and is the English language version of EN 866-1 : 1997 *Biological systems for testing sterilizers and sterilization processes — Part 1: General requirements*, published by the European Committee for Standardization (CEN).

Cross-references

Publication referred to	Corresponding British Standard
EN 550 : 1994	BS EN 550 : 1994 *Sterilization of medical devices — Validation and routine control of ethylene oxide sterilization*
EN 552 : 1994	BS EN 552 : 1994 *Sterilization of medical devices — Validation and routine control of sterilization by irradiation*
EN 554 : 1994	BS EN 554 : 1994 *Sterilization of medical devices — Validation and routine control of sterilization by moist heat*
EN 868-1 : 1997	BS EN 868 *Packaging materials and systems for medical devices which are to be sterilized* Part 1 : 1997 *General requirements and test methods*
EN 1174-1 : 1996	BS EN 1174 *Sterilization of medical devices — Estimation of the population of micro-organisms on product* Part 1 : 1996 *Requirements*
EN 28601 : 1992	BS EN 28601 : 1992 *Specification for representation of dates and times in information interchange*

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

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN title page, pages 2 to 10, an inside back cover and a back cover.

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 866-1

February 1997

ICS 11.080

Descriptors: Medical equipment, sterilizers, bioassay, manufacturing, biological indicators, specifications, preparation, packing, labelling

English version

Biological systems for testing sterilizers and sterilization processes — Part 1: General requirements

Systèmes biologiques pour l'essai des stérilisateurs et les procédés de stérilisation — Partie 1: Exigences générales

Biologische Systeme für die Prüfung von Sterilisatoren und Sterilisationsverfahren — Teil 1: Allgemeine Anforderungen

This European Standard was approved by CEN on 1997-01-10. CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart 36, B-1050 Brussels

Foreword

This European Standard has been prepared by Technical Committee CEN/TC 102, Sterilizers for medical purposes, the secretariat of which is held by DIN.

This Standard is one of a series of European Standards concerned with biological systems for testing sterilizers. These European Standards are:

EN 866-1 *Biological systems for testing sterilizers and sterilization processes — Part 1: General requirements*

EN 866-2 *Biological systems for testing sterilizers and sterilization processes — Part 2: Particular systems for use in ethylene oxide sterilizers*

EN 866-3 *Biological systems for testing sterilizers and sterilization processes — Part 3: Particular systems for use in moist heat sterilizers*

prEN 866-4 *Biological systems for testing sterilizers and sterilization processes — Part 4: Particular systems for irradiation sterilizers*

prEN 866-5 *Biological systems for testing sterilizers and sterilization processes — Part 5: Particular systems for low-temperature steam formaldehyde sterilizers*

prEN 866-6 *Biological systems for testing sterilizers and sterilization processes — Part 6: Particular systems for dry heat sterilizers*

prEN 866-7 *Biological systems for testing sterilizers and sterilization processes — Part 7: Particular requirements for self-contained biological indicator systems for use in moist heat sterilizers*

prEN 866-8 *Biological systems for testing sterilizers and sterilization processes — Part 8: Particular requirements for self-contained biological indicator systems for use in ethylene oxide sterilizers*

In addition CEN/TC 102 Working Group 7 has prepared a series of European Standards describing non-biological indicators for use in sterilizers. These European Standards are:

EN 867-1 *Non-biological systems for use in sterilizers — Part 1: General requirements*

EN 867-2 *Non-biological systems for use in sterilizers — Part 2: Process indicators (Class A)*

EN 867-3 *Non-biological systems for use in sterilizers — Part 3: Specification for Class B indicators for use in the Bowie and Dick test*

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 1997, and conflicting national standards shall be withdrawn at the latest by August 1997.

This European Standard has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s).

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Contents

Introduction

European Standards for sterilizers (EN 285 and prEN 1422) and for the validation and process control of sterilization (EN 550 and EN 554) describe performance tests for sterilizers and methods of validation and routine control, respectively.

This standard specifies the general requirements for biological indicators, and subsequent Parts specify the particular requirements for biological indicators for defined sterilization processes. The use of the indicators specified in this standard is described in EN 550 and EN 554.

The use of a biological system for testing a sterilization process does not allow necessarily the same level of sensitivity in response to inadequate levels of all the critical variables of the process.

The biological indicators specified in this standard are not intended for use in any process other than that stated by the manufacturer. The use of a biological indicator in a process other than that stated by the manufacturer can give dangerously misleading results.

The performance of a biological indicator can be affected by the conditions of storage prior to use, the methods of use, and the techniques employed after exposure to the process. For these reasons, the recommendations of the manufacturer for storage and use should be followed and biological indicators should be transferred to the specified recovery conditions as soon as possible after exposure to the process. Biological indicators should not be used beyond any expiry date stated by the manufacturer.

Biological indicators should always be used in combination with appropriate physical and/or chemical monitoring in demonstrating the efficacy of a sterilization process. When a physico-chemical variable of a sterilization process is outside its specified limits, a sterilization cycle should always be regarded as unsatisfactory, irrespective of the results obtained from the biological indicators. See also EN 550 and EN 554.

1 Scope

This Part of EN 866 specifies general requirements for the manufacture of biological systems to be used in testing sterilizers and sterilization processes.

The requirements of this Part of EN 866 apply to all biological systems specified in subsequent Parts of EN 866, unless the requirement is modified or added to by a subsequent Part, in which case the requirement of the particular Part will apply.

2 Normative references

This European Standard incorporates, by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references, the latest edition of the publication referred to applies.

EN 550	*Sterilization of medical devices — Validation and routine control of ethylene oxide sterilization*
EN 552	*Sterilization of medical devices — Validation and routine control of sterilization by irradiation*
EN 554	*Sterilization of medical devices — Validation and routine control of sterilization by moist heat*
EN 868-1	*Packaging materials and systems for medical devices which are to be sterilized — Part 1: General requirements — Requirements and test methods*
EN 1174-1	*Sterilization of medical devices — Estimation of the population of micro-organisms on product — Part 1: Requirements*
EN 28601 : 1992	*Data elements and interchange formats — Information interchange — Representation of dates and times* (ISO 8601 : 1988 and technical corrigendum 1 : 1991)

3 Definitions

For the purposes of this standard, the following definitions apply.

3.1 biological indicator

An inoculated carrier contained within its primary pack ready for use (see figure C.1 in annex C).

3.2 biological systems

Those systems which depend for their function on the demonstration of viability of a test organism.

NOTE. This demonstration of viability can be achieved by any method suitable for indicating, qualitatively or quantitatively, either the metabolic activity or the replication of the organism. Tests based on physical or chemical detection of change in a particular chemical entity, whether or not this was originally derived or isolated from a biological system (e.g. an enzyme) are considered in the series EN 867 *Non-biological systems for use in sterilizers*.

3.3 carrier

The supporting material on which the test organisms are deposited (see figure C.1 in annex C).

3.4 culture collection number

The unique identification allocated by the recognized culture collection.

3.5 culture conditions

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

3.6 D value (decimal reduction value)

The time in minutes, or the absorbed irradiation dose in kilograys, required to secure inactivation of 90 % of the test organisms under stated exposure conditions.

3.7 inactivation

The loss of the ability of the test organisms to germinate, outgrow and/or multiply under culture conditions.

3.8 inoculated carrier

A carrier on which a defined number of test organisms has been deposited (see figure C.1 in annex C).

3.9 primary pack

The packaging system which protects the inoculated carrier from damage and contamination without preventing penetration of the sterilizing agent(s) (see figure C.1 in annex C).

3.10 process challenge device

An object which simulates the worst case of conditions as they are given for the sterilizing agent(s) in the items of the goods to be sterilized (see figure C.1 in annex C.)

NOTE 1. A device is so constituted that a biological indicator can be put in the place most difficult to reach by the sterilizing agent(s). The design of the process challenge device depends on the kind of goods to be sterilized and the sterilization procedure. The biological indicator should not interfere with the function of the process challenge device.

NOTE 2. In some process challenge devices an inoculated carrier can be used instead of a biological indicator.

3.11 recognized culture collection

An international depository authority under the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for the Purpose of Patent and Regulations.

3.12 resistometer

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

3.13 secondary pack

The container in which biological indicators are packed for transport and storage.

3.14 spores

Bacterial endospores.

3.15 survivor curve

A graphical representation of inactivation against increasing exposure to stated conditions.

3.16 test organism

Micro-organisms used for the manufacture of inoculated carriers.

3.17 total count

The number of test organisms in unit volume of a suspension, estimated by direct counting using light microscopy.

3.18 viable count

The number of viable test organisms in unit volume of a suspension, estimated by growth of discrete colonies under the stated culture conditions.

3.19 nominal number

The theoretical number of micro-organisms based on the viable count.

4 General requirements

4.1 Manufacturing controls

4.1.1 The manufacturer shall establish, document and maintain a formal quality system to cover all operations required by this standard.

NOTE. European standards of the ISO 9000 series and the EN 45000 series describe requirements for quality systems for manufacture and testing.

4.1.2 Traceability of manufacturing components (e.g. carrier, culture media and packaging material) shall be maintained.

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

4.1.4 The procedures and methods in this standard shall be carried out by suitably trained and experienced microbiology laboratory staff.

4.2 Labelling

Where a date is required on labelling or information supplied with the product, this shall be expressed in accordance with EN 28601.

4.3 Test methods

The test methods specified in this standard are reference methods. When alternative methods are used, these shall be defined, validated and have proven correlation with the reference method.

4.4 Culture conditions

The culture conditions shall be demonstrated as being capable of recovering an inoculum of between 10 and 100 test organisms. The suspension of test organisms used shall be of the same strain and prepared in the same manner as the organisms to be used for inoculation of carriers. The population shall be determined by viable count of the same suspension used to provide the inoculum.

5 Test organisms and their preparation for the manufacture of inoculated carriers

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

The test organism shall be of a strain suitable for handling without special containment facilities.

NOTE. When the strain of the test organism is not already lodged with a recognized culture collection, the manufacturer should do so.

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

– traceable to the reference culture lodged with the recognized culture collection (see note 1); and

– verified as to its identity and purity (see note 2).

NOTE 1. The method(s) used for the maintenance of cultures of the test organism should be designed and maintained to ensure that the cultures are protected from contamination and induced changes in their inherent properties.

NOTE 2. Verification tests are specific for each strain of test organism and should be documented and validated by the manufacturer.

5.3 The culture medium and incubation conditions used for preparation of the test organism suspension shall be defined by the manufacturer.

These culture conditions shall produce consistently test organism suspensions meeting the performance requirements of this standard and the particular performance requirements in the relevant subsequent Parts of this standard.

5.4 After growth and, where required, sporulation, the test organisms shall be removed from the culture medium.

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

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

5.5 The viable count of the suspension shall be determined.

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

5.6 The container and conditions for storage of suspensions of test organisms, together with their expiry date, shall be defined by the manufacturer. The conditions shall be monitored during storage. The container and conditions shall maintain consistently test organism suspensions meeting the performance requirements of this standard and the particular performance requirements in the relevant subsequent parts of this standard.

5.7 If the test organism suspension is distributed for the inoculation of carriers by a third party, each container shall be labelled with the following information:

a) the name of the test organism;

b) the culture collection number;

c) the nominal volume of the suspension in millilitres;

d) the viable count;

e) the recommended storage conditions;

f) the expiry date;

g) a unique code from which the manufacturing history can be traced;

h) the manufacturer's name and address or other means of identification.

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

6 Carriers and their inoculation

6.1 The material of which the carrier is made shall withstand exposure to the sterilization process for which it is intended without distortion, melting, corrosion or other failure which would impair its utility. Compliance is tested by observation of carrier material exposed to the limiting values for the range and rate of change of chemical and physical variables of the sterilization process. These limits are given in the relevant subsequent parts of this standard.

NOTE 1. The carrier should be sufficiently robust to withstand transport in the primary and secondary pack and handling at the point of use without breakage.

NOTE 2. The design of the carrier should be such that:

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

b) it is appropriate to be located in a process challenge device.

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

Compliance shall be tested in accordance with the method described in annex A.

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

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

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

Prior to inoculation, the carrier shall be free from micro-organisms which can affect adversely the performance of the product as specified in the subsequent Parts of this standard.

Compliance shall be demonstrated by either:

a) sterilizing the carrier in accordance with EN 550, EN 552 or EN 554; or

b) estimating the bioburden in accordance in EN 1174-1 and demonstrating that numbers, types and interactions of the micro-organisms will not result in non-compliance with the requirement.

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

6.7 The conditions under which inoculation is carried out shall be specified, validated and controlled to ensure that the inoculated carrier remains free from micro-organisms, other than the test organism, which can affect adversely the performance of the product as specified in the subsequent parts of this standard.

6.8 The conditions for storage of inoculated carriers and their expiry date shall be defined by the manufacturer. These conditions shall be monitored during storage. These conditions shall maintain consistently inoculated carriers meeting the performance requirements of this standard and the particular performance requirements in the relevant subsequent parts of this standard.

7 Packaging and labelling of inoculated carriers for distribution

7.1 Where the inoculated carriers are distributed for use by a third party, they shall be packaged to prevent damage to the test organism bearing surface of individual inoculated carriers.

7.2 Each package containing a number of inoculated carriers shall be accompanied by the following information:

a) name of test organism;

b) culture collection number;

c) the nominal number of test organisms per inoculated carrier;

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

e) the number of inoculated carriers;

f) the recommended storage conditions;

g) the expiry date;

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

i) the sterilization process for which the inoculated carrier is designed;

j) directions for use; this shall include the culture conditions to be used after exposure to the sterilization process;

k) the resistance of the test organisms and the method used to determine the resistance;

l) instructions for the disposal of inoculated carriers.

8 Preparation of biological indicators from inoculated carriers

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

8.2 The conditions under which packaging is carried out shall be specified and controlled to ensure that the inoculated carrier remains free from micro-organisms other than the test organism.

8.3 The primary pack shall comply with the requirements of en 868-1.

8.4 Each primary pack shall be labelled with the following information:

a) name of a test organism;

b) a unique code from which the manufacturing history can be traced;

c) the manufacturer's name or other means of identification.

9 Packaging and labelling of biological indicators

9.1 Biological indicators shall be packed in a secondary pack to prevent damage during transport and storage.

9.2 The secondary pack shall be accompanied by the following information:

a) name of test organism;

b) culture collection number;

c) the nominal number of test organisms per biological indicator;

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

e) the number of biological indicators;

f) the recommended storage conditions;

g) the expiry date;

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

i) the sterilization process for which the biological indicator is designed;

j) directions for use; this shall include the culture conditions to be used after exposure to the sterilization process;

k) the resistance of the test organisms and the method used to determine the resistance;

l) instructions for the disposal of biological indicators.

10 Determination of test organism resistance

Each batch of biological indicators or inoculated carriers shall be tested to demonstrate conformance with the performance requirements specified in the relevant subsequent Part of this standard, including resistance to the sterilization process.

The test method in annex B shall be used as a reference method.

NOTE 1. Individual batches can be tested by other methods of demonstrated equivalence.

This shall include determination of the D value, both by the construction of a survivor curve by direct enumeration, and by most-probable-number (MPN) determination.

The D values obtained by both methods shall be within the range specified in the particular Part of this standard.

NOTE 2. The ideal survivor curve is linear over the full range of inactivation. In practice, deviations from this ideal occur but should be maintained within acceptable limits. Construction of a survivor curve by direct enumeration establishes the resistance for surviving populations greater than 5×10^1, whereas the MPN method establishes the resistance for surviving populations below this 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.

Annex A (normative)
Determination of growth inhibition by carriers exposed to the sterilization process

A.1 Materials

A.1.1 *Suspension of test organisms*, of the same strain and prepared in the same manner as the organisms to be used for inoculation of carriers. The suspension shall be of known population, determined by viable count, to permit dispensing of aliquots with a population of between 10 and 100 viable organisms.

A.1.2 *Resistometer*, complying with the relevant subsequent Part of this standard.

A.1.3 *Growth medium*, as specified in the culture conditions.

A.1.4 *Incubator*, set and monitored to the temperature specified in the culture conditions.

A.2 Procedure

A.2.1 Take a representative sample of 12 uninoculated carriers, and divide it into six groups of two. Prepare nine containers of growth medium.

A.2.2 Prepare three packs, each containing two carriers. They shall be packed in the material used in the manufacture of biological indicators and then exposed to the sterilization process.

A.2.3 The operational conditions of the resistometer shall be set to the values specified in the relevant subsequent Part of this standard.

A.2.4 After exposure to the process, as soon as possible but in any case within 2 h of the end of the process, unwrap the carriers and aseptically transfer them to the growth medium without intermediate treatment. Place the contents of a pack of two carriers in each of three containers of growth medium previously incubated to the incubation temperature.

A.2.5 Record the time taken to complete the transfer.

A.2.6 Then incubate the growth medium at the stated temperature for 2 h to allow any inhibitory substances to desorb from the carriers. Remove the growth medium from the incubator and inoculate it with a volume of the test organism suspension calculated to contain between 10 and 100 viable organisms. Return the inoculated media to the incubator and incubate for the time stated by the manufacturer for the recovery of biological indicators under normal conditions of use.

A.2.7 ***Carrier control***

Place the two carriers from each pack in each of the three containers of growth medium, heat for 2 h, inoculate with between 10 and 100 test organisms, and incubate for the stated recovery period in the same manner as described above.

A.2.8 ***Growth medium control***

Incubate three containers of growth medium, containing no carriers, for 2 h, inoculate with between 10 and 100 test organisms and incubate for the stated recovery period in the same manner as described above.

A.2.9 At the end of the stated recovery period, remove all nine containers from the incubator and examine for viable organisms in accordance with the manufacturer's instructions.

A.2.10 Report results as 'growth' or 'no growth' of the test organism.

A.3 Interpretation of results

A.3.1 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 indicate failure to control the population of the test organism inoculum, or inappropriate recovery conditions (growth medium, temperature etc.).

A.3.2 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, but where growth was obtained in the growth control medium, can indicate that the material of which the carrier is made is itself inhibitory to the growth of the test organism.

A.3.3 If 'no growth' occurs in one or more of the three tests on carriers exposed to the process, the carrier shall not be regarded as suitable for the manufacture of inoculated carriers or biological indicators.

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

Annex B (normative)
Determination of test organism resistance

B.1 D value determination by an MPN method

B.1.1 Test samples shall be subjected to graded exposures to the defined exposure conditions with all process variables except times remaining constant. Not fewer than twenty inoculated carriers shall be used for each exposure time.

NOTE. Details of the performance requirements for exposure apparatus are given in the relevant subsequent Parts of this standard.

B.1.2 After exposure, the test samples shall be assayed using the stated culture conditions.

B.1.3 A minimum of seven exposure conditions shall be used and shall include at least:

a) one set of samples in which all tested samples show growth;

b) four sets of samples in which a fraction of the samples show growth;

c) two sets of samples in which no growth is observed.

B.1.4 *Calculation of D value*

In the calculation given below, the data generated by the method described in **B.2.1** to **B.2.3** inclusive is codified as follows:

Table B.1 Data for calculation of D value

Time of exposure to sterilant	Number of test samples exposed	Number of test samples showing no growth
t_1	n_1	r_1
t_2	n_2	r_2
t_3	n_3	r_3
t_4	n_4	r_4
t_5	n_5	r_5
t_6	n_6	r_6
t_7	n_7	r_7

NOTE 1. t_1 is the shortest exposure time to sterilant and all test samples show growth; thus $r_1 = 0$. Times of exposure to sterilant t_2 to t_5 are increasing exposure times in the quantal region. Exposure times t_6 and t_7 are the two exposure times at which all samples show no growth; thus $n_6 = r_6$ and $n_7 = r_7$.

For times of exposure to sterilant t_1 to t_6, the factors x and y are calculated as shown:

$$x_i = \frac{t_i + t_{i+1}}{2}$$

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

NOTE 2. r_i is the number of test samples out of the number exposed (n_i) showing no growth at exposure time t_i.

At t_i, all test samples show growth, and so:

$$y_i = \frac{r_{i+1}}{n_{i+1}}$$

From the calculated values of x_i and y_i above, the value μ_i can be calculated for each exposure time (t_i), as follows:

$$\mu_i = x_i\, y_i$$

The mean time to attain no growth, $\overline{\mu}$, from any of the test samples can then be calculated as the sum of μ_i for each exposure time t_1 to t_6:

$$\overline{\mu} = \sum_{i=1}^{i=6} \mu_i$$

NOTE 3. 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 time to attain no growth ($\overline{\mu}$) can be calculated from the equation:

$$\overline{\mu} = t_6 - \frac{d}{2} - \frac{d}{n} \sum_{i=6}^{i=1} r_i$$

The mean D value, $\overline{D}$, can be calculated from the equation:

$$\overline{D} = \frac{\overline{\mu}}{0{,}2507 + \log_{10} N_0}$$

where N_0 is the initial inoculum of test organisms per test sample.

B.2 D value determination by survivor curve method

B.2.1 Test samples shall be subjected to graded exposures to the defined exposure conditions with all process variables except time remaining constant. A minimum of five exposure conditions shall be used and shall include:

a) no exposure to the sterilant;

b) reduction of the viable population to not more than 0,01 % of the original inoculum over the range of exposure times employed.

NOTE. Details of the performance requirements for exposure apparatus are given in the relevant subsequent Parts of this standard.

B.2.2 Not less than three runs shall be performed. Not less than three inoculated carriers shall be used for each exposure time in each run.

B.2.3 After exposure, the test samples shall be treated to remove the test organisms from the carrier and a viable count assay performed using the culture conditions and methods stated by the manufacturer.

NOTE. The method used to remove test organisms from the carrier depends on the carrier material and should be validated.

B.2.4 Using all the data obtained, plot the $\log_{10}$ of the surviving population against time in minutes and determine the best fit rectilinear curve by regression analysis using the method of least squares. Calculate the reciprocal of the slope of the line obtained which is equal to the D value in minutes.

NOTE. The correlation coefficient of the regression line should be determined and should lie within the range 0,80 to 1,0.

Annex C (informative)
Relationship between the components of biological test systems

NOTE. In some process challenges an inoculated carrier can be used instead of a biological indicator.

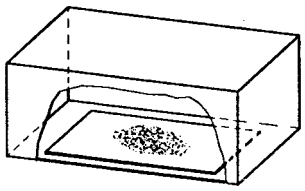
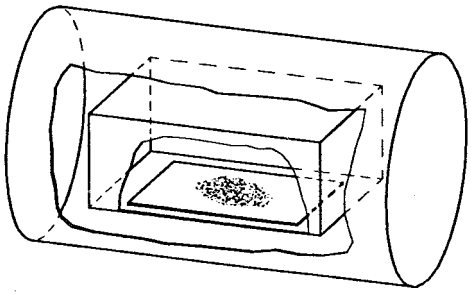
Illustration	Components	Description
	Test organisms + carrier	Inoculated carrier
	Inoculated carrier + primary	Biological indicator
	Biological indicator + process challenge device	Process challenge

Figure C.1 Components of biological test systems

Annex D (informative)
Bibliography

EN 285 *Sterilization — Steam sterilizers — Large sterilizers*

prEN 1422 *Sterilizers for medical purposes — Ethylene oxide sterilizers — Specifications*

EN 45001 *General criteria for the operation of testing laboratories*

EN 45014 *General criteria for suppliers' declaration of conformity*

EN ISO 9001 *Quality systems — Model for quality assurance in design/development, production, installation and servicing* (ISO 9001 : 1994)

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

EN ISO 9003 *Quality systems — Model for quality assurance in final inspection and test* (ISO 9003 : 1994)

List of references

See national foreword.

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