BRITISH STANDARD

BS EN
866-2 : 1998

Biological systems for testing sterilizers and sterilization processes

Part 2. Particular systems for use in ethylene oxide sterilizers

The European Standard EN 866-2 : 1997 has the status of a British Standard

ICS 11.080

Committees responsible for this British Standard

The preparation of this British Standard was entrusted by Technical Committee LBI/35, Sterilizers, autoclaves and disinfectors, to Subcommittee LBI/35/3, Sterilization indicators, upon which the following bodies were represented:

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

This British Standard, having been prepared under the direction of the Sector Board for Materials and Chemicals, was published under the authority of the Standards Board and comes into effect on 15 March 1998

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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 866-2 : 1997 *Biological systems for testing sterilizers and sterilization processes — Part 2: Particular systems for use in ethylene oxide sterilizers*, published by the European Committee for Standardization (CEN).

Cross-reference

Publication referred to	Corresponding British Standard
EN 866-1 : 1997	BS EN 866 *Biological systems for testing sterilizers and sterilization processes* Part 1 : 1997 *General requirements*

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 6, an inside back cover and a back cover.

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EUROPEAN STANDARD

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EN 866-2

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ICS 11.080

Descriptors: Medical equipment, sterilizers, sterilization, ethylene oxide, bioassay, specifications, biological indicators

English version

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

Systèmes biologiques pour l'essai des stérilisateurs et les procédés de stérilisation — Partie 2: Systèmes particuliers destinés à être utilisés dans des stérilisateurs à l'oxyde d'éthylène

Biologische Systeme für die Prüfung von Sterilisatoren und Sterilisationsverfahren — Teil 2: Spezielle Systeme für den Gebrauch in Ethylenoxid-Sterilisatoren

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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 use in irradiation sterilizers*

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

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

This standard specifies the performance requirements for biological indicators supplied ready for use, and for suspensions of test organisms supplied either for the preparation of biological indicators or for the inoculation of product for use in validation studies on, and routine monitoring of, ethylene oxide sterilization processes. The use of the indicators specified in this standard is described in EN 550.

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

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 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 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).

1 Scope

This Part of EN 866 specifies requirements for inoculated carriers and biological indicators intended for use in assessing the performance of sterilizers and sterilization processes employing ethylene oxide gas as the sterilant. These are intended for use in sterilizers employing pure ethylene oxide gas or admixtures of the gas with diluent gases, over a sterilizing temperature range of 20 °C to 65 °C.

NOTE. prEN 1422 specifies the performance and test requirements for ethylene oxide sterilizers. EN 550 specifies the requirements for the validation and routine monitoring of ethylene oxide sterilization.

2 Normative references

This European Standard incorporates by dated or undated references 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 866-1 : 1997 *Biological systems for testing sterilizers and sterilization processes — Part 1: General requirements*

3 Definitions

For the purposes of this standard, the definitions given in EN 866-1 apply.

4 General requirements

The requirements of EN 866-1 shall apply.

5 Test organisms

The test organism shall be spores of *Bacillus subtilis var. niger* or other strains or organisms of demonstrated equivalent performance as required by this standard.

NOTE. *Bacillus subtilis* NCTC 10073, DSM 2277, ATCC 9372 or CIP 7718 have been found to be suitable.

6 Population of test organisms

6.1 Replicate determinations of the viable count on the same batch of suspension shall be within $\pm$ 35 % of the nominal population.

6.2 The number of recoverable test organisms on each biological indicator shall be controlled during manufacture to be either within $\pm$ 50 % of the nominal population stated by the manufacturer, or within the minimum and maximum populations stated by the manufacturer.

6.3 Retrospective determination of the count shall be made by performing a viable count under the culture conditions on a suspension of test organisms obtained by physical removal of the test organisms from the carrier through ultrasonication, shaking with glass beads, or other appropriate validated methods. Counts obtained shall be regarded as acceptable if they are within −50 % and +300 % of the nominal population stated by the manufacturer or the mid-point between the minimum and maximum populations stated by the manufacturer.

NOTE. Guidance on the selection and validation of methods for the removal of micro-organisms from the carrier is given in EN 1174-2.

6.4 For inoculated carriers or biological indicators intended for use in routine monitoring, the nominal number of spores shall be not less than 1×10^6 per unit and shall be stated in increments not greater than $0{,}1 \times 10^6$.

NOTE. Inoculated carriers and/or biological indicators supplied for other purposes, e.g. qualification, validation and other specific tests, may require other nominal populations.

7 Carriers

7.1 The suitability of the carrier for use in ethylene oxide sterilization processes shall be demonstrated in accordance with the requirements of **6.1** and **6.2** of EN 866-1 : 1997 and annex A of this standard.

7.2 The exposure conditions to be used to establish compliance shall be:

– temperature: not less than 55 °C;

– relative humidity: not less than 70 %;

– gas concentration: not less than 800 mg/l;

– exposure time: not less than 6 h.

NOTE. These conditions have been selected to represent a realistic challenge to the carrier whilst remaining within the practical limits of an ethylene oxide sterilization process.

8 Resistance

8.1 General

The manufacturer shall state the D value of each batch of biological indicators or inoculated carriers to an accuracy of ± 0,5 min.

8.2 Biological indicators and inoculated carriers intended for use in routine monitoring

8.2.1 The D values obtained for the spore population on the inoculated carriers shall be not less than 12,5 min when exposed to (600 ± 30) mg/l ethylene oxide at (30 ± 1) °C and (60 ± 10) % relative humidity, and/or not less than 2,5 min when exposed to (600 ± 30) mg/l ethylene oxide at (54 ± 1) °C and (60 ± 10) % relative humidity, determined in accordance with the method given in annex A. (See **10.2** of EN 866-1 : 1997).

NOTE. A temperature coefficient of inactivation of the test organism of not less than 2 can be used to relate these D values to other temperatures when all other conditions remain constant.

8.2.2 The D value obtained by the two methods shall be such that the higher value obtained does not exceed the lower value by more than 50 % of the lower value.

8.3 Biological indicators and inoculated carriers intended for use in validation, qualification and other specific tests

NOTE. Biological indicators and inoculated carriers intended for use in validation, qualification and other specific tests have no specific requirements for the population or resistance of test organisms, to allow users flexibility in devising test programs. The D value and population are determined and stated (see **6.3** and **6.4**, **8.1**, **8.3**a, **8.3**b).

When the purchaser specifies requirements other than those in **8.2** for biological indicators and inoculated carriers intended for use in validation, qualification and other specific tests, the following shall apply.

a) The D values shall be determined by exposure to (600 ± 30) mg/l ethylene oxide at (30 ± 1) °C and (60 ± 10) % relative humidity, and by exposure to (600 ± 30) mg/l ethylene oxide at (54 ± 1) °C and (60 ± 10) % relative humidity, in accordance with the methods given in annex A.

b) The D value obtained shall be such that the higher value obtained does not exceed the lower value by more than 50 % of the lower value. Both D values shall be stated.

Annex A (normative)

Method for the determination of resistance to ethylene oxide sterilization

A.1 Apparatus: Ethylene oxide biological indicator resistometer

A.1.1 The equipment shall be capable of maintaining the conditions given in table A.1, within the limits given, for exposure periods of between 1 min and 120 min to an accuracy of ± 10 s. In addition, the equipment shall be capable of sustaining an exposure of not less than 6 h.

Table A.1 Conditions

Variable	For resistance studies (see clause **8**)	For carrier studies (see clause **7**)
Ethylene oxide	(600 ± 30) mg/l	Not less than 800 mg/l
Temperature	(30 ± 1) °C or (54 ± 1) °C	Not less than 55 °C
Relative humidity	(60 ± 10) %	Not less than 70 %

NOTE 1. Forced circulation may be required to maintain the conditions in the resistometer chamber uniform within the specified limits.

NOTE 2. Resistometers intended for use with mixtures of ethylene oxide and inert gases may be required to withstand high internal pressures, e.g. 650 kPa.

A.1.2 The equipment shall be provided with means to evacuate the reaction chamber to less than 10 kPa within 2 min, to permit adequate air removal prior to admission of the sterilant and to exhaust the sterilant at the end of the exposure period. Air admitted at the end of the cycle shall be filtered through a filter having the ability to remove not less than 99,9 % of 0,5 μm particles.

A.1.3 The time to achieve the required gas concentration from commencement of gas admission shall not exceed 60 s, and the time to exhaust the gas to 10 kPa at the end of the exposure period shall not exceed 60 s.

A.1.4 The chamber and door shall be provided with means to maintain the temperature of the inner surfaces of the chamber at the required operating temperature.

A.1.5 The supply of ethylene oxide gas to the chamber shall be filtered, evaporated to gas phase and preheated to ensure that neither liquid ethylene oxide nor particles of polymer are admitted to the chamber.

A.1.6 The equipment shall be capable of automatic operation and shall be provided with a system for recording temperature, pressure and humidity within the chamber, this system being independent of the control function and such that the limits of error on the recording equipment do not exceed 50 % of the tolerance allowed for each control variable.

For example, the chamber temperature shall be required to be controlled within ± 1 K, and thus the maximum allowable error limit on the temperature recorder is ± 0,5 K.

A.1.7 At the end of the exposure period, the pressure in the resistometer chamber shall be reduced to 10 kPa or less in a period not exceeding 1 min, and the chamber shall return to ambient pressure in not more than 20 s.

A.1.8 The resistometer shall be provided with test connections.

NOTE. Suitable test connections are described in prEN 1422.

A.2 Procedure

A.2.1 *Operation of the resistometer*

A.2.1.1 Preheat the resistometer chamber to the chosen operating temperature.

A.2.1.2 Load the inoculated carriers onto a suitable sample holder.

A.2.1.3 Place the loaded sample holder in the chamber, close the chamber and initiate the test cycle.

A.2.1.4 Carry out the following sequence of operations under automatic control.

a) Evacuate the chamber to (10 ± 0,4) kPa.

b) Admit sufficient water vapour to raise the relative humidity in the chamber to (60 ± 10) % and maintain these conditions for a period of 28 min to 30 min (see prEN 1422, sterilization cycle stage 3: conditioning stage).

c) Admit ethylene oxide to the chamber to obtain a concentration of (600 ± 30) mg/l within 60 s.

d) For the 0 min exposure time, no ethylene oxide shall be admitted.

e) Maintain these conditions for the required exposure period.

f) At the end of the exposure period, evacuate the chamber to (10 ± 0,4) kPa within 60 s and then admit filtered air, or an inert gas such as nitrogen, to ambient pressure.

g) Repeat stage f) a further four times.

A.2.1.5 At the end of the above cycle, remove the sample holder from the chamber within 30 s of the completion of the cycle.

A.2.2 ***Survivor curve method for determination of D value***

A.2.2.1 As soon as possible, but in any case within 2 h, transfer the exposed inoculated carrier into sterile distilled water. Physically remove the spores from the carriers into sterile distilled water by ultrasonication. Record the time taken to complete the transfer.

A.2.2.2 Determine the viable count of the suspension obtained, using the recovery medium and conditions stated by the manufacturer.

A.2.3 ***Most-probable-number method for determination of D value***

A.2.3.1 As soon as possible, but in any case within 2 h, aseptically transfer each inoculated carrier into a container of recovery medium. Record the time taken to complete the transfer.

A.2.3.2 Use the recovery medium stated by the manufacturer and incubate in accordance with the manufacturer's instructions.

A.3 Determination of resistance

Determine the resistance by both the survivor curve method and the MPN method. (See annex B of EN 866-1 : 1997).

Annex B (informative)
Bibliography

EN 550 *Sterilization of medical devices — Validation and routine control of ethylene oxide sterilization*

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

EN 1174-2 *Medical devices — Estimation of the population of micro-organisms on product — Part 2: Guidance*

List of references

See national foreword.

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