List of Titles

American National Standard

ANSI/AAMI/ISO 11135:1994

Medical devices—Validation and routine control of ethylene oxide sterilization

AAMI Association for the Advancement of Medical Instrumentation

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Association for the Advancement of Medical Instrumentation
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11135 Industrial Ethylene Oxide Sterilization, Validation and Routine Control

American National Standard

ANSI/AAMI/ISO 11135—1994
(Revision of ANSI/AAMI ST27—1988)

Medical devices — Validation and routine control of ethylene oxide sterilization

Approved 7 February 1994 by
Association for the Advancement of Medical Instrumentation

Approved 24 March 1994 by
American National Standards Institute, Inc.

Abstract:

This standard establishes requirements and guidance for validation and routine control of ethylene oxide sterilization processes for medical devices.

Committee representation

Association for the Advancement of Medical Instrumentation

The adoption of this International Standard as an American National Standard was approved by the AAMI Industrial Ethylene Oxide Sterilization Working Group, under the auspices of the AAMI Sterilization Standards Committee. Committee approval of the recommended practice does not necessarily imply that all committee, sub-committee, and working group members voted for its approval.

The **AAMI Sterilization Standards Committee** has the following members:

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Carl W. Bruch, PhD
Virginia C. Chamberlain, PhD

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NOTE—Participation by federal agency representatives in the development of this standard does not constitute endorsement by the federal government or any of its agencies.

Background of ANSI/AAMI adoption of ISO 11135:1994

Medical devices—Validation and routine control of ethylene oxide sterilization

The International Organization for Standardization (ISO) is a worldwide federation of national standards bodies. The United States is one of the ISO members that took an active role in the development of this standard.

ISO 11135 was developed by ISO Technical Committee 198 to fill a need for an international standard for industrial ethylene oxide sterilization of medical devices. U.S. participation in ISO/TC 198 is organized through the U.S. Technical Advisory Group for ISO/TC 198, administered by the Association for the Advancement of Medical Instrumentation. The United States made a considerable contribution to this standard.

This International Standard is based on a draft standard prepared by the European Standardization Committee (CEN) and also reflects the requirements of the previous edition (1988) of the American National Standard, Guideline for Industrial Ethylene Oxide Sterilization of Medical Devices (ANSI/AAMI ST27).

AAMI and ANSI procedures require that standards be reviewed and, if necessary, revised every five years to reflect technological advances that may have occurred since publication. AAMI encourages its committees to harmonize their work with international standards as much as possible. As part of their review of

ANSI/AAMI ST27, the AAMI Industrial Ethylene Oxide Sterilization Working Group examined this corresponding international standard to determine to what extent the documents could be harmonized. During this review, the Working Group decided to adopt ISO 11135 verbatim as the ANSI/AAMI revision. While the ANSI/AAMI standard provides extensive guidance, this international standard addresses mainly requirements. The Working Group is developing a technical information report that will provide additional/alternate guidance to industrial users of ethylene oxide sterilization.

The concepts incorporated in this standard should not be considered inflexible or static. This standard, like any other, must be reviewed and updated periodically to assimilate progressive technological developments. To remain relevant, it must be modified as technological advances are made and as new data comes to light.

Suggestions for improving this standard are invited. Comments and suggested revisions should be sent to Standards Department, AAMI, 3330 Washington Boulevard, Suite 400, Arlington, VA 22201.

NOTE—Beginning with the ISO foreword on page vi, this American National Standard is identical to ISO 11135:1994.

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75% of the member bodies casting a vote.

International Standard ISO 11135 was prepared by Technical Committee ISO/TC 198, Sterilization of health care products.

Annexes A, B, C and D of this International Standard are for information only.

Introduction

International Standards require, when it is necessary to supply a sterile product item, that adventitious microbiological contamination of medical devices from all sources is minimized by all practical means. Even so, product items produced under standard manufacturing conditions in accordance with ISO Quality Systems Standards may well, prior to sterilization, have microorganisms on them, albeit in low numbers. Such product items are nonsterile. The purpose of sterilization processing is to deactivate the microbiological contaminants and thereby transform the nonsterile items into sterile ones.

The deactivation of microorganisms by physical and chemical agents used to sterilize medical devices follows an exponential law; inevitably this means that there is always a finite probability that a microorganism may survive regardless of the extent of treatment applied. For a given treatment, the probability of survival is determined by the number and types of microorganisms and the environment in which the organisms exist before and during treatment. It follows that the sterility of any one item in a population of items subjected to sterilization can only be expressed in terms of the probability of the existence of a nonsterile item in that population.

Requirements for the quality system for the design/development, production, supply, installation and servicing are given in the ISO 9000 series.

The ISO 9000 series of Standards designates certain processes used in manufacture as "special" in that the

results cannot be fully verified by subsequent inspection and testing of the product. Sterilization is an example of a special process because efficacy cannot be verified by inspection and testing of the product. For this reason, sterilization processes need to be validated before use and the performance of the process needs to be monitored routinely. The manufacture of a sterile medical device requires attention to product and package characteristics, and to sterilization methods, facilities and controls.

It is important to be aware that exposure to a properly validated and accurately controlled sterilization process is not the only factor associated with the provision of reliable assurance that the product is sterile and suitable for its intended use. Attention should also be given to a number of factors including the microbiological status (bioburden) of incoming raw materials and their subsequent storage, and to the control of the environment in which the product is manufactured, assembled and packaged.

This International Standard contains requirements and offers guidance (as given in the annexes) for the validation and routine monitoring of sterilization by gaseous ethylene oxide. The validation of sterilization procedures presupposes that the sterilization equipment complies with appropriate specifications.

NOTE 1 The requirements are the obligatory parts of this standard with which compliance has to be achieved. The guidance given in the Informative Annexes is not obligatory and it is not provided as a check list for auditors.

The guidance included in the annexes provides explanations as well as methods which are accepted as being suitable for achieving compliance with the requirements. This guidance is provided in order to assist in obtaining a uniform understanding and implementation of this International Standard. Methods other than those given in the guidance may be used. However, these methods need to be demonstrated to be effective in achieving compliance with the requirements of this International Standard.

Medical devices—Validation and routine control of ethylene oxide sterilization

1 Scope

1.1 This International Standard establishes requirements and guidance for validation and routine control of ethylene oxide sterilization processes for medical devices.

Particular attention is drawn to the need for specific testing for safety, quality and efficacy, possibly exceeding the requirements of 4.2, which may be necessary for a specific product.

NOTE 2 Although this International Standard has been written for medical device sterilization, it may also apply to other health care products.

1.2 It does not cover the quality assurance system which is essential to control all stages of manufacture which include the sterilization process.

1.3 It does not cover operator safety (for further information, see IEC 1010-2).

Ethylene oxide is toxic, flammable and explosive. Attention is drawn to the existence in some countries of regulations laying down safety requirements for handling ethylene oxide and for premises in which it is used.

Attention is drawn to the existence in some countries of statutory regulations laying down limits for the level of ethylene oxide residues within medical devices and products.

1.4 It does not cover sterilization either by the technology of injecting ethylene oxide or its mixtures directly into individual product packages or continuous sterilization processes.

1.5 It does not cover analytical methods for determining levels of residual ethylene oxide and/or its reaction products (see ISO 10993-7).

1.6 It does not cover products that are affected adversely by ethylene oxide or by other ethylene oxide residuals produced in the processes described.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 9001:1987, *Quality systems — Model for quality assurance in design/development, production, installation and servicing.*

ISO 9002:1987, *Quality systems — Model for quality assurance in production and installation.*

ISO 9004:1987, *Quality management and quality system elements — Guidelines.*

ISO 10993-7:—[1)], *Biological evaluation of medical devices — Part 7: Ethylene oxide sterilization residuals.*

ISO 11138-1:—[1)], *Sterilization of health care products — Biological indicators — Part 1: General.*

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 aeration: Part of the sterilization process during which ethylene oxide and/or its reaction products desorb from the medical device until predetermined levels are reached.

NOTE 3 This may be performed within the sterilizer and/or in a separate chamber or room.

3.2 aeration area: Either a chamber or a room in which aeration occurs.

3.3 biological indicator (BI): Inoculated carrier contained within its primary pack providing a known resistance to the relevant process.

3.4 calibration: Comparison of a measurement system or device of unknown accuracy to a measurement system or device of known accuracy (traceable to national standards) to detect, correlate, report, or eliminate by adjustment, any variation from the required performance limits of the unverified measurement system or device.

3.5 chamber: Enclosed area which only accommodates sufficient product to fill the sterilizer.

3.6 commissioning; installation qualification: Obtaining and documenting evidence that equipment has been provided and installed in accordance with its specifications and that it functions within predetermined limits when operated in accordance with operational instructions. (See also validation.)

3.7 conditioning: Treatment of product within the sterilization cycle, but prior to sterilant admission, to attain a predetermined temperature and relative humidity. This part of the sterilization cycle may be carried out either at atmospheric pressure or under vacuum. (See also preconditioning.)

3.8 cycle completion: That point after completion of the sterilization cycle at which the sterilization load is ready to be removed from the chamber.

3.9 exposure time: Time for which the sterilizer chamber is maintained within the specified range for temperature, sterilant concentration, pressure and humidity.

3.10 flushing: Procedure by which the sterilant is removed from the load and chamber by either

a) multiple alternate admissions of filtered air or inert gas and evacuations of the chamber; or

b) continuous passage of filtered air or inert gas through the load and chamber.

3.11 inoculated carrier: Carrier on which a defined number of test organisms has been deposited.

3.12 medical device: Any instrument, apparatus, appliance, material or other article, whether used alone or in combination, including the software necessary for its proper application intended by the manufacturer to be used for human beings for the purposes of:

–diagnosis, prevention, monitoring, treatment or alleviation of disease;

–diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap;

–investigation, replacement or modification of the anatomy or of a physiological process;

–control of conception;

and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means.

3.13 parametric release: Declaring product as sterile, based on physical and/or chemical process data rather than on the basis of sample testing or biological indicator results.

3.14 performance qualification: Obtaining and documenting evidence that the equipment as commissioned will produce acceptable product when operated in accordance with the process specification. (See also validation.)

3.15 preconditioning: Treatment of product prior to the sterilization cycle in a room or chamber to attain specified limits for temperature and relative humidity. (See also conditioning.)

NOTE 4 This part of the sterilization cycle may be carried out either at atmospheric pressure or under vacuum.

3.16 preconditioning area: Either a chamber or a room in which preconditioning occurs.

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

NOTES—

5 The device is so constituted that a biological indicator can be arranged in the place most difficult for the sterilant to reach. 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.

6 In some process challenge devices an inoculated carrier may be used in place of a biological indicator.

3.18 process development: Documented program of studies which is performed in order to define the sterilization process based upon the product/packaging/loading pattern and/or equipment limitations.

3.19 product compatibility: Ability of the sterilization process to achieve the intended results without detrimental effect on the product.

3.20 reference load: Specified sterilization load made up to represent the most difficult combination of products to be sterilized.

3.21 revalidation: Set of documented procedures to confirm an established validation.

3.22 room: Enclosed area capable of holding more product than can be accommodated in the sterilizer(s) at any one time.

3.23 sterilant injection stage: Stage beginning with the first introduction of sterilant into the chamber and ending whenever the set operating pressure has been attained.

3.24 sterilant injection time: Duration of the sterilant injection stage.

3.25 sterilant removal time: Portion of the sterilization cycle in which sterilant is removed from the chamber and sterilization load, but not necessarily desorbed from individual products. (See also aeration.)

3.26 sterility: State of being free from viable microorganisms. (See sterilization.)

NOTE 7 In practice no such absolute statement regarding the absence of microorganisms can be proven.

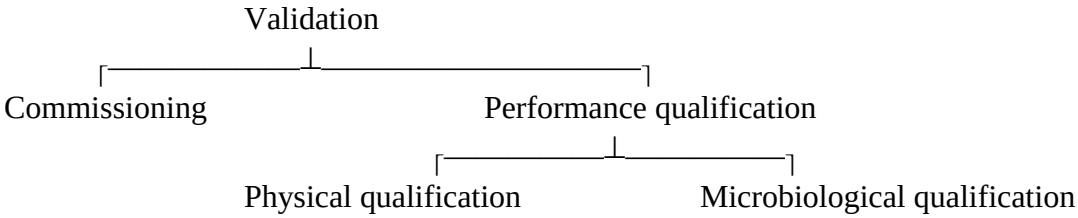
3.27 sterile: Free from viable microorganisms. (See sterilization and note 7.)

3.28 sterilization: Validated process used to render a product free of all forms of viable microorganisms.

NOTE 8 In a sterilization process, the nature of microbial death is described by an exponential function. Therefore, the presence of viable microorganisms on any individual item can be expressed in terms of probability. While this probability may be reduced to a very low number, it can never be reduced to zero. The probability can be expressed as a sterility assurance level (SAL).

3.29 sterility assurance level; SAL: Probability of a viable microorganism being present on a product unit after sterilization.

NOTE 9 SAL is normally expressed as 10^{-n}.



3.30 sterilization cycle: Treatment in a sealed chamber comprising air removal, conditioning (if used), injection of sterilant, exposure to ethylene oxide, removal of ethylene oxide and flushing (if used).

3.31 sterilization load: Goods that are to be or have been sterilized simultaneously in the same sterilization chamber.

NOTE 10 The sterilization load may include more than one manufacturing batch or lot.

3.32 sterilization process: All treatments which are required to accomplish sterilization to include preconditioning (if used), the sterilization cycle and aeration.

3.33 usable sterilizer chamber volume: Space inside the sterilizer chamber which is not restricted by fixed or mobile parts (loading units, pallets, etc.) and which is consequently available to accept the sterilization load. This is expressed in terms of height, width and depth.

3.34 validation: Documented procedure for obtaining, recording and interpreting the results needed to show that a process will consistently yield a product complying with predetermined specifications.

NOTE 11 Validation is considered as a total process which consists of commissioning and performance qualification. The relationship between these terms is illustrated above.

4 General

Medical devices to be sterilized shall be manufactured under conditions that ensure that their bioburden is consistently low. Employing a quality system complying with ISO 9001 or ISO 9002 meets this requirement.

The documented procedures and instructions required by this International Standard shall be implemented effectively according to the requirements of ISO 9001 or ISO 9002.

4.1 Personnel

Responsibility for the maintenance of equipment (see 4.4.1), for the validation and routine control of ethylene oxide sterilization and for the release of product shall be assigned to qualified personnel as specified in subclauses 4.1.2.2 and 4.18 or ISO 9001:1987 or in subclauses 4.1.2.2 and 4.17 of ISO 9002:1987.

4.2 Process development and product compatibility

4.2.1 Prior to the introduction of a new or altered product, package, loading pattern or sterilization process, the sterilization process to be validated shall be defined and documented.

A demonstration of equivalence to previously validated product, package or loading pattern shall be considered to meet this requirement. Any demonstration of equivalence shall be documented.

4.2.2 Product and packaging shall be designed to allow removal of air and penetration of steam and ethylene oxide. The location within the product at which sterilization is most difficult to achieve shall be identified.

4.2.3 It shall have been demonstrated that the specified sterilization process does not affect the correct functioning of the product and its packaging.

4.2.4 If resterilization is to be permitted, the effects of such processing shall be evaluated.

4.3 Sterilization process

The sterilization process shall include preconditioning and/or conditioning, sterilization cycle and aeration.

4.3.1 Preconditioning and/or conditioning

Preconditioning and/or conditioning treatments shall be performed under controlled conditions for a defined period of time to achieve specified temperature and relative humidity within the load (see A.3.1).

Humidity during conditioning shall be generated by the introduction of steam into the sterilizer.

4.3.2 Sterilization cycle

The sterilization cycle shall include:

a) air removal;

b) conditioning (if used);

c) sterilant injection;

d) maintenance of specified conditions for the exposure time;

e) sterilant removal;

f) flushing (if used); and

g) air admission to atmospheric pressure.

4.3.3 Aeration

Product shall be retained under specified conditions for a defined period for aeration. (See also 5.3.4.)

Aeration may be performed within the sterilizer and/or in a separate chamber or room.

4.4 Equipment

4.4.1 The specification for the equipment to be used for ethylene oxide sterilization, including the preconditioning area, shall be documented.

NOTE 12 Such a specification for a sterilizer design may be established by local or international regulators or by a relevant standards organization.

4.4.2 The conditions used for storage of sterilant prior to and during use shall ensure that its quality and composition remains within specification.

4.5 Calibration

An effective system shall be established, documented and maintained for the calibration of all controlling, indicating and recording instruments used for validation and routine control of the sterilization process. This system shall comply with the requirements of either subclause 4.12 of ISO 9001:1987 or subclause 4.11 of ISO 9002:1987.

4.6 Maintenance

4.6.1 Preventative maintenance shall be planned and performed in accordance with documented procedures. The procedure for each planned maintenance task and the frequency at which it is to be carried out shall be specified and documented.

4.6.2 Equipment (see 4.4.1) shall not be used to process medical devices until all maintenance tasks have been satisfactorily completed and recorded.

4.6.3 Records of maintenance shall be retained as specified in subclause 4.16 of ISO 9001:1987 or in subclause 4.15 of ISO 9002:1987.

4.6.4 The maintenance scheme, maintenance procedures and maintenance records shall be reviewed periodically by a designated person (see 4.1).

5 Validation

5.1 General

Procedures for validation shall be documented and records of validation shall be retained as specified in subclause 4.16 of ISO 9001:1987 or in subclause 4.15 of ISO 9002:1987.

5.2 Commissioning

5.2.1 Commissioning shall demonstrate that the equipment specifications for the preconditioning (if used), sterilization and aeration equipment are met.

5.2.2 Commissioning shall commence with the calibration of all instrumentation for controlling, indicating and recording the sterilization process.

5.3 Performance qualification — physical

5.3.1 Physical performance qualification shall be performed on the introduction of new or altered products, packaging, loading patterns, equipment or process parameters, unless equivalence to a previously validated product, packaging or loading pattern combination has been demonstrated.

The demonstration of equivalence shall be documented.

5.3.2 Product used for physical performance qualification shall be packaged as it will be routinely presented for

sterilization.

5.3.3 The maximum elapsed time between the completion of preconditioning (if used) and the commencement of the sterilization cycle shall be established and documented.

5.3.4 The physical performance qualification shall demonstrate

a) that at the end of the defined preconditioning time, the sterilization load is within the temperature and humidity ranges documented in the preconditioning specification;

b) the correlation between humidity and the increase in pressure on steam admission;

c) that at the admission of the sterilant to the chamber the sterilization load is within the temperature and humidity ranges documented in the sterilization process specification;

d) that gaseous sterilant has been admitted to the sterilizer chamber;

e) that the temperature and humidity and where applicable other parameters are within the ranges documented in the sterilization process specification;

f) that physical conditions specified for the sterilization load are maintained for the entire exposure time; and

g) that during aeration, the sterilization load is within the specified temperature range.

5.3.5 The levels of residual ethylene oxide and/or its reaction products after aeration in accordance with the documented procedures shall be determined to demonstrate that the levels after aeration are below specified limits.

5.4 Performance qualification — microbiological

5.4.1 Microbiological performance qualification shall be performed on the introduction of new or altered products, packaging, loading pattern, equipment or process parameters, unless equivalence to a previously validated product, package or loading pattern has been demonstrated. The demonstration of equivalence shall be documented.

5.4.2 The appropriateness of the biological indicators shall be established and documented.

5.4.3 Product used for microbiological performance qualification shall be packaged as it will be routinely presented for sterilization.

5.4.4 The microbiological performance qualification shall demonstrate the adequacy of the process for the sterilization of product by the inactivation of biological indicators complying with ISO 11138-1.

These indicators shall be placed at representative positions throughout the sterilization load under the cycle conditions selected to deliver less lethality then those used routinely such that on application of the specified sterilization cycle, assurance of sterility is attained.

5.4.5 If a process challenge device designed to simulate the product is to be used for routine monitoring in combination with indicators for ethylene oxide sterilization, the appropriateness of this process challenge device shall be demonstrated.

5.4.6 Indicators for ethylene oxide sterilization shall be positioned within the sterilization load prior to preconditioning (if used), and remain in position during the sterilization cycle.

5.4.7 The bioburden of the product shall be established and documented. A future International Standard will cover microbiological methods of validation and routine control.

5.5 Certification of validation

5.5.1 A validation report shall be documented. The report shall be signed by persons designated as responsible for preparing, reviewing and accepting this report. The validation report shall be retained as specified in subclause 4.16 of ISO 9001:1987 or in subclause 4.15 of ISO 9002:1987.

5.5.2 The validation report shall contain or reference specific validated product and the documented specification for the ethylene oxide sterilization process. The validation report shall also include the value and tolerances for the following.

5.5.2.1 Preconditioning (if used):

a) time, temperature, humidity;

b) minimum temperature of product permitted to enter preconditioning;

c) loading pattern and separation of product within the preconditioning area;

d) sterilization load temperature and humidity; and

e) maximum elapsed time between removal of the load from preconditioning and commencement of the sterilization cycle.

5.5.2.2 Conditioning, if used (see 4.3.1):

a) if used, the initial vacuum level and time taken to achieve it;

b) holding time under vacuum;

c) time, temperature, pressure and humidity; and

d) temperature and humidity of the sterilization load.

5.5.2.3 Sterilization:

a) sterilant injection pressure rise, sterilant injection time and final pressure;

b) ethylene oxide concentration determined independently from the increase in pressure, using at least one of the following:

1) mass of sterilant used,

2) volume of sterilant used,

3) direct analysis of chamber atmosphere;

c) chamber temperature;

d) exposure time; and

e) temperature of the sterilization load.

5.5.2.4 Aeration:

a) time and temperature;

b) pressure changes (if any) within the chamber and/or room (see 4.3.3);

c) rate of change of air or other gas;

d) temperature of the sterilization load; and

e) loading pattern and separation of product within the chamber and/or room.

5.6 Revalidation

5.6.1 The validation and any subsequent revalidation data shall be reviewed at least annually and the extent of revalidation determined and documented. Procedures for the review of validation and revalidation shall be documented and records of revalidation shall be retained.

5.6.2 A revalidation report shall be documented. The report shall be signed by the same functionary that prepared, reviewed and accepted the original validation report (see 5.5).

6 Process control and monitoring

6.1 Data shall be recorded and retained for each sterilization cycle to demonstrate that the sterilization process specification has been met. These data shall include at least the following:

a) temperature and humidity within the preconditioning area (if used), monitored and recorded from a position which can be related to that at which it is most difficult to achieve the specified conditions;

b) time of commencement and of removal of load from preconditioning (if used) of each sterilization load;

c) time of commencement of the sterilization cycle of each sterilization load;

d) temperature and pressure in the chamber during the sterilization cycle measured from a representative position within the chamber;

e) evidence that the gaseous sterilant has been admitted to the sterilizer chamber;

f) a measure of the quantity of ethylene oxide used or the concentration of ethylene oxide in the chamber;

g) exposure time;

h) time, temperature, pressure changes (if any) and/or the operation of the air supply (if used) during aeration; and

i) the results of testing indicators for ethylene oxide sterilization (see 6.3).

6.2 All records shall be retained as specified in subclause 4.16 of ISO 9001:1987 or in subclause 4.15 of ISO 9002:1987.

6.3 Indicators for ethylene oxide sterilization, recovery media and culture conditions used to monitor the sterilization process shall comply with ISO 11138-1.

6.4 At stipulated intervals the bioburden shall be estimated.

7 Product release

7.1 Conventional product release

7.1.1 The criteria for designating conformance of the sterilization process used for a particular sterilization load shall be documented. These criteria shall include

a) conformance to the physical cycle specification;

b) no growth of the test organism from any of the processed indicators for ethylene oxide sterilization (see 6.3) following incubation.

7.1.2 Product shall be considered as nonconforming and handled in accordance with subclauses 4.13 and 4.14 of ISO 9001:1987 if either

a) a physical cycle variable is outside the documented tolerances; or

b) any culture of a processed biological indicator shows growth of the test organism.

7.2 Parametric release

NOTE 13 The contents of this subclause supplement and/or modify the requirements in clauses 4 to 7.1 when parametric release is to be used routinely in the operation of the sterilization process.

7.2.1 Performance qualification — microbiological

The following requirement is given in addition to those in 5.4.

The microbiological qualification shall be performed by determining the lethality of the cycle by either method A (see 7.2.1.1) or method B (see 7.2.1.2).

If other chambers which deliver the same process parameters are to be used for this sterilization process, these chambers, having undergone full physical performance qualification, shall be qualified either

a) in the same manner as the original chamber; or

b) using a reduced microbiological performance qualification which demonstrates the delivery of the required level of microbiological lethality.

7.2.1.1 Method A: survivor curve construction

The lethality of the sterilization cycle shall be determined by construction of a survivor curve using direct enumeration of survivors.

At least five points employing graded exposure times to ethylene oxide, with all other process parameters except time remaining constant, should be included on the survivor curve. The initial count (i.e. the time zero on the survivor curve) should be determined on biological indicators exposed to all stages prior to ethylene oxide injection.

The data enable the calculation of the time of exposure to ethylene oxide needed to achieve a particular probability of survival of the test organism.

7.2.1.2 Method B: fraction-negative method

Indicators for ethylene oxide sterilization shall be exposed to graded exposures of ethylene oxide with all parameters except time remaining constant. After exposure, the test samples are assayed by direct immersion into an appropriate culture medium. The samples are scored as to the proportion of the samples showing no growth after incubation. A minimum of seven exposure conditions should be used covering:

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

b) at least four sets in which a fraction of the samples show growth (quantal region);

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

The D-value may be calculated from the results obtained using the method described in B.7. The exposure time required to achieve a specified probability of the survival of the test organism shall be calculated from this D-value.

7.2.2 Certification of validation

The requirement specified in 5.5.2 is replaced by the following.

The validation report shall contain or reference the documented specification for the ethylene oxide sterilization process. This specification shall include details of the load configuration as well as the values and tolerances for the following.

7.2.2.1 Preconditioning, if used (see 4.3.1):

a) time, temperature and humidity;

b) minimum temperature of product permitted to enter preconditioning;

c) loading pattern and separation of product within the preconditioning area;

d) temperature and humidity of the sterilization load; and

e) maximum elapsed time between removal of the load from the preconditioning and commencement of the sterilization cycle.

7.2.2.2 Conditioning, if used (see 4.3.1):

a) if used, the initial vacuum level and time taken to achieve it;

b) holding time under vacuum;

c) time, temperature, pressure, humidity; and

d) temperature and humidity of the sterilization load.

7.2.2.3 Sterilization:

a) sterilant injection pressure rise, sterilant injection time and final pressure;

b) ethylene oxide concentration, determined from direct analysis of chamber atmosphere;

c) chamber temperature;

d) temperature of the sterilization load;

e) number of sterilant additions during the exposure time (when applicable);

f) exposure time.

7.2.2.4 Aeration:

a) time and temperature;

b) pressure changes (if any) within the chamber and/or room;

c) rate of changes of air or other gas; and

d) temperature of the sterilization load.

7.2.3 Revalidation

The following requirement is in addition to those given in 5.6.

Revalidation of the sterilization process shall include microbiological requalification.

7.2.4 Routine control and monitoring

The requirement specified in 6.1 is replaced by the following.

Data shall be recorded and retained for each sterilization cycle to demonstrate that the sterilization process specification has been met. These data shall include at least the following:

a) temperature and humidity within the preconditioning area (if used), monitored and recorded from a position which can be related to that at which it is most difficult to achieve the specified conditions;

b) temperature within the sterilization load during preconditioning;

c) time of commencement and removal of the sterilization load from preconditioning (if used) for each sterilization load;

d) time of commencement of the sterilization cycle of each sterilization load;

e) elapsed time between removal of the sterilization load from preconditioning (if used) and the commencement of the sterilization cycle;

f) temperature within the sterilization load during the sterilization cycle;

g) humidity during conditioning (if used) as determined by direct measurement;

h) pressure in the chamber during the sterilization cycle;

i) temperature in the chamber from a minimum of two points;

j) evidence that gaseous sterilant has been admitted to the sterilizer chamber;

k) concentration of ethylene oxide in the chamber determined by analysis;

l) exposure time;

m) time, temperature, pressure changes (if any) and/or the operation of the air supply (if used) during aeration;

n) an indication that the circulation system (if used) was operational during the sterilization cycle.

The requirement in 6.3 does not apply when parametric release is used.

The requirements specified in 6.2 and 6.4 apply.

7.2.5 Product release

The requirement in 7.1 does not apply if parametric release is used.

Product shall be considered as nonconforming and handled in accordance with subclauses 4.13 and 4.14 of ISO 9001:1987 if a physical cycle variable is outside the documented tolerances.

Annex A
(informative)

General aspects of sterilization

For ethylene oxide sterilization the following general aspects should be carefully considered.

NOTE 14 The clauses in the body of this International Standard to which the guidance in these annexes specifically applies are indicated by the number of the relevant clause in square brackets.

A.1 Personnel

The level of qualification, training and experience required by personnel at various levels will depend upon the activities being performed. General guidance on training as part of the overall system of quality assurance is given in clause 18 of ISO 9004:1987.

Personnel with the following responsibilities should receive training and have the necessary qualification:

a) microbiological testing;

b) installation of equipment;

c) equipment maintenance;

d) physical performance qualification;

e) routine sterilizer operation;

f) calibration;

g) process design;

h) equipment specification;

or other areas as applicable.

A.2 Process development and product compatibility [4.2]

A.2.1 The development of a sterilization process for a particular medical device needs to establish a process which is both effective and compatible with the medical device. Therefore, initial investigations into product compatibility, together with experimentation to identify and/or optimize the sterilization process, may be undertaken whilst the product is in the design phase.

NOTE 15 Requirements for a quality system which includes design of medical devices are specified in ISO 9001.

A.2.2 During an ethylene oxide sterilization process, products can be subjected to environmental stresses such as vacuum and pressure changes, elevated temperature and changes in humidity. The product may also react with ethylene oxide and/or diluent gases used. The product design should ensure that functionality and safety are not compromised by exposure to the anticipated range of sterilization conditions. Furthermore, a high moisture content and changes in pressure may affect the strength of package seals with a consequent loss of integrity.

A.2.3 The selection of the sterilization process which is to be used for medical devices should include consideration of all factors which influence the efficacy of the process. The following may be taken into account:

a) the availability of sterilization equipment;

b) the range of conditions which can be achieved with the available sterilizing equipment;

c) sterilization processes already in use for other products;

d) requirements for levels of residual ethylene oxide and/or its reaction products; and

e) the results of process development experiments (see A.2.4).

A.2.4 A process development exercise may consist of a number of elements:

a) determination of time required to achieve specified conditions of temperature and humidity during preconditioning (if preconditioning is to be used);

b) determination of the limits for the variables of the sterilization process (see 4.3 and A.3);

c) estimation of the bioburden on product in which the challenge presented to the sterilization cycle by the bioburden should be established and the appropriateness of the biological indicator to be used for performance qualification and routine monitoring should be confirmed (see note 16); and

d) a determination of the minimum aeration time at specified conditions to achieve sufficient outgassing so that the ethylene oxide and/or its reaction products are at or below those levels established in ISO 10993-7.

NOTE 16 Requirements for, and guidance on, bioburden estimation will form the subject of a future

International Standard.

As a result of the process development activities, a sterilization process can be defined. The appropriateness of this sterilization process is demonstrated in the performance qualification studies (see 5.3 and B.3, and 5.4 and B.4).

A.3 Process [4.3]

A.3.1 Preconditioning and/or conditioning [4.3.1]

A.3.1.1 The resistance of microorganisms to deactivation by ethylene oxide is affected by their water content. For this reason it is common practice to control and monitor the humidity of the atmosphere to which the product is exposed in order to attempt to equilibrate the water content of the microorganisms with the local conditions. Before commencing the sterilization cycle, it is usual to precondition product at a defined temperature and humidity. Such preconditioning can reduce the duration of the sterilization cycle.

Preconditioning areas should be separate from the sterilizer, assembly and packaging areas.

Preconditioning may be performed in the sterilizer chamber prior to, but not as part of, the sterilization cycle or in a separate preconditioning area. It is common to use such a separate conditioning area for this purpose. Preconditioning areas should be easily cleanable and of durable finish.

The design and construction of the preconditioning area should provide facilities for segregation and identification of different sterilization loads, and facilities for controlling ingress and egress of product and personnel. The preconditioning area should be in close proximity to the sterilizer(s) to facilitate rapid transfer of the product.

It is recommended that the preconditioning area should have assisted air circulation around the sterilization load and throughout the area.

A.3.1.2 The length of time that access doors are left open should be limited. If separate doors are provided for access of personnel they should be self-closing. Means to alert the operator when the doors have been left open should be provided. Auditory and/or visual alarms activated after a preset delay may be provided.

A.3.1.3 Humidification by steam injection is preferred (see 4.3.1) because humidifiers which operate by dispersion of unheated water as an aerosol (e.g. spinning disc humidifiers and nebulizers) can be potent sources of microbial contamination. Where humidification during preconditioning cannot be performed by introduction of steam, it should be generated with microbiological control of the incoming water and generating equipment so as to prevent additional product contamination. Steam of the quality required for steam sterilizers (see EN 285 produced by CEN/TC 102) is suitable for humidification during preconditioning and conditioning.

A chamber relative humidity in excess of 30% is commonly used to humidify the load. The specified relative humidity will depend on the product to be sterilized. Consideration should be given to the potential product and package damage that excessive relative humidity can cause.

Product heating and humidification is used to establish reproducible product temperature and moisture content prior to gas exposure. Studies establishing minimum residence time ensure that the required conditions are reached. Where applicable a maximum time between removal of the load from the preconditioning and the start of the sterilization cycle needs to be established. A transfer time of 60 minutes or less is common practice.

A.3.1.4 The temperature and humidity of the preconditioning area should be such that the temperature and humidity of the sterilization load entering the sterilizer are neither so low as to cause condensation and unduly long heat-up times, nor so high that temperature control of the sterilizer cycle is compromised.

At the end of preconditioning, the measured temperature and humidity ranges within the sterilization load

should not exceed ± 5°C and ± 15% humidity. The actual temperature and humidity ranges should be demonstrated during physical performance qualification (see 5.3 and B.3).

A.3.2 Sterilization cycle [4.3.2]

A.3.2.1 Physical performance factors within the sterilization process which have to be considered include:

a) depth and rate of attainment of vacuum;

b) chamber leak rate (performed either under vacuum for subatmospheric cycles or under vacuum and at pressure for superatmospheric cycles);

c) during the conditioning phase, pressure rise on injection of steam;

d) pressure rise and rate of attainment of specified pressure on admission of sterilant and correlation of factors with which it is intended to monitor ethylene oxide concentration;

e) depth and rate of attainment of vacuum used to remove sterilant;

f) pressure rise and rate of attainment of pressure on admission of air (or any other gas used during this stage of the sterilization cycle);

g) number of times these last two stages are repeated and any variations in successive repetitions.

A.3.2.2 To achieve reproducible ethylene oxide distribution throughout the sterilizer chamber and sterilization load, it is necessary to control residual chamber air content prior to sterilant introduction because ethylene oxide and air do not mix well in static situations. Air removal employing deep evacuation is common where pure ethylene oxide or flammable gas mixtures are used and the use of non-flammable ethylene oxide/diluent sterilant mixtures is common for products that cannot withstand deep vacuum air removal. Air removal by evacuation is common practice but air removal by gas displacement may be practiced with special safety precautions and validation of the purging conditions required to establish the required gas concentration.

A recorded temperature range within an empty chamber during gas exposure of less than or equal to ±3°C of the required set point should be obtained. Throughout the exposure time, the sterilization load should attain the minimum specified temperature and the temperature range across the product load should be less than or equal to 10°C at any given time during sterilant exposure.

A.3.3 Aeration [4.3.3]

Residues of ethylene oxide and its reaction products may be hazardous. It is essential for the manufacturer to be aware of the possible occurrence of residues in the product. Temperature, dwell time, forced air circulation, loading characteristics, product and packaging materials all affect the efficiency of aeration.

Aeration may be performed within the sterilizer, in a separate area, or a combination of both.

A.4 Equipment [4.4]

A.4.1 Areas used for storage of cylinders, tanks or cartridges of ethylene oxide or sterilant gas mixtures should be secure and ventilated.

NOTE 17 Attention is drawn to the existence in some countries of regulations concerning ethylene oxide storage.

Where ambient conditions are subject to temperature variation greater than the range recommended by the supplier, storage areas for the containers of ethylene oxide should include provision for temperature control.

If the ethylene oxide supply to the sterilizer is from a bulk storage tank which is periodically replenished, the tank should be equipped with means to remove samples for analysis, means to empty the tank completely of

ethylene oxide and provision for cleaning in the event of inadvertent contamination or excessive accumulation of polymers.

A.4.2 Independent monitoring of humidity during the conditioning stage of the sterilization cycle is the preferred method of monitoring conditioning. Other methods of measuring humidity within a sterilizer include interpreting its value by monitoring the pressure rise during the admission of steam. Care is required in the use of pressure rise to ensure that it can be correctly related to the humidity.

A.4.3 The system for admission of sterilant to the sterilizer should be equipped with a vaporizer to prevent liquid ethylene oxide being admitted to the sterilizer chamber.

A.4.4 The temperature of the ethylene oxide gas flowing from the vaporizer to the sterilizer chamber should be measured to demonstrate that gaseous ethylene oxide has been produced. This may control an automatic shut-off valve to interrupt the supply of ethylene oxide when the temperature falls below a predetermined value, to prevent liquid ethylene oxide from entering the sterilizer chamber. Where there are sufficient data generated during validation to demonstrate correlation of the temperature of the sterilant gas entering the chamber with vaporizer control parameters such as temperature and sterilant flowrates, routine control of the vaporizer can be used to ensure that ethylene oxide entering the chamber is completely in the gaseous phase.

The homogeneity of conditions within the chamber should be achieved by forced circulation (see A.3.2).

A.4.5 A minimum of two probes to measure chamber temperature should be used. At least one of these probes should be positioned within the chamber at the coldest location determined during commissioning or at another correlated location. Locating the second temperature probe in the same area as the first probe is located allows functioning of the recording system to be verified.

A.5 Calibration and maintenance [4.5]

A.5.1 Leak tests should be performed on all chambers as part of the planned preventative maintenance program irrespective of the cycle employed. If there is an automatic leak test as part of the operating cycle, the correct function of this automatic system should be confirmed at specified intervals.

A.5.2 Replacement of filters for air admitted to the sterilizer chamber should be included in the planned preventative maintenance schedule. The frequency of replacement of such filters will depend on local operating conditions and should be specified. The specified intervals should be determined on the basis of the recommendations of the manufacturer of the filter, experience of local operating conditions and sterilizer performance. Cleaning/replacement of the internal surfaces of the vaporizer should be included in a planned preventative maintenance schedule.

A.5.3 The selection of humidity sensors for ethylene oxide sterilization processes requires special care. Factors which need to be considered include the following.

a) If the performance of the sensor is adversely affected by adsorption of ethylene oxide, provision should be made either to isolate the sensor from the chamber atmosphere prior to the admission of ethylene oxide or to remove the sensor as appropriate for degassing.

b) If sensors are removed and degassed, they should be recalibrated at a minimum of two points representative of the range to be measured.

Annex B
(informative)
Validation

B.1 General [5.1]

Validation is considered a total process which consists of commissioning and performance qualification. The relationship between these terms is illustrated in note 11.

Commissioning is associated with demonstrating that the equipment conforms to specification, and performance qualification with demonstrating that acceptable product will be produced when the commissioned equipment is used in accordance with documented procedures.

B.2 Commissioning [5.2]

B.2.1 Preconditioning

Commissioning is performed with the preconditioning area empty in order to establish that the design criteria are met.

The pattern of air circulation throughout the area to be occupied by the sterilization load(s) should be determined. This may be performed by smoke tests in combination with calculation of air change rates and anemometric determinations.

Temperature and humidity should be monitored throughout the preconditioning area over a period long enough to demonstrate that values are representative. The temperature and humidity in a number of locations distributed throughout the preconditioning area should be determined. Locations for monitoring should be selected to include any positions likely to be at extremes of the preconditioning area specification and for these locations at least two temperature probes and one humidity sensor should be used. (From practical experience, the use of one temperature probe and one humidity sensor for nominal 2.5 m^3 of the preconditioning area has been found to give an adequate profile of the empty area.)

B.2.2 Conditioning

Product which has been preconditioned may lose moisture during the vacuum stage of the sterilization cycle and so steam injection may be used during conditioning to maintain moisture levels.

Commissioning of conditioning is usually performed concurrently with the general commissioning of the sterilizer (see B.2.3.)

B.2.3 Sterilization

B.2.3.1 Commissioning is performed with the sterilizer chamber empty to establish the operational limits for factors affecting the efficacy of sterilization. The data obtained are then used for performance qualification.

If inert gases are used instead of ethylene oxide, account should be taken of the differences in the relative heat capacity when assessing the results.

B.2.3.2 The temperature profile of the internal surfaces of the empty chamber should be obtained by attaching temperature sensors directly to the chamber walls. In addition, the temperature profile of the empty chamber space should be determined. The number of temperature sensors used should provide a complete temperature profile of the internal surfaces and the empty chamber space. This number of sensors will depend upon the design of the sterilizer and the sterilization process specification. A common qualification practice for these temperature measurements is to use the following numbers of sensors:

a) for chambers with usable sterilizer chamber volume of 5 m^3 and less, at least 10 sensors, evenly distributed;

b) for chambers with usable sterilizer chamber volume larger than 5 m^3, at least one additional position should be measured for each additional 1 m^3 of chamber volume;

c) for chambers with usable sterilizer chamber volumes larger than 10 m^3, at least 20 temperature sensors.

Temperature sensors should be located in those positions which are likely to represent the maximum temperature differential such as positions near unheated portions of the chamber or door, and positions near steam or gas entry ports. The remaining temperature sensors should be distributed evenly throughout the sterilizer.

B.2.3.3 The physical performance factors of the sterilization process should be determined for the empty chamber. These factors include:

a) depth and rate of attainment of vacuum;

b) chamber leak rate (performed either under vacuum for subatmospheric cycles or under vacuum and at pressure for superatmospheric cycles);

c) pressure rise on injection of steam during the conditioning phase;

d) pressure rise and rate of attainment on admission of ethylene oxide and correlation of factors with which it is intended to monitor sterilant concentration;

e) depth and rate of attainment of vacuum used to remove ethylene oxide;

f) pressure rise and rate of attainment of pressure on admission of air (or other gases);

g) number of times these last two stages are repeated and any variations in successive repetitions.

The commissioning should also determine the performance of associated ancillary systems. For example, the quality of the steam supplied, the capability of the sterilant vaporizer to achieve a minimum gas input temperature, the reliability of the filtered air and water supplies to the sterilizer and the capability of the steam generator to maintain supplies of the required quality under maximum sterilization load conditions should be demonstrated.

Replicate cycles should be carried out to demonstrate the repeatability of control.

B.2.4 Aeration

The temperature profile of the aeration area should be determined in the same manner as recommended for preconditioning areas (see guidance on commissioning of preconditioning earlier). The air flowrates and air flow patterns through the area should also be determined.

B.2.5 Repeating of commissioning

In the event of

a) engineering work having been undertaken which may affect the sterilizing equipment, or

b) the sterilizing equipment having been unused for a period which may have affected the performance of critical components,

the sterilizing equipment should be commissioned again. A formal review should then be undertaken and documented to decide whether the performance qualification exercise should be repeated.

If the performance of the sterilizer is found to be outside the tolerances of the existing sterilization process specification during recommissioning, the cause should be investigated.

B.2.6 Initial microbial challenge

Initial biological indicator tests may be carried out in the empty chamber simultaneously with physical testing. The inclusion of such testing during commissioning may provide information on the performance of the sterilizer prior to performance qualification, but the data generated cannot be related to final product sterility.

When using biological indicators in an empty chamber, it is important to expose the biological indicators to the proposed preconditioning and conditioning stages of the sterilization process.

B.3 Performance qualification — physical [5.3]

Examples of significant changes in product, packaging or process which should be qualified include:

– packaging,

– product design,

– sterilization load configuration or density,

– sterilizing equipment,

– process cycle.

The effects of such changes on all stages of the sterilization process including preconditioning and aeration should be determined.

Results obtained from commissioning should be used to identify features needing particular investigation during performance qualification.

B.3.1 Preconditioning

B.3.1.1 Performance qualification should be performed with the preconditioning area loaded to the specified maximum and in typical partially loaded states. Performance qualification should be carried out with the loading patterns and pallet separations specified in the documented procedures.

B.3.1.2 Product at or, in some cases, below the minimum temperature specified for product to enter preconditioning should be used for performance qualification studies. If product temperature may vary prior to preconditioning, for example because of transport for sterilization at a remote facility, it may be necessary to make allowance for the range of product temperature which may be experienced.

The temperature and humidity profile within the sterilization load should be obtained over the period of time required for the sterilization load to attain the minimum predetermined temperature and humidity. The temperatures and humidities obtained within the sterilization load after the maximum permitted period of preconditioning should also be established to confirm that conditions within the specification will be attained when the maximum permitted preconditioning time is used. The number of sensors used should provide a complete profile of the sterilization load. This number of sensors will depend upon the design of the preconditioning area, the commissioning data, and the sterilization process specification. A common qualification practice for these measurements is to use the following numbers of sensors:

a) five temperature probes and two humidity sensors for sterilization loads of nominal volume less than 2.5 m^3;

b) for volumes of product in excess of 2.5 m^3, two temperature probes and one humidity sensor within each nominal 2.5 m^3 of product;

c) for larger preconditioning areas holding more than 50 m^3 of nominal volume of product, probes need not be located in each nominal 2.5 m^3 of product but the profile should include sufficient locations to demonstrate the attainment of the required conditions throughout each sterilization load.

Temperature and humidity sensors should be located within the unit container and any other packaging intended to be placed in the sterilizer.

B.3.2 Conditioning

The guidance on physical performance qualification of preconditioning (see B.3) applies also to the performance qualification of conditioning. A common qualification practice for these measurements is to use the following number of probes:

a) for chambers with usable sterilizer chamber volume of 5 m^3 and less, at least 10 sensors evenly distributed;

b) for chambers with usable sterilizer chamber volume larger than 5 m^3, at least one additional position should be measured for each additional 1 m^3 of chamber volume;

c) for chambers with usable sterilizer chamber volumes larger than 10 m^3, at least 20 temperature sensors.

B.3.3 Sterilization

To ensure that the adequacy of the conditioning process is demonstrated, product used for these performance qualification studies should be at or below the minimum temperature specified for product being loaded into the sterilizer. When preconditioning is used, the product should be preconditioned for the minimum specified time.

The loading pattern or patterns should be documented for each sterilizer. The combination of products permitted within the loading pattern should be documented. If sufficient knowledge of the relative difficulty in sterilizing each type of product, and the effect of the cycle on each type of product (e.g. absorption of ethylene oxide) already exists, reference load(s) may be specified and used for validation purposes.

New products should be compared with this reference load and if judged to present greater difficulty in sterilization, should be subjected to a full performance qualification study.

Temperature profiles of the sterilization load should be determined for each loading pattern and for the reference load. From practical experience it has been found that the use of the same number of probes as for the temperature distribution in the empty chamber during commissioning provides an adequate profile.

The location of probes throughout the sterilization load should be selected to determine the maximum temperature variation and take into account hot or cold spots located during commissioning.

Physical performance factors should be determined for the specified loading patterns in order to prepare the operating specification. These factors should include the ones cited in B.2.3.

B.3.4 Aeration

NOTE 18 The definition of acceptable levels of ethylene oxide and reaction products are outside the scope of this International Standard: product biocompatibility is covered in ISO 10993.

The temperature within the sterilization load during the aeration process should be measured over the period of time required for the sterilization load temperature to stabilize.

B.4 Performance qualification—microbiological [5.4]

Examples of significant changes in product, packaging or process which should be qualified include:

– packaging,

– product design,

– sterilization load configuration or density,

– sterilizing equipment,

– process cycle.

The effects of such changes on all stages of the sterilization process including preconditioning and aeration should be determined.

Results obtained from commissioning and physical performance qualification should be used to identify critical features for particular investigation during microbiological performance qualification.

The appropriateness of the biological indicator may be shown in a number of ways. The method chosen will depend upon the bioburden estimate and the extent to which the bioburden has been characterized. No single method can be recommended for all products: the following methods may be used as indicated.

a) When the bioburden estimate is accompanied by microbial identifications: the D-values can be determined or obtained from the literature for the resistant portion of this population. The time required to deactivate the bioburden to a specified sterility assurance level can be compared to that of the biological indicator to confirm the appropriateness of the biological indicator.

b) When microbial identifications are not performed and the bioburden is low (e.g. less than 100): the appropriateness of the biological indicator can be shown by inspection, in that the entire bioburden population would need to have a D-value which is 1.5 to twice that of the biological indicator in order to present a greater challenge than the biological indicator. Resistance of this magnitude for naturally occurring bioburden is not supported by the literature.

c) When microbial identifications are not performed and the bioburden estimate is high: the appropriateness of the biological indicator should be determined by exposure to sublethal (partial) cycles. In these studies, the relative inactivation rates can be compared through testing.

Biological indicators should be placed in the part of the product which is the most difficult to sterilize. If the design of the product is such that a biological indicator cannot be accommodated in the part most difficult to sterilize, the product should be inoculated with the spore suspension to provide a known number of viable spores. The spore suspension, materials and techniques used should comply with ISO 11138-1.

It is important to achieve an even distribution of spores on the surface of product which is to be inoculated. The surface characteristics of the product will affect the distribution of spores and may lead to a difference in resistance behavior compared with other challenge systems.

Biological indicators or inoculated product should be evenly distributed in the sterilization load but distribution should include those locations where sterilization conditions are the most difficult to achieve. The number of biological indicators used for microbiological performance qualification should demonstrate microbial deactivation throughout the sterilization load. The locations used may be the same as those selected for temperature monitoring, and further insight into process efficacy may be gained by placing two biological indicators near each thermocouple location. A common qualification practice for these microbiological tests is to use the following numbers of biological indicators:

a) for usable chamber volumes up to 5 m^3, at least 20;

b) for usable sterilizer chamber volumes between 5 m^3 and 10 m^3, the number of biological indicators should be increased by two for every additional 1 m^3;

c) for usable sterilizer chamber volumes above 10 m^3, the number of biological indicators should be increased again by two for every additional 2 m^3.

Microbiological performance qualification should be carried out using one of the following general approaches.

NOTE 19 When determining the exposure times during Methods A or B below, account should be taken of presterilization microbiological contamination levels on products. The accuracy and precision of the

method(s) used to determine presterilization microbiological contamination also needs to be considered when using these methods to determine exposure time.

B.4.1 Method A: survivor curve construction

The method described in 7.2.1.1 may be used as a guide.

B.4.2 Method B: fraction-negative method

The method described in 7.2.1.2 may be used as a guide.

B.4.3 Method C: half-cycle method

This method involves determination of the minimum time of exposure to ethylene oxide, with all other process parameters except time remaining constant, at which there are no survivors. Two further experiments should be performed to confirm the minimum time. Both should show no growth from the biological indicators. The specified exposure time should be at least double this minimum time. A cycle of short duration from which survivors can be recovered should also be run to demonstrate the adequacy of the recovery technique.

The conditions used for recovery of biological indicators in validation studies should be established and documented. The incubation period should take account of the possibility of delayed outgrowth of spores which have been exposed to ethylene oxide.

B.5 Certification of validation [5.5]

The validation report should include or reference the following:

a) details of products sterilized (including packaging and load patterns in the sterilizer);

b) the specification of the sterilizer;

c) the commissioning data;

d) the records, physical and biological, of all performance qualification runs;

e) an indication that all gauges, recorders etc., were calibrated at the time of the performance qualification;

f) provision for future review and revalidation;

g) the validation protocol(s);

h) the documented procedures used;

i) training manuals and records of all personnel involved;

j) documented operating procedures including process control limits; and

k) maintenance and calibration procedures.

On completion of the validation program, the test results should be compiled into a test report. The validation of a product/packaging/loading pattern combination for a particular sterilizer thus will become certified by the approval of the person(s) designated in the manufacturers quality system (in accordance with subclauses 4.1.2.2 and 4.18 of ISO 9001:1987).

B.6 Revalidation [5.6]

B.6.1 Revalidation should be performed to confirm that inadvertent process changes have not been made and to demonstrate that the original validation report remains valid. Revalidation will include elements of recommissioning and requalifications. Typically the requalification would be performed for the reference

load or for a sample product type. However, if recommissioning or requalification detected a process change the commissioning and performance qualification may need to be performed again.

B.6.2 Previous validation and revalidation results should be considered in establishing the revalidation protocol. Single recommissioning and requalification cycles are typically performed. Data from revalidation should be compared with records of the original validation (and any subsequent revalidation) to confirm that the original performance has been retained. This comparison is facilitated by a common format for validation and revalidation reports.

B.6.3 In revalidation the following should be included:

B.6.3.1 Recommissioning:

a) confirmation of calibration status of all instrumentation;

b) chamber leak rate test (see A.4);

c) determination of performance of associated ancillary systems such as steam generators and sterilant vaporizers (see B.2);

d) empty chamber temperature profiles (see B.2);

e) determination of physical performances factors for the empty chamber such as:

1) depth and rate of attainment of vacuum,

2) pressure rise on injection of steam during the conditioning phase,

3) pressure rise and rate of attainment on admission of sterilant;

4) correlation of pressure rise with other method(s) used to monitor ethylene oxide concentration (see B.3),

5) depth and rate of attainment of vacuum used to remove ethylene oxide,

6) pressure rise and rate of attainment of pressure on admission of air (or other gases),

7) number of repetitions of 5) and 6) and any variations in successive repetitions (see B.2);

f) audit of maintenance and calibration records.

B.6.3.2 Requalification:

a) determination of sterilization load temperature and humidity profile during preconditioning (see 5.3.2);

b) determination of sterilization load temperature and humidity profile during conditioning (see 5.3.3);

c) determination of sterilization load temperature profile during sterilant exposure (see 5.3.4);

d) determination of physical performance factors for the loaded chamber such as:

1) depth and rate of attainment of vacuum,

2) pressure rise on injection of steam during the conditioning phase,

3) correlation of pressure rise during conditioning with other method(s) used to monitor chamber humidity,

4) pressure rise and rate of attainment on admission of sterilant,

5) correlation of pressure rise with other method(s) used to monitor ethylene oxide concentration

(see C.3),

6) depth and rate of attainment used to remove ethylene oxide,

7) pressure rise and rate of attainment of pressure on admission of air,

8) number of repetitions of 5) and 6) and any variations in successive repetitions (see B.3);

e) determination of sterilization load temperature profile air flowrates during aeration (see B.3);

f) microbiological performance qualification (see B.4).

B.7 Method for calculating D-values using microbiological performance qualification method B

NOTE 20 The details of calculating D-values using the microbiological performance qualification method B given in this annex is based on the method described by Pflug, I.J. and Holcomb, R.G., Principles of Thermal Destruction of Micro-organisms, in *Disinfection, Sterilization and Preservation*, 3rd edition (1983) ed. S.S. Block, Lea and Febiger, Philadelphia.

The performance of method B in accordance with 5.4 will generate the following data:

Time of exposure to sterilant	**Number of test samples exposed**	**Number of test samples showing no growth**
t_1	n_1	0
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	n_7

Time t_1 is the shortest exposure time to sterilant, and all test samples show growth. 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 test samples show no growth.

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

$$x_i = \frac{ti + ti + 1}{2}$$

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

At t_1, 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(mean time to sterility) can be calculated for each

time of exposure (t_i) as follows:

$$\mu_i = x_i y_i$$

The mean time to attain no growth, û, from any of the test samples then can be calculated as the sum of μ_i for each time of exposure t_1 to t_6:

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

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

$$\overline{D} = \frac{\hat{\mu}}{0.2507 + \log_{10} N_0}$$

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

For the purposes of calculating the sterilization period using this method, D_{calc}, the upper 95% confidence level for $\overline{D}$ should be used. This can be calculated from the equation:

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

where V is derived as follows:

$$a = 0.25 \sum_{i=2}^{i=6} (t_{i+1} - t_{i-1})^2 \left(\frac{r_i (n_i - r_i)}{n_i^{\,2} (n_i - 1)} \right)$$

Annex C
(informative)
Process control and monitoring [6]

An operating specification documenting the procedures for routine operation of the sterilization process should be prepared.

C.1 Preconditioning

The reference position for routine monitoring of temperature and relative humidity during preconditioning should be the location at which it is most difficult to achieve the desired conditions. Data for this routine monitoring should be reviewed for acceptability before product is released for sterilization.

The ambient temperature of products entering the preconditioning area should be at or above the minimum temperature specified during validation (see 5.3). It is not generally necessary routinely to determine the temperature of product prior to preconditioning where the conditions of storage are known. Special provision may be necessary for storage of product prior to preconditioning if it has been transported for sterilization through extremes of climatic conditions. The loading pattern and separation of product should conform to those determined during validation. For each sterilization load, the entry and exit times from the preconditioning area should be recorded. Records should be retained and related to sterilization documentation. All goods within the preconditioning area should be identified so that they can be related to sterilization documentation.

Preconditioning areas should be cleaned according to a documented schedule, and records of cleaning

should be kept. The environment of the preconditioning area should be controlled to minimize microbiological contamination. Particular care should be taken to avoid the growth of fungi. For each sterilization load processed, legible records of elapsed time, temperature and humidity achieved during preconditioning should be retained.

C.2 Conditioning

Continuous monitoring and recording of humidity during conditioning can also be used to provide additional data on this phase of the sterilization cycle.

C.3 Sterilization

C.3.1 The pressure rise at sterilant injection provides an indirect measure of the ethylene oxide concentration in the sterilizer chamber. As ethylene oxide concentration is a key variable affecting the efficacy of the sterilization process, it is considered essential that a separate second system for documenting that the pressure rise is in fact due to ethylene oxide admission is provided. This second system may be any one of

a) mass loss of the sterilant supply;

b) volume of sterilant used; or

c) direct measurement of ethylene oxide concentration within the chamber.

Direct ethylene oxide gas concentration may be accomplished by gas chromatography or infrared analysis. Consideration should be given to the safety hazards associated with systems used to sample and analyze explosive gas mixtures. The use of a system which will also allow determination of chamber water content as a measure of humidity may be advantageous. The accuracy and precision of the analytical method should be known and the analytical equipment included in the calibration program (see 4.5).

C.3.2 The number of biological indicators for routine use should provide sufficient indicators to be distributed throughout the sterilization load. A common practice for routine microbiological monitoring is to use the following numbers of biological indicators:

a) for usable sterilizer chamber volumes up to 5 m^3, at least 10;

b) for usable sterilizer chamber volumes between 5 m^3 and 10 m^3, the number of biological indicators should be increased by one for every additional 1 m^3;

c) for usable sterilizer chamber volumes above 10 m^3, the number of biological indicators should be increased again by one for every additional 2 m^3.

The number of biological indicators selected for monitoring a defined sterilization process will be dependent upon the validation data obtained. In particular, the partial loading of sterilizer chambers requires a specific validation exercise (see 5.3.1 and 5.4.1). A rational choice needs to be made for the number of biological indicators to be used in validated partial loads.

Biological indicators should be located in those positions found during qualification to be the most difficult to sterilize, and the balance distributed uniformly throughout the sterilization load. Biological indicators should be placed in the sterilization load, or within test pieces placed within the sterilization load, prior to preconditioning. Biological indicators should, whenever possible, be removed from the sterilization load and cultured as soon as possible on completion of the cycle. Any effects of delayed recovery, and in particular exposure to residual ethylene oxide, should be determined.

NOTE 21 Attention is drawn to the existence of statutory regulations existing in some countries on personnel exposure to ethylene oxide.

Observations of growth from biological indicators not attributable to failure to meet physical process specifications should be analyzed; this can lead to a need for the validation to be repeated.

Annex D
(informative)
Product release [7]

D.1 Conventional product release [7.1]

In conventional product release, the physical sterilization process variables and results of biological indicator incubation are reviewed to assess the suitability of the sterilization process.

Failure to meet the physical specification or growth from a biological indicator after culture should lead to the sterilization load being placed in quarantine and the cause of failure investigated. The investigation should be documented and the handling of product should be in accordance with the documented procedures for control of nonconforming product required by the applicable International Standard in the ISO 9000 series. Guidance on the review and disposition of nonconforming product is contained in EN***** (CEN/TC 205 WG 5).

If the physical sterilization process variables are below the minimum tolerances of the specification or growth of the test organisms observed, the sterilization load should not be released, even on concession; product should be either resterilized or scrapped.

If a sterilization load is resterilized, the sterilization procedure should have been validated (see also 4.2). The suitability of the product and packaging for resterilization, and the effect of repeated exposure to the sterilization process on product function and levels of residual ethylene oxide and/or reaction products need to be considered. Records of the original sterilization should be traceable from the resterilization records.

D.2 Parametric release [7.2]

If a sterilization cycle operating within specified tolerances has been demonstrated to be both effective and reproducible, confirmation that the process parameters were within tolerance is taken as evidence of the reliability of the cycle. Parametric release is the declaration of adequacy of sterilization of product based on measurement and evaluation of physical parameters, rather than the results of sample testing or exposure of biological indicators.

The science underlying sterilization processing requires a comprehensive understanding of the kinetics of microbial deactivation on exposure to the sterilizing agent. In the case of ethylene oxide sterilization, there are many factors which influence microbial deactivation. As a result of the interrelationship of these many factors, in practice the use of a system of parametric release for ethylene oxide sterilization will require a greater knowledge and more control of the sterilization parameters. Furthermore, because of the influence of the sterilization load, parametric release is only possible with closely defined and validated sterilization loads and load configurations. The sterilization load and load configuration should therefore be regarded as process parameters.

The contents of 7.2 supplement and/or modify clauses 4 to 7.1 when parametric release is to be used routinely in the operation of the sterilization process.

D.2.1 Sterilization [7.2.2.3]

The guidance in A.3.2 is modified to incorporate the following.

The temperature within the sterilization load during the period of exposure to ethylene oxide is regarded as satisfactory if it can be kept within a range of $^{0}_{+3}$ °C of the minimum specified temperature of the

sterilization cycle (see figure D.1.)

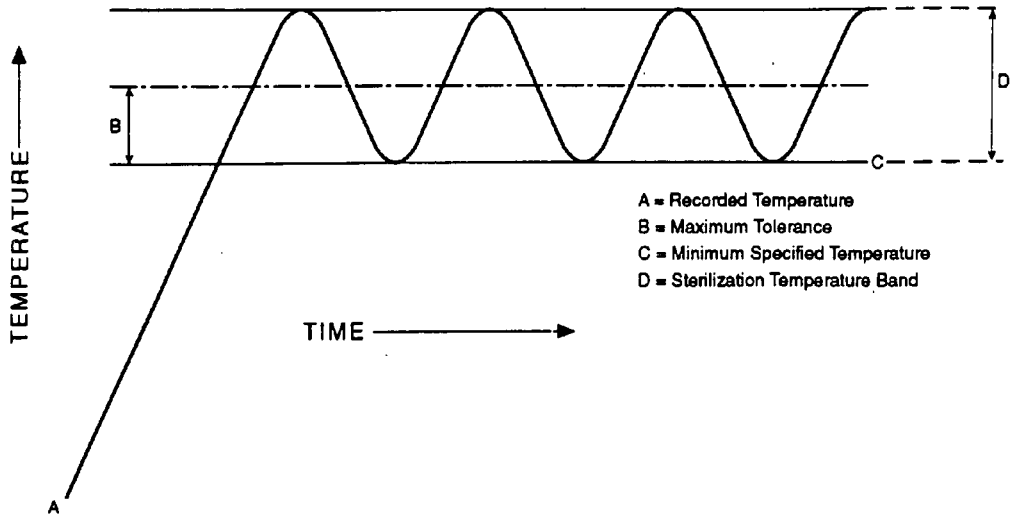


Figure D.1 Illustration of temperature range

D.2.2 Aeration [7.2.2.4]

The following guidance is additional to A.3.3.

A validated, well defined and monitored degassing procedure should be followed as part of any ethylene oxide process using parametric release because there is no requirement for the delay inherent in the use of biological monitors. Degassing can be performed within the sterilizer or in a separate area, although a combination of both is commonly used.

D.2.3 Equipment

The following guidance is additional to A.4.

The homogeneity of conditions within the sterilizer chamber should be achieved by forced circulation. The gas circulation system should be equipped with a monitoring device that indicates when circulation is ineffective. Devices which monitor "power on" to the fan or pump are not sufficient; it is necessary to demonstrate that the required gas flow is being maintained.

D.2.4 Performance qualification — microbiological

The microbiological qualification of a sterilization process to be used in combination with parametric release should provide data on the kinetics of microbial deactivation. Proper application of methods A or B can provide these data, but method C does not provide sufficient information on deactivation kinetics and so should not be used when parametric release is to be operated.