List of Titles

American National Standard

ANSI/AAMI/ISO 14160:1998

Sterilization of single-use medical devices incorporating materials of animal origin— Validation and routine control of sterilization by liquid chemical sterilants

AAMI Association for the Advancement of Medical Instrumentation

American National Standard ANSI/AAMI/ISO 14160—1998

Sterilization of single-use medical devices incorporating materials of animal origin—Validation and routine control of sterilization by liquid chemical sterilants

Approved 31 July 1998 by
Association for the Advancement of Medical Instrumentation

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Abstract: This standard specifies requirements for the development, validation, process control and monitoring of the sterilization, by the use of liquid chemical sterilants, of single-use medical devices comprising, in whole or in part, materials of animal origin.

Keywords: calibration, certification, manufacturing, monitoring, performance, process, qualification

List of Titles

AAMI Standard

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Contents

Committee representation

Association for the Advancement of Medical Instrumentation

Sterilization Standards Committee

The adoption of ISO 14160, First edition, 1998-03-15 as an AAMI Standard was initiated by the U.S. TAG for ISO/TC 198 and the AAMI Liquid Chemical Sterilization Working Group, which also functions as the U.S. Technical Advisory Group to the relevant work in the International Organization for Standardization (ISO). U.S. representatives from the AAMI Liquid Chemical Sterilization Working Group (U.S. Sub-TAG for ISO/TC 198/WG 10, Liquid Chemical Sterilization) played an active role in developing the International Standard.

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

Cochairpersons:	Virginia C. Chamberlain, PhD
	William E. Young
Members:	Carl W. Bruch, PhD, Consultant, Hudson, WI
	Virginia C. Chamberlain, PhD, Consultant, Hendersonville, NC
	Neal E. Danielson, D's Enterprise
	Judith Dowler, Medical Devices Bureau, Health Canada, Ottawa, Canada
	Frank B. Engley, Jr., PhD, University of Missouri, Columbia, MO
	Victoria Hitchins, PhD, U.S. Food and Drug Administration/Center for Devices and Radiological Health
	Robert Morrissey, PhD, Johnson & Johnson
	S. Richard Nusbaum, Pennsylvania Engineering Co.
	Barry F.J. Page, Consultant, Garner, NC
	Marimargaret Reichert, RN, Reichert Consulting
	Janet K. Schultz, MSN RN, Jan Schultz and Associates
	James Whitbourne, Sterilization Technical Services
	James L. Whitby, MA MB FRCP, University of Western Ontario, London, ON
	William E. Young, Baxter Healthcare Corporation

The **Liquid Chemical Sterilization Working Group** has the following members:

Cochairpersons:	Damon P. Jones
	Charles Roberts, MS
Members:	Terri Antonucci, Steris Corporation.
	Susanna F. Barrett, U.S. Food and Drug Administration/Center for Devices and Radiological Health
	Mary Bruch, Mary Bruch Micro-Reg
	Virginia C. Chamberlain, PhD, Consultant, Hendersonville, NC
	Adolph D'Amico, Gulf Stream Medical, Inc.
	Judith Dowler, Medical Devices Bureau, Health Canada, Ottawa, Canada
	James M. Gibson, Jr., J.M. Gibson Associates
	H. Phillip Hovnanian, PhD, PE, Biomedical Devices
	Damon P. Jones, Medtronic, Inc.
	Susan L. Jordan, PhD, Union Carbide Corporation
	Christine Loshbaugh, Baxter Healthcare Corporation
	Patrick McCormick, Bausch & Lomb, Inc.
	Sallie McLaughlin, Sulzercarbomedics
	Gregg Mosely, Biotest Laboratories
	Barry F.J. Page, Consultant, Garner, NC
	Charles D. Paige, VA Central Office
	Marimargaret Reichert, RN MA, Reichert Consulting
	Charles Roberts, MS, Johnson & Johnson
	Gabriel A. Rodriguez, Sherwood/Davis & Geck

Alternates: Martin S. Favero, PhD, Johnson & Johnson Medical, Inc.
Christopher Fricker, PhD, Steris Corporation
Louis M. Glasgow, Bausch & Lomb, Inc.
Candace McManus, U.S. Food and Drug Administration/Center for Devices and Radiological Health

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 AAMI adoption of ISO 14160:1998

Sterilization of single-use medical devices incorporating materials of animal origin — Validation and routine control of sterilization by liquid chemical sterilants

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 14160 was developed by ISO Technical Committee 198, *Sterilization of health care products,* to fill a need for an international standard for sterilization by liquid chemical steriliants of medical devices incorporating materials of animal origin. 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 on behalf of the American National Standards Institute (ANSI). The United States made a considerable contribution to this standard.

AAMI encourages its committees to harmonize their work with international standards as much as possible. Upon review of ISO 14160, the AAMI Sterilization Standards Committee and the AAMI Liquid Chemical Working Group decided to adopt ISO 14160 verbatim as an AAMI Standard.

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 22201-4598.

NOTE—Beginning with the ISO foreword on page vii, this AAMI Standard is identical to ISO 14160:1998.

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 14160 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products.*

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

Introduction

A sterile product item is one which is free of viable microorganisms. International standards require, when it is necessary to supply a sterile product item, that adventitious microbiological contamination of a medical device from all sources prior to sterilization be minimized by all practical means. Even so, product items produced under defined manufacturing conditions in accordance with the requirements for quality systems for medical devices (see ISO 13485 and ISO 13488) can, prior to sterilization, have microorganisms on them, albeit in low numbers. Such product items are non-sterile. The purpose of sterilization processing is to inactivate the microbiological contaminants and thereby transform the non-sterile items into sterile ones.

The inactivation of a pure culture of microorganisms by physical and/or chemical agents used to sterilize medical devices often approximates an exponential relationship; inevitably this means that there is always a finite probability that a microorganism can 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 by the environment in which the organisms exist during treatment. It follows that the sterility of any one item in a population of items subjected to sterilization processing cannot be guaranteed and the sterility of the processed population of items has to be defined in terms of the probability of there being a viable microorganism present on the device.

Generic requirements for the quality system for the design/development, production, installation and servicing are given in the ISO 9000 family of standards and in ISO 13485 and ISO 13488. The ISO 9000 series of standards designates certain processes used in manufacture as "special" if the results cannot be fully verified by subsequent inspection and testing of the product. Sterilization is an example of a special process because process efficacy cannot be verified by inspection and testing of the product. For this reason, sterilization processes have to be validated before use, the performance of the process monitored routinely and the equipment maintained.

It is important to be aware that the 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 in this respect suitable for its intended use. Attention has also to be given to a number of factors, including the microbiological status (bioburden) of incoming raw materials and/or components, their subsequent storage, and to the control of the environment in which the product is manufactured, assembled and packaged.

The agents for sterilization used most frequently for medical devices are moist heat, dry heat, irradiation and ethylene oxide. While some devices containing animal tissues may be compatible with these commonly applied methods of sterilization (for example catgut sutures are usually sterilized by irradiation), other devices, such as biological heart valves or tissue patches, are not compatible with conventional sterilization processes. It has been recognized that other sterilizing agents might have to be used in these exceptional circumstances. Liquid chemical sterilants have been widely used in such instances and, in common with the other sterilization methods, the efficacy of the process needs to be demonstrated and recorded before it is adopted for routine use.

This International Standard contains requirements for the validation and routine monitoring of sterilization of single-use medical devices containing materials of animal origin by exposure to liquid chemical sterilants; guidance on the application of this International Standard is given in annex A. Manufacturing processes for medical devices containing animal tissues frequently include exposure to chemical agents which can in themselves reduce significantly the bioburden on the medical device. Following the manufacturing process, a medical device is exposed to a defined sterilization process; the requirements for validation and routine control described in this International Standard apply only to this defined sterilization process and do not take account of the lethal effects of other bioburden reduction steps.

NOTE — The guidance given in annex A is not obligatory and it is not provided as a check list for auditors.

AAMI Standard AAMI/ISO 14160—1998

Sterilization of single-use medical devices incorporating materials of animal origin — Validation and routine control of sterilization by liquid chemical sterilants

1 Scope

This International Standard specifies requirements for the development, validation, process control and monitoring of the sterilization, by the use of liquid chemical sterilants, of single-use medical devices comprising, in whole or in part, materials of animal origin.

This International Standard does not apply to material of human origin.

This International Standard does not describe a quality assurance system for the control of all stages of manufacture.

NOTE 1 Attention is drawn to the standards for quality systems (see ISO 9001 and ISO 13485 or ISO 9002 and ISO 13488) which can be used in the control of all stages of manufacture including the sterilization process.

This International Standard does not describe tests to establish the effects of any chosen sterilization method upon the fitness for use of the medical device.

NOTE 2 Such testing is a crucial part of the design and development of a medical device.

This International Standard does not describe methods for the validation of the inactivation of viruses.

NOTE 3 In developing a method for processing medical devices containing materials of animal origin, consideration of the effects of liquid chemical sterilization on potential viral contaminants will also be necessary because of the source of materials used in the manufacture of these particular medical devices. The importance of validation of viral inactivation for processes within the scope of this International Standard is recognized. This aspect is excluded from this International Standard; a separate European Standard is in preparation (EN 12442-3).

NOTE 4 Liquid chemical sterilants traditionally employed to sterilize animal tissues in medical devices may not be effective in inactivating the causative agents of transmissible spongiform encephalopathies such as bovine spongiform encephalopathy (BSE), or scrapie. Satisfactory validation in accordance with this International Standard should not be assumed to demonstrate inactivation of infective agents of this type.

This International Standard does not cover the level of residual sterilant within medical devices.

NOTE 5 ISO 14538 is concerned with this issue.

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, 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 9001:1994, *Quality systems — Model for quality assurance in design, development, production, installation and servicing.*

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

ISO 11138-1:1994, *Sterilization of health care products — Biological indicators — Part 1: General.*

ISO 11737-1:1995, *Sterilization of health care products — Microbiological methods — Part 1: Estimation of the population of microorganisms on product.*

NOTE — The relationship between International Standards and European Standards is given in annex B.

3 Definitions

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

3.1
batch
defined quantity of bulk, intermediate, or finished product that is intended or purported to be uniform in character and quality, and which has been produced during a defined cycle of manufacture

3.2
bioburden
population of viable microorganisms on a product and/or a package

3.3
carrier
supporting material on which test organisms are deposited

3.4
commissioning
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

3.5
decimal reduction value
D-value
time (expressed in minutes) or irradiation dose (expressed in kilograys) required to achieve inactivation of 90% of a population of the test organism under stated exposure conditions

3.6
exposure time
time for which the medical device is exposed at the specified temperature and sterilant concentration

3.7
inactivation
process resulting in the loss of the ability of microorganisms to grow and/or multiply

NOTE — For the purpose of this International Standard, microorganisms comprise sporing and non-sporing bacteria, viruses, fungi and protozoa.

3.8
inoculated carrier
carrier on which a defined number of test organisms has been deposited

3.9
liquid chemical sterilant
defined formulation of chemicals in a solution or liquid form which is applied to achieve sterility

3.10
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 purpose 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

3.11
performance qualification
obtaining and documenting evidence that the equipment as commissioned will produce acceptable product when operated in accordance with the process specification

3.12
presterilization count
viable count obtained prior to sterilization

3.13
product compatibility
ability of the sterilization process to achieve the intended results without detrimental effect on the product

3.14
process development
documented programme of studies which are performed in order to define the sterilization process based upon the product/packaging/loading pattern and/or equipment limitations

3.15
revalidation
repetition of part or all of the validation test requirements for the purpose of reconfirming process reliability

3.16
sterility
the state of being free from viable microorganisms

NOTE — In practice no such absolute statement regarding the absence of microorganisms can be proven (see 3.18 sterilization).

3.17
sterile
free from viable microorganisms

NOTE — In practice no such absolute statement regarding the absence of microorganisms can be proven (see 3.18 sterilization).

3.18
sterilization
validated process used to render a product free of all forms of viable microorganisms

NOTE — 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) normally expressed in the form 10^{-n}.

3.19
storage solution
liquid in which a medical device in its final form is presented for use

3.20
validation
documented procedure for obtaining, recording and interpreting the data required to show that a process will consistently yield product complying with predetermined specifications

NOTE — For sterilization by liquid chemical sterilants, validation is considered as a total programme, which consists of commissioning and performance qualification.

3.21
viable count
number of microorganisms estimated by growth of discrete colonies under the stated culture condition

NOTE — A discrete colony may not necessarily originate from a single viable microorganism.

4 General

4.1 Control of manufacturing

The manufacturing process shall be established and controlled to maintain the presterilization count below a specified limit.

NOTE 1 Employing a quality system complying with ISO 13485 or ISO 13488 meets this requirement.

A documented system shall be established and maintained to control the sourcing of raw materials of animal origin.

NOTE 2 A European Standard on sourcing, controls, collection and handling (EN 12442-2) is under preparation.

The documented procedures and instructions required by this International Standard shall be implemented effectively. Documentation and records shall be reviewed and approved by designated personnel (see 4.2).

4.2 Personnel

Responsibility for the maintenance of equipment (see 4.4), for the validation (see clause 5) and routine control (see clause 6) of sterilization by exposure to liquid chemical sterilants and for the release of product shall be assigned to qualified personnel as specified in ISO 9001 or in ISO 9002.

4.3 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 ISO 9001 or ISO 9002.

4.4 Maintenance of equipment

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.

Equipment shall not be used to process medical devices unless all maintenance tasks have been satisfactorily completed and recorded.

Records of maintenance shall be retained as specified in ISO 9001 or ISO 9002.

The maintenance scheme, maintenance procedures and maintenance records shall be reviewed periodically by a designated person (see 4.2).

4.5 Process development and product compatibility

4.5.1 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.

NOTE — The specified sterilization process may comprise separate treatments with more than one liquid chemical sterilant.

4.5.2 Product and packaging shall be designed to allow contact with liquid chemical sterilant and so that residues of the liquid chemical sterilant are below levels as specified by the manufacturer. The location within the product at which sterilization is most difficult to achieve shall be identified.

4.5.3 It shall have been demonstrated and documented that the sterilization process does not affect adversely the fitness for use of the product or its packaging. If resterilization is to be permitted, the effects of such processing shall be evaluated and documented.

5 Validation

5.1 General

Procedures for validation shall be documented and records of each validation shall be retained (see 5.4.1).

5.2 Commissioning

Commissioning shall demonstrate that the specifications for equipment used for the sterilization process are met.

5.3 Performance qualification

5.3.1 The performance qualification shall demonstrate that the sterilization process has:

a) appropriate lethal activity against a representative range of microorganisms (see 5.3.5, 5.3.6 and A.5);

b) defined processing parameters (e.g., time, temperature, liquid chemical sterilant concentration, pH) which are capable of control throughout the process.

5.3.2 For the performance qualification, the part of the product which is most difficult to sterilize, as defined according to 4.5.2, shall be taken into consideration during the performance qualification.

5.3.3 The presterilization count of the product shall be established as described in ISO 11737-1.

5.3.4 Before performance qualification is undertaken, a method shall be validated for the neutralization of the liquid chemical sterilant prior to culturing survivors. The method shall not in itself adversely influence the ability to interpret the results.

5.3.5 The combination of conditions with the lowest microbicidal activity within the process specification shall be identified and this combination of conditions shall be used in the performance qualification.

5.3.6 Microbiological performance qualification shall include the following three stages:

a) A screening test to identify microorganisms with a high resistance to the process (see A.4.2.3).

b) Determination of inactivation kinetics.

This consists of the construction of log survival curves for the microorganisms identified as having a high resistance to the process. The inactivation curve shall include a minimum of 5 points covering at least a thousand-fold reduction in numbers (see also A.4.2.4.1 and A.4.2.5). If the product does not allow the above-mentioned procedure, the MPN method as specified in annex A.4.2.4.2 may be used. This shall be rationalized and documented.

Microorganisms shall be presented to the process on carrier material(s) representative of the medical device;

c) Assessment of inactivation of the microorganisms from the presterilization count as they are induced to grow onto carriers of tissue.

The range of microorganisms employed, in addition to isolates from the bioburden, shall include microorganisms with a known high resistance to the sterilization process and, in any event, resistance equivalent to spores of *Bacillus subtilis* (see table A.2) complying with ISO 11138-1.

NOTE — In the design of such experiments, consideration should be given to the level of organic and/or inorganic contamination and variation between replicate experiments.

5.3.7 Within the sterilization process, the exposure time shall not be less than $D[6 + \log_{10}(100+B)]$ where D is the D-value of the most resistant microorganism identified during performance qualification and B is the value of the bioburden estimated as described in ISO 11737-1.

NOTE — The extended treatment specified by this clause provides a probability of at least 1×10^{-6} of microorganisms surviving treatment. EN 556 specifies this is a requirement for terminally sterilized devices labeled sterile (see annex C).

5.3.8 If the medical device is subjected to an aseptic transfer following the completion of sterilization process:

a) processes used for the sterilization of components for manufacture (e.g. containers, storage solutions) shall be validated and routinely controlled in accordance with the appropriate International Standard;

b) transfer procedures after exposure to the liquid chemical sterilant shall be validated in accordance with ISO 13408 (see A.4.2.7).

5.4 Certification of validation

5.4.1 A validation report containing the results of all validation exercises 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 ISO 9001 or in ISO 9002.

5.4.2 The validation report shall contain or reference the documented process specification for liquid chemical sterilization. The process specification shall specify the medical device for which the validation has been performed and shall detail, including values and tolerances where appropriate, the following:

a) frequency and method(s) for bioburden estimations, together with action limits;

b) specification for the environment in which the liquid chemical sterilant and containers are prepared, and aseptic transfers (see 5.3.8) are undertaken;

c) training and certification criteria for approval of personnel to be authorized to undertake aseptic transfers (see 5.3.8);

d) method of ensuring the absence of viable microorganisms from the liquid chemical sterilant solution(s) (see A.6);

e) formulation of the liquid chemical sterilant, including the specification of its constituents;

f) pH of the liquid chemical sterilant;

g) residual activity required for the liquid chemical sterilant after the sterilization process in terms of chemical concentration and/or microbicidal activity;

h) specification of the exposure vessel in which products come into contact with the liquid chemical sterilant, including materials of construction, size, and details of any pretreatment to be applied;

i) number of products to be sterilized per unit volume of liquid chemical sterilant;

j) exposure time;

k) temperature to be used for sterilization;

l) any other critical process variable(s) determined during process development;

m) method of sterilizing any storage solution in which the product is presented after sterilization (see 5.3.8).

5.4.3 In cases where a validation for a specific device is also judged valid for other devices, the justification for this shall be documented.

5.5 Revalidation

5.5.1 The validation and any subsequent revalidation data shall be reviewed at least annually and a rationale shall be prepared and documented whether or not revalidation is required.

A revalidation exercise shall be undertaken unless sufficient data have been generated to demonstrate the continued appropriateness of the sterilization process. Procedures for the review of validation and revalidation data shall be documented and records of revalidation shall be retained.

5.5.2 A revalidation report shall be documented. The report shall be signed by the persons designated by the same functions/organizations that prepared, reviewed and accepted the original validation report (see 5.4.1).

6 Process control and monitoring

6.1 At stipulated intervals, the bioburden shall be estimated as described in ISO 11737-1. If a microorganism that has not been studied in the original performance qualification is isolated during routine estimation of the presterilization count, the exercise in 5.3.5 shall be performed with this microorganism.

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

a) variables monitored during sterilization of final container(s), if appropriate;

b) variables monitored during sterilization of storage solution, if appropriate;

c) initial chemical concentration(s) and pH of liquid chemical sterilant;

d) parameters monitored during preparation of liquid chemical sterilant;

e) results of integrity tests on any filters used to sterilize solutions, if appropriate;

f) exposure time;

g) temperature during the exposure time;

h) results of environmental monitoring during aseptic transfer, if appropriate;

i) identities of personnel who are:

   1) preparing storage solutions and liquid chemical sterilant solutions;

   2) controlling the sterilization process; and

   3) performing aseptic transfer, if appropriate;

j) numbers (or other unique identification) of products processed.

6.3 For each batch of medical devices subjected to liquid chemical sterilization, the following shall be examined for the presence of viable microorganisms.

a) chemical sterilant solutions;

b) storage solutions, if applicable;

c) at least one of the following:

   1) finished product;

   2) product which has been rejected but subjected to the complete manufacturing process; or

   3) isolated pieces of animal tissue, justified as being representative of the medical device, which have been subjected to the complete manufacturing process.

6.4 For each batch of medical devices, a portion of either:

   a) liquid chemical sterilant solution, or

   b) liquid chemical sterilant solution remaining following the sterilization process

shall be challenged under the same conditions as the batch of medical devices by a carrier of the animal tissue inoculated with a microorganism complying with ISO 11138-1 and containing at least 10^6 organisms with a known high resistance to the sterilization process, as identified during performance qualification (see 5.3).

NOTE — When performing the test, appropriate precautions should be taken to minimize the risk of contamination of the manufacturing equipment.

6.5 If a bulk liquid chemical sterilization process is used for which it is not appropriate to introduce test organisms into the sterilization vessel or manufacturing environment; and

a) the relationship between the complete chemical composition of the liquid chemical sterilant and microbicidal activity of the liquid chemical sterilant has been demonstrated; and

b) the sterilization process has been applied for a significant period for at least 30 batches of medical devices during which time no failures have been detected in accordance with 6.4,

then testing specified in 6.4 may be discontinued and complete chemical analysis capable of demonstrating compliance with the limits of the process specification shall be performed on the liquid chemical sterilant remaining at the end of the sterilization process.

NOTE — The figure of 30 batches is based on the assumption of a binomial distribution of failures to achieve a confidence level of 0.95 (α:0.05) and a confidence interval of 0.9.

6.6 The results from 6.2, 6.3 and either 6.4 or 6.5 shall form part of the evidence permitting release of product as sterile and shall be retained as part of the sterilization records.

6.7 All records shall be retained as specified in ISO 9001 or in ISO 9002.

7 Product release from sterilization

7.1 The criteria for designating a given sterilization process as conforming shall be documented. These criteria shall include:

a) conformance to the process specification(s); and

b) no growth in microbiological testing (see 6.3 and 6.4) following incubation.

7.2 A given sterilization process shall be considered as non-conforming and non-conforming product shall be handled as specified in ISO 9001 or in ISO 9002 if:

a) a process variable is outside the documented tolerances; or,

b) any microbiological test (see 6.3 and 6.4) shows growth following incubation.

Annex A
(informative)

Guidance

A.1 Introduction

This guidance is not intended as a checklist for assessing compliance with this International Standard.

It provides explanations as well as methods that are accepted as being suitable for achieving compliance with the specified requirements. This guidance is provided to assist in obtaining a uniform understanding and implementation of ISO 14160. Methods other than those given in the guidance may be used. However these methods should be demonstrated as being effective in achieving compliance with the requirements of ISO 14160.

The guidance is not intended to be exhaustive but is offered in order to highlight important aspects to which attention should be given. It provides examples of how to meet the requirements, recognizing that other methods that achieve the same ends are equally acceptable. It also gives general advice on how to meet the requirements and draws attention to aspects of the requirements that might not be readily apparent to those unfamiliar with the sterilization of medical devices.

The subclauses in this International Standard to which the guidance in this annex specifically applies are indicated by the number of the relevant subclause in square brackets.

A.2 Personnel [4.2]

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 ISO 9004-1.

Personnel with the following responsibilities should possess particular qualifications and training:

— microbiological testing;

— veterinary microbiology;

— chemical analysis and formulation;

— installation of equipment;

— equipment maintenance;

— physical performance qualification;

— routine sterilizer operation;

— calibration;

— process design;

— equipment specification;

— aseptic processing.

A.3 Process development and product compatibility [4.5]

A.3.1 The development of a sterilization process for a particular medical device needs to establish a process that 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 while the product is in the design phase.

Requirements for a quality system that include design of medical devices are specified in ISO 9001 and ISO 13485.

A.3.2 During a liquid chemical sterilization process, products can be subjected to environmental stresses. The product could also react with the liquid chemical sterilants used. The product design should ensure that functionality and safety are not compromised by exposure to the anticipated range of sterilization conditions.

A.3.3 The selection of the sterilization process that 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 (see also NOTE 5 in clause 1):

a) availability of sterilization equipment;

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

c) sterilization processes already in use for other products;

d) requirements for levels of residual chemicals and/or their reaction products;

e) results of process development experiments;

f) possibility that the use of a liquid chemical sterilant leads to proliferation of resistant microorganisms.

A.3.4 A process development exercise may consist of a number of elements:

a) determination of the critical process variables and the limits for these variables within the sterilization process;

b) estimation of the bioburden on product from which the challenge presented to the sterilization process should be established;

c) appropriateness of the inoculated carrier to be used for performance qualification and routine monitoring should be confirmed.

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).

A.4 Validation [5]

A.4.1 Estimation of bioburden during process validation [5.3.3]

The guidance in this annex is provided in addition to that in ISO 11737-1 because of the particular difficulties in performing bioburden estimations of animal tissues.

The objective of bioburden estimations is threefold:

— to establish the nature of contaminating microorganisms present on the product;

— to establish the number of microorganisms present on the product;

— to establish the extent of variation and thus the consistency of the contamination by comparing the numbers found on consecutive batches.

Bioburden estimations should be carried out on:

— starting materials of animal origin;

— materials after each significant processing stage;

— product immediately prior to sterilization (i.e. presterilization counts);

Any method for estimating bioburden will only indicate the presence of a limited proportion of the numbers and species of microorganisms present. Bioburden values should therefore be corrected for errors in efficiency of removal of microorganisms from product and effectiveness of the applied culture conditions in detecting those microorganisms that have been removed. Estimates of recovery efficiencies may vary and can be extremely low for animal tissues. As a conservative approach, the equation for calculation of exposure time in 5.3.7 includes an additional safety factor of 100 to the estimate of the bioburden to compensate for these limitations.

Attention should be paid to the selection of appropriate media and incubation conditions for enumeration. In particular, the requirements for the isolation of microorganisms that might be associated with materials of animal origin should be considered. The media in table A.1 could be suitable in some instances.

Table A.1 — Summary of media, incubation conditions and organisms suitable for growth promotion

Medium[1)]	Possible incubation Conditions [3)]	Challenge organisms to demonstrate ability to support growth [4)]
1. Tryptone soya broth and tryptone soya agar	Aerobic 30 ºC-35 °C	*Bacillus subtilis* *Klebsiella pneumoniae* *Pseudomonas aeruginosa/diminuta* *Staphylococcus epidermidis*
2. Nutrient broth and nutrient agar	**a.** Aerobic 30 ºC-35 °C	*Bacillus subtilis* *Klebsiella pneumoniae* *Pseudomonas aeruginosa/diminuta*
	b. Aerobic 20 ºC-25 °C	*Saccharomyces cerevisiae*
3. Lowenstein-Jensen(1)	Aerobic 30 ºC-35 °C	*Trichophyton rubrum* *Mycobacterium phlei (3 weeks)*
4. Blood agar	**a.** Aerobic 30 ºC-35 °C	*Bacillus subtilis* *Klebsiella pneumoniae* *Pseudomonas aeruginosa/diminuta* *Staphylococcus epidermidis* *Enterococcus faecalis*
	b. Anaerobic 30 ºC-35 °C	*Clostridium sporogenes*
5. Potato dextrose agar	Aerobic 20 ºC-25 °C	*Saccharomyces cerevisiae* *Trichophyton rubrum*
6. Robertson's cooked meat(2)	Aerobic 30 ºC-35 °C	*Clostridium sporogenes*

1 Media should not incorporate colour change indicator dyes (which are frequently inhibitory to microbial growth) except in special circumstances, e.g. L-J medium. The detection of growth in liquid media should be made visually by either by turbidity or by special density/microscopy, and where necessary confirmed by subculture on appropriate solid media.

2 Alternative media with reducing properties suitable for detection of anaerobic growth may be substituted.

3 All incubation for growth promotion should be for a minimum period of two weeks except L-J medium which should be for a minimum period of three weeks. Temperature should be controlled within tolerances selected from within the range given. Injured organisms may require longer incubation period.

4 Each batch of a medium should be tested to demonstrate that the medium is capable of recovering 10-100 organisms per inoculum. (A batch should be regarded as a single preparation from one lot of raw materials autoclaved at one time). Testing should be done with defined strains from recognized type culture collection.

If an inoculum of 10 organisms is used, this will rarely lead to the recovery of 10 colony forming units (cfu) on solid media, as

(a) there will be a difference between total and viable count, and
(b) the precise number of organisms in the inoculum will vary about a mean of 10.

Recovery of one or more colony forming unit of the inoculate organism (or visible growth in liquid medium) would be regarded as satisfactory evidence of the medium's ability to support growth of the test organism. In testing liquid media, small volumes should be employed (10 cm^3) if dilution inhibition is to be avoided.

A.4.2 Performance qualification [5.3]

A.4.2.1 General

The selection of species of microorganisms to be used in the validation of a liquid chemical sterilization process for material of animal origin should take into account all of the following criteria:

— microbial species which could be present as a result of the source of the animal tissue;

— microbial species isolated during the estimation of bioburden on product;

— microbial species isolated from the environment in which the animal tissue is harvested and the manufacturing environment in which the final medical device is produced;

— microbial species which have a demonstrated high resistance to the liquid chemical sterilant or which can be expected to have an increased resistance;

— a range of microbial species.

Some examples of microorganisms which have been employed are given in table A.2. The microorganisms listed have previously demonstrated significant resistance to liquid chemical sterilant with animal tissues. The table is provided as guidance only and is not intended to be a comprehensive list of microorganisms that have to be evaluated.

The three stages of microbiological performance qualification — screening, construction of survival curves and assessment of inactivation on carriers of tissues — are considered in A.4.2.3, A.4.2.4 and A.4.2.5, respectively.

A.4.2.2 Neutralization [5.3.4]

Before commencing a microbiological performance qualification, it is necessary to ensure that the results of qualification experiments are not influenced adversely by microbicidal or microbiostatic effects due to carry-over of the liquid chemical sterilant into the recovery system. The effects of microbicidal or microbiostatic substances can be reduced by dilution, removed by filtration or inactivated by reaction with a neutralizing agent.

The choice of neutralization system will be influenced by the composition of the liquid chemical sterilant and the effectiveness of the chosen system should be demonstrated prior to the commencement of performance qualification.

A.4.2.3 Screening of microbial isolates [5.3.6(a)]

A.4.2.3.1 General

As it is often impractical to carry out inactivation studies on all isolates obtained from the product, a screening test should be employed. By exposing samples of tissue to conditions less severe than those used for processing, the more resistant isolates can be quickly segregated and used in the inactivation studies. Any isolate found to be more resistant than the reference microorganisms selected for the validation exercise (see table A.2) should be fully identified.

Due to the unique applications of liquid chemicals for use in sterilization processes, it is necessary to be vigilant in detecting, screening and testing microorganisms found to be present and which could pose a significant resistance to the sterilization process. A risk exists that new or altered microorganisms could be introduced during the manufacturing process and could possess a resistance to the sterilization process that is greater than the original test and validation microorganism(s).

Therefore, an ongoing procedure for screening and evaluating the resistance of microorganisms encountered in the manufacturing process and environment should be established (microbial isolate screening procedure) (see also A.5.).

The microbial screening process should be conducted to ensure that new or modified microorganisms are detected and evaluated in a timely manner.

The microbial isolate screening procedure should incorporate three phases. These phases are:

a) microbial isolate collection;

b) microbial isolate identification;

c) challenge testing (screening).

A.4.2.3.2 Microbial isolate collection

Microbial isolates should be collected from the manufacturing process and environment in which the medical device is produced. Collection should concentrate initially on micro-organisms that are known to exist on the product prior to sterilization (bioburden isolates). In addition to product bioburden, isolates should be collected from the manufacturing environment in which the product is produced. This environment may include, but is not limited to, process solutions, work surfaces, water purification systems, raw materials, and personnel.

Table A.2 — Examples of microorganisms which have been used for assessment of the activity of specific liquid chemical sterilants

Species	Culture Collection No.		
Spores of:	**ATCC[1)]**	**NCTC[2)]**	**NCIMB[3)]**
Clostridium sporogenes	3584	-	10696
Bacillus subtilis	6051 9372	3610	3610
Bacillus pumilus	27142	10327	10692
Chaetomium globosum	6205	-	-
Microascus cinereus	16594	-	-
Vegetative cells of :			
Mycobacterium chelonae	35752	946	1474
Methylobacterium extorquens	43645	-	9399
Trichosporium beigelii	22310	-	-

NOTE — Similar or equivalent cultures held by Culture Collections registered under the Budapest Convention may be used.

1) American Type Culture Collection
2) National Collection of Type Cultures
3) National Collection of Industrial and Marine Bacteria

A.4.2.3.3 Microbial isolates characterization

Microbial isolates collected for evaluation should be characterized and/or identified for future reference. Characterization should include, at a minimum, colony morphology, cellular morphology, Gram reaction and growth rate description. When possible, identification of the species or subspecies is preferred.

A.4.2.3.4 Challenge testing (screening)

Challenge testing of the microbial isolates should be conducted as indicated in 5.3.6. Microbial isolates which demonstrate a significant resistance to the sterilization process during initial challenge testing should be evaluated fully and compared to the microorganism(s) used for initial process qualification and validation studies. The relative resistance of the challenge microbial isolates should be evaluated with regards to the overall sterilization process.

One approach to initial challenge testing has been to expose a suspension containing at least 10^5 of the isolated microorganisms to the liquid chemical sterilant at the minimum conditions of the process specification for a time equal to the D-value of the most resistant microorganism used in performance qualification. If, following this exposure, survivors are detected, this indicates that the resistance of the isolate is greater by 20% or more than that of the most resistant microorganism used in performance qualification. In this situation, the isolate should be subjected to full characterization and detailed investigation of its inactivation kinetics.

A.4.2.4 Studies into microbial inactivation kinetics [5.3.6]

A.4.2.4.1 General

Performance qualification is required to provide an inactivation curve for each microorganism with a minimum of five points covering at least at thousandfold reduction in numbers. Triplicate determinations are commonly employed at each point and should show reproducibility within a limit of twice the standard deviation.

The D-value of the most resistant microorganism used in the test procedures can be calculated from the results obtained. The calculation of D-value is only possible if the log survivor curve (the plot of number of survivors on a logarithmic scale against time of exposure) is linear. Where deviations from linearity occur it can become difficult to predict a satisfactory sterilization process. Such deviations should be further investigated to better characterize the inactivation kinetics.

In order to estimate the D-value when low surviving fractions of microorganisms are recoverable, a Most Probable Number (MPN) determination can be performed for the three microorganisms which demonstrate the greatest resistance to the sterilization process. This D-value may be used as an indication of the acceptability of the calculated exposure time.

The determination of D-values by the MPN method is described in Annex A of ISO 11135 and are discussed further in A.4.2.4.2.

A.4.2.4.2 Investigational phases

Validation of liquid chemical sterilization processes should be conducted so that the possible interactions of the test microorganism(s) with the medical device can be evaluated. This can be accomplished by determining the inactivation kinetics of the test microorganism(s) cultured with, or inoculated onto, the medical device.

Conducting such evaluations requires the investigator to perform four essential phases of investigation. These essential phases are:

— determining components which represent the greatest challenge to liquid chemical sterilant;

— defining the method for establishing the microorganism(s) on the medical device or selected component;

— validating the method of recovery/detection of the test microorganism from the medical device or selected component;

— determining the inactivation kinetics of the test organism(s) in the presence of the medical device or selected component.

Test methods may be conducted by either direct enumeration to establish the number of survivors over time (survivor curve) or by Most Probable Number (MPN) estimation (e.g. Stumbo, Murphy, Cochran or Spearman Karber methods) [17]. Survivor curve construction is preferred, as this method establishes sufficient data to allow the determination of the inactivation kinetics of the test microorganism over time in the presence of the medical device or selected component. MPN methods could be necessary if the medical device or component does not allow consistent recovery of the surviving population over time (i.e. the medical device or carrier cannot be macerated to allow estimation of the surviving microbial population).

While direct enumeration and construction of a survivor curve provides greater information about the inactivation kinetics of the test organism, they can also require more time and resources to perform. Direct enumeration procedures attempt to remove all viable test organisms after predetermined times of liquid chemical sterilant exposure. Removal can be accomplished as for bioburden estimation (ISO 11737-1), for example by homogenizing the test component and preparing appropriate dilutions for survivor enumerations.

Homogenizing the test component may be performed by aseptic maceration or by blending the test component (i.e. animal tissue) in sterile diluting fluid. Serial dilutions of the homogeny are prepared and appropriate dilutions enumerated by standard counting methods (see ISO 11737-1).

A.4.2.5 Use of carriers of tissue

A.4.2.5.1 General

Using the microorganism that has demonstrated the highest resistance to the liquid chemical sterilant in suspension tests, the test organism inactivation kinetics should be evaluated in the presence of the test component. The study design should include controlled ("worst case") exposures of the inoculated or cultured test component in the liquid chemical sterilant against time. Samples should be removed at predetermined time intervals to allow for estimation of the surviving population by the validated recovery method.

Upon completion of the inactivation kinetics study, a survivor curve, plotting estimated survivors on a logarithmic scale against time, should be prepared. The D-value of the test organism in the presence of the test component and worst-case sterilization conditions can then be established. The evaluation of the inactivation kinetics should be performed a minimum of three times to demonstrate reproducibility.

A.4.2.5.2 Choice of carrier

Studies into microbial inactivation kinetics should be conducted on carrier materials that pose the greatest challenge to the sterilization process. Selection of the carrier should take into consideration the contact and/or interaction of the liquid chemical sterilant with the carrier (e.g. hydrophobic, filamentous material can be a greater challenge than smooth, hydrophilic surfaces).

If the choice of carrier is not readily apparent, screening tests to identify the most challenging carrier should be performed.

A.4.2.5.3 Establishing microorganism(s) on the test component (carrier)

The method of establishing viable microorganisms on the carrier prior to liquid chemical sterilant exposures is paramount in creating a simulation that will assess the sterilization process effectively and in a manner that estimates challenges to the sterilization processes as they occur in practice. Two methods can be used to establish a viable microorganism on the test component; direct inoculation or cultivation in simulated manufacturing conditions.

Direct inoculation utilizes a viable spore or cell suspension that is applied to the carrier immediately prior to liquid chemical sterilant exposure. Consideration should be given to the time that the inoculum is allowed to penetrate and adhere to the carrier prior to liquid chemical sterilant exposure. In addition, any bactericidal effect of the carrier should also be taken into account.

Cultivation of microorganism(s) in simulated manufacturing conditions should be utilized, and is preferred when possible over inoculation, when the selected microorganism is capable of growth in/on the product during normal manufacturing conditions. A culture of the carrier(s) with the test organism should be established. Under such conditions, the level of viable microorganisms present in/on the product must be at least 1000 colony forming units and uniform from one component to the next in a single culture system prior to liquid chemical sterilant exposure. Data to demonstrate appropriate minimum population and uniformity should be collected immediately prior to inactivation kinetic studies.

The method for recovering viable microorganisms from the carrier after liquid chemical sterilant exposure should be defined and validated. The validation should demonstrate that the method chosen recovers the surviving organisms in a reproducible manner.

A.4.2.6 Effect of organic materials

For some products, contamination by organic material can be a contributory factor in limiting process efficacy. The investigations should include evaluations of solutions containing appropriate organic material (e.g. serum, albumin etc.) or a sterile macerated suspension of tissue. The type and concentration of organic material should be documented.

For products where drying in the presence of organic material can occur, inoculated carriers comprising microorganisms dried in suitable organic material should also be used. Such inoculated carriers should include preparations of microorganisms resistant to drying, such as *Clostridium sporogenes* spores, *Bacillus subtilis* spores, *Enterococcus faecalis*, *Mycobacterium chelonae* and *Candida albicans* (see tables A.1 and A.2).

A.4.2.7 Validation of aseptic process [5.3.8]

The aseptic transfer of product from the sterilant to the final sterile container also requires validation. Included within this validation exercise should be the following:

a) process used to sterilize the empty containers in accordance with the requirements of the International Standard for the sterilization process used (see ISO 11134, ISO 11135, and ISO 11137);

b) process used to sterilize any storage solution into which the product is aseptically transferred;

b) NOTE — Filtration sterilization should be undertaken in accordance with ISO 13408-1.

c) physical and microbiological monitoring of the environment in which the aseptic transfer is to be performed;

d) aseptic transfer technique using media trials to simulate the environmental exposure likely to occur during the transfer from sterilant solution to final container.

A.5 Ongoing microbiological monitoring [6.1]

Microbial isolation and challenge testing should be conducted on a routine basis. The goal of such testing is to detect possible changes to the microorganism(s) that can be present during the sterilization process. Since validation procedures are established to evaluate the sterilization process against a given range of microorganisms, ongoing testing should be performed to provide evidence that the microorganisms presented to the sterilization process are not or have not become more resistant than those used during original validation studies (see also A.4.2.3.1).

A.6 Process control and monitoring [6]

A sterilization process employing liquid chemical sterilants usually involves a number of phases:

— preparation of the liquid chemical sterilant;

— exposure of the product to the sterilant at a controlled temperature for a specified time.

If it is not a terminal sterilization process, there are two additional phases:

— preparation and sterilization of the primary package and any storage solution in which product is to be presented;

— aseptic transfer of the product from the sterilant into its primary pack.

The preparation of the sterilant requires careful control. Records of the constituents, such as batch numbers and quantities, should be retained and the final concentration of the active ingredient(s) should be confirmed by assay. Frequently, sterilant solutions are filtered prior to use in order to remove any microorganisms and other impurities carried over from the components of the sterilant. Filters used for such processes should be tested for their integrity after use.

The sterilization process is required to be carried out in exposure vessels of defined specification under temperature-controlled conditions.

In order to assess the routine acceptability of a process, the composition of the sterilant should be checked after product has been removed. Such checks may be chemical or microbiological. A chemical check could be an assay to confirm that the composition was within specification following completion of the process. A microbiological check, for example, could be exposure of an inoculated carrier to the sterilant to demonstrate continued microbicidal efficacy.

Following exposure, product can be transferred aseptically to its final container. During this transfer, the environment in the vicinity should be monitored microbiologically.

A list of personnel who have been qualified to undertake aseptic transfers should be established and maintained. This approved list should be kept under constant review and personnel should be requalified at defined intervals. The qualification and requalification typically takes the form of media transfers and parallels the approach of "broth fills" used to qualify filtration sterilization and aseptic processes.

If product is to be presented in a storage solution, this should be sterilized prior to use. If aseptic processing is used, ISO 13408-1 should be followed.

Final product testing is generally of limited value in determining sterility of a batch of product. However, in this particular application, certain gross failures may be detected by testing for microbiological contamination after completion of the process (see 6.3). When conducting such microbiological tests, it is important to ensure the removal of any residual microbicidally active sterilant or storage solution (see A.4.2.2).

Annex B
(informative)

References to European Standards with their relevant equivalents

B.1 Identical

ISO standard	European standard
ISO 9001	EN 9001
ISO 9002	EN 9002
ISO 9004-1	EN 9004-1

B.2 Technically equivalent

ISO standard	European standard
ISO 11135	EN 550
ISO 11737-1	EN 1174-1

B.3 Related

ISO standard	European standard
ISO 11134	EN 554
ISO 11137	EN 552
ISO 11138-1	EN 866
ISO 13408-1	EN XXXXX[1)]
ISO 13485	EN 46001
ISO 13488	EN 46002

[1)] To be published.

B.4 No international standard available

EN 556	*Sterilization of medical devices — Requirements for medical devices labelled "Sterile"*
EN 12442-1[1)]	*Animal tissues and their derivatives utilized in the manufacture of medical devices — Part 1: Analysis and management of risks*
prEN 12442-2[1)]	*Animal tissues and their derivatives utilized in the manufacture of medical devices — Part 2: Sourcing, controls, collection and handling*
prEN 12442-3[1)]	*Animal tissues and their derivatives utilized in the manufacture of medical devices — Part 3: Elimination and/or inactivation of viruses and other transmissible agents — Determination of non-viability*

[1)] To be published.

Annex C
(informative)

Bibliography

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[2] ISO 11134: 1994, *Sterilization of health care products — Requirements for validation and routine control — Industrial moist heat sterilization.*

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

[4] ISO 11137: 1995, *Sterilization of health care products — Requirements for validation and routine control — Radiation sterilization.*

[5] ISO 13408-1:—[1)], *Aseptic processing of health care products.*

[6] ISO 13485: 1996, *Quality systems — Medical devices — Particular requirements for the application of ISO 9001.*

[7] ISO 13488: 1996, *Quality systems — Medical devices — Particular requirements for the application of ISO 9002.*

[8] ISO 14538:—[1)], *Biological evaluation of medical devices — Methods for the establishment of permissible limits for sterilization and process residues in medical devices using health-based risk assessment.*

[9] EN 556, *Sterilization of medical devices — Requirements for medical devices labelled "Sterile".*

[10] EN 12442-1:—[1)], *Animal tissues and their derivatives utilized in the manufacture of medical devices — Part 1: Analysis and management of risks.*

[11] EN 12442-2—[1)], *Animal tissues and their derivatives utilized in the manufacture of medical devices — Part 2: Sourcing, controls, collection and handling.*

[12] EN 12442-3—[1)], *Animal tissues and their derivatives utilized in the manufacture of medical devices — Part 3: Elimination and/or inactivation of viruses and other transmissible agents — Determination of non-viability.*

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[1] To be published.