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Sterilization of health care products — Biological indicators — Guidance for the selection, use and interpretation of results

Stérilisation des produits de santé — Indicateurs biologiques — Directives générales pour la sélection, l'utilisation et l'interprétation des résultats

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Foreword

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

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 14161 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

This International Standard provides guidance regarding the selection, use and interpretation of results of biological indicators when used to develop, validate and monitor sterilization processes. The procedures described in this document are of a general nature and do not, of themselves, constitute a comprehensive development, validation, or monitoring programme with regard to the sterilization of health care products. The intent of this International Standard is not to mandate the use of biological indicators in a process, but, if they are used, to provide guidance for their proper selection and use, to avoid misleading results.

Biological indicators are not intended for use in any process other than that specified by the manufacturer on the product labelling. The use of an inappropriate biological indicator can give misleading results. In this International Standard, the user will find guidance on selection of the correct biological indicator for their particular sterilization process and critical parameters as well as guidance on its appropriate use.

The user should select a biological indicator that is appropriate for the particular process to be employed. There are wide variations in sterilization processes and biological indicator manufacturers are not able to foresee all possible uses of their product. Manufacturers, therefore, label biological indicators according to their intended use. It is the responsibility of the users of biological indicators to select, use, recover and interpret the results as appropriate for the particular sterilization process used.

Biological indicators should always be used in combination with physical and/or chemical measurements in demonstrating the efficacy of a sterilizing process. When a physical and/or chemical variable of a sterilization process is outside its specified limits, cycle parameters should be evaluated. It should be noted that measurements, which need to be evaluated, may be made during the cycle in the context of the overall cycle. Systems and/or procedures should be established to evaluate any deviations from the cycle process limits, and reasons for accepting any deviation should be fully documented.

The performance of a biological indicator can be adversely affected by the conditions of storage and transport prior to its use, the use of the biological indicator, the sterilizer operating parameters, or the techniques employed after exposure to the process. For these reasons, the recommendations of the biological indicator manufacturer for storage and use should be followed. After exposure, biological indicators should be aseptically transferred and subjected to the validated recovery conditions as specified by the biological indicator manufacturer.

It should be noted that biological indicators are not intended to indicate that the products, nor any other load being sterilized, are sterile. Biological indicators are utilized to test the effectiveness of a given sterilization process and employed equipment by assessing microbial lethality according to the concept of sterility assurance level. Suitably trained personnel should conduct these studies.

Sterilization of health care products — Biological indicators — Guidance for the selection, use and interpretation of results

1 Scope

This International Standard provides guidance for the selection, use and interpretation of results from application of biological indicators when used in the development, validation and routine monitoring of sterilization processes. This International Standard applies to biological indicators for which International Standards exist.

NOTE 1 See for example the ISO 11138 series.

NOTE 2 The general information provided in this International Standard may have useful application for processes and biological indicators not currently addressed by existing International Standards, e.g. new and developing sterilization processes.

This International Standard does not consider those processes that rely solely on physical removal of microorganisms, e.g. filtration.

This International Standard is not intended to apply to combination processes, using for example washer disinfectors or flushing and steaming of pipelines.

This International Standard is not intended to apply to liquid sterilization processes.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to apply. Members of ISO and IEC maintain registers of currently valid International Standards.

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

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

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

ISO 11138-2:1994, *Sterilization of health care products — Biological indicators — Part 2: Biological indicators for ethylene oxide sterilization.*

ISO 11138-3:1995, *Sterilization of health care products — Biological indicators — Part 3: Biological indicators for moist heat sterilization.*

ISO 11737-1:1995, *Sterilization of medical devices — Microbiological methods — Part 1: Estimation of population of microorganisms on product.*

ISO 13683:1997, *Sterilization of health care products — Requirements for validation and routine control of moist heat sterilization in health care facilities.*

ISO 14937, *Sterilization of health care products — General criteria for characterization of a sterilizing agent and development, validation and routine control of a sterilization process.*

3 Terms and definitions

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

3.1
accreditation
procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks

NOTE 1 See reference [3].

NOTE 2 Accreditation does not itself qualify the laboratory to approve any particular product. However, accreditation may be relevant to approval and certification authorities when they decide whether or not to accept data produced by a given laboratory in connection with their own activities.

3.2
aseptic technique
conditions and procedures used to exclude the introduction of microbial contamination

3.3
bioburden
population of viable microorganisms on or in a product and/or package

3.4
biological indicator
BI
inoculated carrier contained within its primary pack ready for use and providing a defined resistance to the specified sterilization process

NOTE See ISO 11138-1.

3.5
***D*-value**
D_{10} value
time or radiation dose required to achieve inactivation of 90 % of a population of the test microorganism under stated exposure conditions

NOTE See ISO 11138-1.

3.6
inoculated carrier
carrier on which a defined number of test organisms have been deposited

NOTE 1 See ISO 11138-1.

NOTE 2 The carrier is the supporting material on which test organisms are deposited.

NOTE 3 The test organism is a microorganism used for the manufacture of inoculated carriers.

3.7
inoculation
transferral of a defined microbial entity into or on an item

3.8
log reduction
LR
reduction in number of viable microorganisms, expressed in $\log_{10}$ units, after fractional exposure to a sterilization cycle

3.9
process challenge device
PCD
item which is deemed to present one of the greatest challenges to the effective performance of the sterilizing agent(s) in the collection of items to be sterilized

NOTE 1 The item is so constituted that a biological indicator can be placed in the position that is most difficult for the sterilizing agent to reach.

NOTE 2 The design of the process challenge device depends on the type of goods to be sterilized and the sterilization procedure.

NOTE 3 The biological indicator should not interfere with the function of the process challenge device.

NOTE 4 In some process challenge devices an inoculated carrier may be used in place of a biological indicator.

3.10
process challenge location
PCL
site that simulates “worst case” conditions as they are given for sterilizing agent(s) in the goods to be sterilized

NOTE 1 The site is so constituted that a biological indicator can be placed in the position that represents a rigorous challenge for the sterilizing agent to reach.

NOTE 2 The site depends on the type of goods to be sterilized and the sterilization process parameters.

NOTE 3 The biological indicator should not interfere with the function of the goods.

NOTE 4 In some sites an inoculated carrier may be used in place of a biological indicator.

3.11
process parameter
specified value for a process variable

NOTE Specifications for a sterilization process include the process parameters and their tolerances.

3.12
resistometer
equipment designed to create defined combinations of the physical and/or chemical variables of a sterilization process within defined limits

NOTE 1 See ISO 11138-1.

NOTE 2 Also referred to as Biological Indicator Evaluator Resistometer (BIER).

3.13
sterilization cycle development
procedure for determination of the appropriate processing parameters and conditions which are consistent with attaining the desired specifications and label claims for a given product or group of products

3.14
sterilization cycle validation
documented procedure for obtaining, recording and interpreting the results required to establish that a process would consistently yield product complying with predetermined specifications

3.15
sterile
free from viable microorganisms

3.16
sterilization
validated process used to render a product free from viable microorganisms

NOTE In a sterilization process, the nature of microbial inactivation 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.

3.17
supplier
organization that provides a product to the customer

NOTE 1 In a contractual situation, the manufacturer may be called the “contractor”.

NOTE 2 The supplier may be, for example, the manufacturer, distributor, vendor, importer, assembler or service organization. The supplier can be either external or internal to the organization. The supplier is a person or business concern that manufactures goods or owns a factory and represents the “first party” (see reference [4]).

3.18
third party
person or body that is recognized as being independent of the parties involved, as concerns the issue in question

NOTE 1 See reference [1].

NOTE 2 Parties involved are usually supplier (“first party”) and purchaser (“second party”) interests.

3.19
user
person or body employing biological indicators for a given purpose

NOTE 1 See reference [4].

NOTE 2 The user is the customer who is the recipient of a product provided by the supplier (see reference [4]). In a contractual situation, the user is called “purchaser”. The user may be the customer, beneficiary or purchaser. The user can be either external or internal to the organization and represents the “second party”.

3.20
***z*-value**
<thermal sterilization process> the change in exposure temperature which corresponds to a 10-fold change in *D*-value

NOTE See ISO 11138-3.

4 General

This guidance International Standard provides information on biological indicators that may apply generally for any sterilization process, including new sterilization processes not yet covered by International Standards.

The use of biological indicators is normally documented in procedures and/or instructions.

NOTE Employing quality systems complying with ISO 13485 or ISO 13488 satisfies this provision (see references [11] and [12]).

Biological indicators that are defined in ISO 11138-1, ISO 11138-2 and ISO 11138-3 give requirements for the manufacture of biological indicator systems where the biological component is a microorganism, such as a bacterial endospore or other microbiological form. The ISO 11138 series gives requirements for biological indicators for use in sterilization processes. These International Standards require that suitably trained personnel carry out the procedures and methods described.

A suitable biological indicator consists of carrier material and packaging, and has a microbiological component that is known to be suitable for handling without special containment facilities. The growth conditions should be well documented and the use of the indicator should be as simple and well described to the user as possible to avoid misinterpretation.

No formal approval system exists internationally for biological indicators that are marketed and used for stated purposes or under stated conditions. Some national regulatory authorities, however, have particular requirements for biological indicators and for the choice and use of biological indicators for the validation and control of products marketed as sterile or sterilized.

A biological indicator represents a microbiological challenge to a sterilization process, and is used to verify that a sterilization process has the ability to inactivate microorganisms that have a known resistance to a referenced sterilization process. Test organisms employed in biological indicators typically have resistance to sterilization which exceeds that of common bioburden microorganisms, although some organisms may exhibit a resistance to sterilization in excess of that of the test organisms. The appropriate biological indicator has a combination of population and resistance that exceeds that of the bioburden. If there is reason to believe that the goods to be processed may be contaminated with particularly resistant organisms, extended sterilization processing, based on the bioburden, may be required.

The user should ensure that the biological indicator has been validated for use with the particular range of sterilization conditions that are used. This may require additional information than that given in the labelling. When biological indicators are used outside reference conditions, the user may require information on the reaction to be expected from the indicator, e.g. the effect of sub-optimal moisture conditions on the biological indicators used in an ethylene oxide process. Users who employ biological indicators for non-standard sterilization techniques should thoroughly characterize the resistance of the biological indicators to the particular sterilization process as compared to a wide range of microorganisms, including any hazardous microorganisms or infectious agents that may constitute a part of the bioburden of the product. The relationship of the response of the biological indicator to process parameters should be clearly demonstrated.

It is incumbent upon those responsible for the sterilization of product to ensure that the type of biological indicator employed to validate and/or routinely monitor a given sterilization process is appropriate for that use.

The manufacturer's recommendations for the use and storage of the biological indicators should always be followed. Failure to do so may compromise the integrity of the biological indicator. If the user removes the inoculated carrier from the biological indicator's primary packaging, changes in the resistance characteristics may occur. Guidance should be sought from the manufacturer on the extent of this change or the user may evaluate changes in the resistance characteristics. The user should document that the performance characteristics of the inoculated carrier are appropriate for their use.

Biological indicators should not be used beyond the expiration date stated by the manufacturer.

Those who employ biological indicators for validation and/or routine monitoring of sterilization should be properly trained in their use. Post-sterilization handling of inoculated carriers and inoculated products should be performed according to validated guidelines or in compliance with the directions provided by the manufacturer of the inoculated carriers. The transfer of microorganisms exposed to the sterilization process to the appropriate recovery medium should employ aseptic technique.

The ISO 11138 series gives requirements for the information that the manufacturer should provide for biological indicators. The information may be provided on the label, as a packet insert, or as a general specification accompanying the biological indicators. These International Standards also include minimum requirements for resistance characteristics. Testing conditions and methods are given as reference methods.

Users of biological indicators come from a wide variety of industries, private enterprises and health care facilities. Users generally are not required to perform resistance assays on biological indicators, but may have differing requirements for their quality assurance systems, which include audits (see 6.2.2).

The verification of resistance characteristics by the user is an alternative to and/or complementary to an audit, when necessary.

5 Characteristics of biological indicators

5.1 General

Biological indicators provide means to assess directly the microbial lethality of a sterilization process (see references [14] and [15]). When used in conjunction with physical and/or chemical process monitors, biological indicators can provide an indication of the effectiveness of a given sterilization process.

A sterilization process should be considered as satisfactory only when the desired physical and/or chemical parameters and microbiological results, as determined by an appropriate sterilization cycle development, validation, and monitoring programme, have been realized. Failure to achieve the desired physical and/or chemical parameters and/or microbiological challenge forms the basis for declaring the sterilization process as nonconforming (see reference [29]).

Biological indicators consist of a defined population of test organisms presented in such a manner as to allow their recovery following sterilization processing. For example, test organisms employed for ethylene oxide sterilization processes can be spores of a suitable strain of *Bacillus subtilis*, as noted in ISO 11138-2. For steam sterilization or moist heat sterilization, the test organisms employed can be spores of a suitable strain of *Bacillus stearothermophilus*, as noted in ISO 11138-3.

The basis of all formulae used to determine biological indicator resistance characteristics such as D-values is that the inactivation reaction follows first-order kinetics, with the requirement that the value for the correlation coefficient for the linearity of the survivor curve be not less than 0,8 (see ISO 11138-1). The strain, the production method, the suspension fluid, the carrier and packaging materials all affect the resistance characteristics of the finished product (see ISO 11138-1).

The design and construction of a biological indicator may result in unique resistance characteristics and may vary depending on whether the biological indicator is intended for use in the development and validation of a sterilization process or for use in routine monitoring. If the design of the biological indicator for use in routine monitoring differs from that employed to validate the sterilization process, the challenge to the process during validation should be capable of correlation with the challenge to the process during routine monitoring (see annex A, Figure A.2).

5.2 Test organism suspension for direct inoculation of products

Direct inoculation of test organisms on or in product may be necessary in cycle development and other studies, when the use of a biological indicator is not feasible. Direct inoculation may be appropriate for assessing factors such as product sterilizability, identification of the more difficult to sterilize locations within the device, and localized microbiological effects, e.g. moist heat versus dry heat environments (see annex D).

NOTE The “most difficult to sterilize” site on a device or within a sterilization load is determined based on experimental and reproducible data derived from the particular sterilization methodology. In practice, the “most difficult to sterilize” site represents those locations that are most likely to provide high resistance to the sterilization process. One should refer to specific sterilization standards (e.g. ISO 11134 and ISO 11135) for guidance in estimating difficult-to-sterilize locations.

To assess the efficacy of sterilization at a particular site or location on the product, the desired species and population of test organisms may be inoculated at those sites that represent a rigorous challenge to the sterilization process. The use of suspensions of test organisms to prepare inoculated carriers or inoculated products requires caution. Inoculation of test organisms onto different materials may alter the resistance characteristics, causing the resistance to be higher or lower due to adhesion of spores to the material as monolayer and/or multilayer, to coating effects, to bacteriostatic or bactericidal effects, etc. Likewise, caution should be exercised with regard to the techniques employed to recover the test organisms following processing in order to ensure an adequate level of recovery from the product (see ISO 11737-1). Methods used for recovery of test organisms should be validated and expressed in terms of percent recovery of the original inoculum (see reference [24]).

For products or materials, the use of direct inoculation with a spore suspension may cause prolonged or decreased survival of spores, in terms of percent recovery of the original inoculum under normal sterilization conditions. Inoculated products may be assayed with either survivor curve (enumeration/direct counting) or fraction-negative analysis (Most Probable Number procedures) (see annex A, Figure A.4). This requires aseptic techniques.

The D-value and, when appropriate, the z-value, are only constant values under determined and defined conditions. The resistance characteristics of a spore suspension provided by a manufacturer or supplier of biological indicators may not correspond to the resistance characteristics for direct product inoculation studies. The resistance characteristics should be validated for the carrier employed (solid carrier material or fluid) as well as for the specific sterilization cycle employed.

5.3 Inoculated carriers

Inoculated carriers consist of a defined population of test organisms inoculated on or in a suitable carrier material. Caution should be exercised to ensure that the integrity of the carrier material selected is sufficient to withstand sterilization processing without degradation and to minimize the loss of the inoculated test organisms during transport and handling.

The resistance characteristics of a test organism in suspension may be considerably changed upon deposition on or in carriers. Several factors may influence the resistance characteristics, such as the surface onto which the suspension is inoculated (e.g. solid materials, viscous products or fluids), the way the spores are dispersed and otherwise treated, the methods of drying, etc.

If an inoculated carrier is removed from the biological indicator primary package to be used for cycle development or cycle validation studies or for process challenge devices for routine process monitoring, it is the responsibility of the user to validate this application. It should be recognized that the resistance of the inoculated carrier (e.g. “naked” carrier) may differ from the resistance of the biological indicator system as labelled, due to hindrance to sterilizing agent penetration by the primary packaging.

The resistance characteristics of an inoculated carrier provided by the manufacturer of biological indicators might not correspond to the resistance characteristics established in direct product inoculation studies.

The carrier material should be evaluated by the biological indicator manufacturer with the sterilizing agent for which the biological indicator is intended, to show that it neither retains nor releases inhibitory substances (e.g. sterilizing agent residuals) to such an extent that the recovery of low numbers of test organisms is inhibited subsequent to processing (see ISO 11138-1 for carrier-material validation).

5.4 Biological indicators

5.4.1 General

The resistance characteristics of biological indicators vary according to the manufacturing methods and the testing conditions. The same lot of biological indicators may also show varying resistance characteristics according to the process and placement within the load in which they are used. The user needs to document the placement of the chosen biological indicators in the sterilizer chamber location, within the product load or a process challenge device (see annex B).

5.4.2 Self-contained biological indicators

Self-contained biological indicators consist either of:

a) an ampoule of growth medium and a carrier inoculated with test organisms contained within an outer vial so that the sterilizing agent obtains access to the inoculated carrier via a tortuous path or filter. After exposure to the sterilization process, the growth medium is brought into contact with the inoculated carrier by breaking the ampoule of growth medium, thereby eliminating the need to aseptically transfer the inoculated carrier to a separate vial of growth medium; or