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Sterilization of health care products — Biological indicators —

Part 7: Guidance for the selection, use and interpretation of results

Stérilisation des produits de santé — Indicateurs biologiques —

Partie 7: 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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

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

This first edition cancels and replaces ISO 14161:2009, which has been technically revised.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document provides guidance regarding the selection, use and interpretation of results of biological indicators 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 document is not to stipulate the use of biological indicators in a process but, if they are used, to provide guidance for their proper selection and use in order to avoid misleading results.

In this document, users will find guidance on selection of the correct biological indicator for their particular sterilization process (see the ISO 11138 series) and critical parameters as well as guidance on its appropriate use.

The selection of an appropriate biological indicator for the particular process used is critical. There is a wide variety of sterilization processes in common use, 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.

The performance of a biological indicator can be adversely affected by the conditions of storage and transport prior to its use, by inappropriate/non-indicated use of the biological indicator or by the sterilizer process parameters. In addition, the incubation procedure used after exposure to the process, including incubation temperature and culture medium type, supplier and specific batch, can affect measured resistance as a function of recovery and growth. For these reasons, the recommendations of the biological indicator manufacturer for transportation, storage and use should be followed. After exposure, the aseptic transfer (if applicable) and incubation of biological indicators as specified by the biological indicator manufacturer is critical for obtaining correct results.

It is important to note that biological indicators are not intended to indicate that the products in the load being sterilized are sterile. Biological indicators are utilized to test the effectiveness of a given sterilization process and the equipment used, by assessing microbial lethality according to the concept of sterility assurance level. Suitable training is necessary for personnel conducting these studies.

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

Sterilization of health care products — Biological indicators —

Part 7: Guidance for the selection, use and interpretation of results

1 Scope

This document 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.

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

It is not applicable to combination processes using, for example, washer-disinfectors or flushing and steaming of pipelines.

It does not specify requirements for the selection and use of biological indicators intended to monitor vaporised hydrogen peroxide processes for isolator and room biodecontamination processes at atmospheric pressure.

It is not applicable to liquid immersion sterilization processes.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
aseptic technique
conditions and procedures used to minimize the risk of the introduction of microbial contamination

[SOURCE: ISO 11139:2018, 3.16]

3.2
bioburden
population of viable microorganisms on or in a product and/or sterile barrier system

[SOURCE: ISO 11139:2018, 3.23]

3.3
biological indicator
BI
test system containing viable microorganisms providing a specified resistance to a specified sterilization process

[SOURCE: ISO 11139:2018, 3.29, modified — "BI" has been added to term.]

3.4
***D* value**
D_{10} value
time or dose required under stated conditions to achieve inactivation of 90 % of a population of the test microorganisms

[SOURCE: ISO 11139:2018, 3.75]

3.5
holding time
period during which process parameters are maintained, within their specified tolerances

[SOURCE: ISO 11139:2018, 3.133]

3.6
inoculated carrier
supporting material on or in which a specified number of viable test microorganisms has been deposited

[SOURCE: ISO 11139:2018, 3.144]

3.7
inoculation
addition of a defined amount of a characterized microbial entity into or on to an item

3.8
log reduction
LR
reduction in number of viable microorganisms

Note 1 to entry: Expressed in log units.

3.9
operational qualification
OQ
process of obtaining and documenting evidence that installed equipment operates within predetermined limits when used in accordance with its operational procedures

[SOURCE: ISO 11139:2018, 3.220.3]

3.10
performance qualification
PQ
process of establishing by objective evidence that the process, under anticipated conditions, consistently produces a product which meets all predetermined requirements

[SOURCE: ISO 11139:2018, 3.220.4]

3.11
process challenge device
PCD
item providing a defined resistance to a cleaning, disinfection, or sterilization process and used to assess performance of the process

[SOURCE: ISO 11139:2018, 3.205]

3.12
process challenge location
PCL
site chosen within a load as the position at which the least microbiological inactivation is expected to be delivered

[SOURCE: ISO 11139:2018, 3.206]

3.13
process parameter
specified value for a process variable

Note 1 to entry: The specification for a process includes the process parameters and their tolerances.

[SOURCE: ISO 11139:2018, 3.211]

3.14
process variable
chemical or physical attribute within a cleaning, disinfection, packaging, or sterilization process, changes in which can alter its effectiveness

EXAMPLE Time, temperature, pressure, concentration, humidity, wavelength.

[SOURCE: ISO 11139:2018, 3.213]

3.15
reference microorganism
microbial strain obtained from a recognized culture collection

[SOURCE: ISO 11139:2018, 3.228]

3.16
resistometer
test equipment designed to create specified combinations of the physical and/or chemical parameters of a sterilization process

[SOURCE: ISO 11139:2018, 3.233]

3.17
spore log reduction
SLR
negative exponent to the base 10 describing the decrease in the number of spores

Note 1 to entry: It is expressed as a logarithm.

[SOURCE: ISO 11139:2018, 3.260]

3.18
sterile
free from viable microorganisms

[SOURCE: ISO 11139:2018, 3.271]

3.19
sterility assurance level
SAL
probability of a single viable microorganism occurring on an item after sterilization

[SOURCE: ISO 11139:2018, 3.275, modified — Note 1 to entry has been deleted.]

3.20
sterilization
validated process used to render product free from viable microorganisms

Note 1 to entry: In a sterilization process, the nature of microbial inactivation is exponential and thus the survival of a microorganism on an individual item can be expressed in terms of probability. While this probability can be reduced to a very low number, it can never be reduced to zero.

[SOURCE: ISO 11139:2018, 3.277]

3.21
survival-kill window
extent of exposure to a sterilization process under specified conditions where there is a transition from all biological indicators showing growth to all biological indicators showing no growth

[SOURCE: ISO 11139:2018, 3.292]

3.22
third party
person or body that is recognized as being independent of the parties involved, as concerns the issue in question

Note 1 to entry: Parties involved are usually supplier ("first party") and purchaser ("second party") interests.

3.23
z **value**
change in temperature of a thermal sterilization or disinfection process that produces a tenfold change in *D* value

Note 1 to entry: It is expressed in degree Celsius (°C).

[SOURCE: ISO 11139:2018, 3.326]

4 General

4.1 This document provides guidance on biological indicators that can apply generally for any sterilization process, including new sterilization processes covered by ISO 14937.

4.2 The use of biological indicators is normally documented in the user's standard operating procedures (SOPs), procedures, or instructions.

NOTE Employing quality management systems such as ISO 13485 usually satisfies this provision.

4.3 Biological indicators should 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, the reason for the sterilizer's inability to achieve its process parameters should be evaluated and the problem corrected. Similarly, when an indicator failure takes place when the physical and/or chemical variables of the sterilization process are within the specified limits, the reason for the sterilizer's failure to inactivate the indicator should be evaluated and the problem corrected. 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.

4.4 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 (e.g. Risk Group 1, WHO, 2004). The growth conditions should be well documented, and the use of the indicator should be as simple and well described as possible to avoid misinterpretation by the user.

4.5 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 microorganisms employed in biological indicators typically have resistance to sterilization which exceeds that of common bioburden microorganisms, although some microorganisms can exhibit a resistance to sterilization in excess of that of the test microorganisms. The appropriate biological indicator provides a challenge to the sterilization process which exceeds that of the bioburden through a combination of population and resistance. If there is reason to believe that the goods to be processed could be contaminated with particularly resistant microorganisms, extended sterilization processing, based on the bioburden, could be required.

4.6 Biological indicators are not intended for use in any sterilization process other than that specified by the biological indicator manufacturer on the product labelling. Microbial species and strains are selected as biological indicator candidates based on their known resistance to the specific method of sterilization. The use of an inappropriate biological indicator can give misleading results.

The user should ensure that the biological indicator has been qualified for use with the particular range of sterilization conditions that are used. This could require information in addition to that given in the labelling. When biological indicators are used outside reference conditions, the user can require information on the reaction 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 outside the manufacturer's labelled recommendations should thoroughly characterize the resistance of the biological indicators to the particular sterilization process. The relationship of the response of the biological indicator to process parameters should be clearly demonstrated.

4.7 The user who is responsible for the sterilization of product should ensure that the type of biological indicator employed to validate and/or routinely monitor a given sterilization process is appropriate for that use.

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

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

4.10 Users who employ biological indicators for sterilization process development, validation and/or routine monitoring of sterilization should be properly trained in their use.

4.11 The time between completion of sterilization process and incubation should be within the manufacturer's stated time or should be justified as described in 8.2.3.

4.12 Transfer of microorganisms exposed to the sterilization process to the appropriate recovery medium should be done using aseptic technique.

4.13 Self-contained biological indicators are specifically designed to eliminate the need for aseptic handling because all of the components required to effect post process incubation are enclosed within the primary packaging which need not be opened (see 5.4).

4.14 The ISO 11138 series gives requirements for the information that the manufacturer shall provide for biological indicators. The information might be provided on the label, as a packet insert or as a general specification accompanying the biological indicators. The ISO 11138 series also includes minimum requirements for resistance characteristics. Testing conditions and methods are given as reference methods.

4.15 Users of biological indicators come from a wide variety of industries, private enterprises and health care facilities. Users are not generally required to perform resistance assays on biological indicators but can have differing requirements for their quality assurance systems, which may include audits by regulators or Notified Bodies (see 6.2.2). If a user wishes to carry out a population and/or resistance determination it is essential that they use the method specified by the manufacturer.

4.16 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

5.1.1 Biological indicators provide means to assess directly the microbial lethality of a sterilization process (see ISO 13485 and Reference [16]). 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.

Biological indicators, in their simplest form, consist of an inoculated carrier in primary packaging. The inoculated carrier can take a variety of forms, including paper strips, threads, metal coupons or other carriers suitable for inoculation. The primary packaging is chosen to permit the sterilizing agent to penetrate to the inoculated carrier while maintaining a sterile barrier after processing.

5.1.2 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 process 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 ISO 13485 and ISO 9001).

5.1.3 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* or *Bacillus atrophaeus*, 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 *Geobacillus stearothermophilus*, as noted in ISO 11138-3. Test organisms other than bacterial spores can be used if they have been shown to provide appropriate resistance to the sterilization process.

5.1.4 The basis of all formulae used to determine biological indicator resistance characteristics such as D values is that the inactivation reaction follows first-order log-linear kinetics, with the requirement that the value for the coefficient of determination, r^2, for the linearity of the survivor curve be not less than 0,8 (see Annexes E and F). The strain of the test organism, the production method, the suspension fluid, the carrier and packaging materials and the testing conditions all affect the resistance characteristics of the biological indicators (see ISO 11138-1).

5.1.5 The design and construction of a biological indicator can result in unique resistance characteristics and can 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 processes, the challenge to the process during validation should be correlated with the challenge to the process during routine monitoring.

5.1.6 Depending upon placement within the load and the specific sterilization process conditions at those discrete locations, biological indicators from the same batch can show different survival capabilities (see 7.2.3). Users of biological indicators should note that 10 indicators spread throughout the load are

not considered replicates due to the differences in lethality that may exist throughout the chamber and load (see Note to 11.3.1).

5.2 Test organism suspension for direct inoculation of products

5.2.1 Direct inoculation of test organisms on or in product can be necessary in process development and other studies when the use of a biological indicator is not feasible. Direct inoculation can be appropriate for assessing factors such as product sterilisability, identification of the most difficult to sterilize locations within the device, and localized microbiological effects, e.g. moist heat versus dry heat environments.

The rationale for the selection of the "most difficult-to-sterilize" site(s) on a product or within a sterilization load should be documented based on experimental data or derived from prior knowledge of 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 17665-1 and ISO 11135) for guidance in determining and selecting difficult-to-sterilize locations.

5.2.2 To assess the efficacy of sterilization at a particular site or location on the product, the desired species and population of test organisms can be inoculated at those sites. The use of suspensions of test organisms to prepare inoculated carriers or inoculated products requires caution. This is because the materials on to which test organisms are inoculated can alter the test organisms' resistance characteristics. The resistance can be higher or lower due to deposition as a monolayer or multilayer (clumping), coating effects, and/or bacteriostatic or bactericidal effects of the material.

5.2.3 The methods used to recover test organisms should be validated to ensure an adequate level of recovery from the product (see ISO 11737-1). Test organism recovery should be expressed in terms of percent recovery of the population of the original inoculum. A change in survival characteristics of test organisms due to inoculation can affect the observed percent recovery of the original inoculum. Inoculated products may be assayed with either survivor curve (enumeration/direct counting) or fraction-negative procedures (see Figure A.4). These assays require aseptic techniques.

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

5.3 Inoculated carriers

5.3.1 Inoculated carriers consist of a defined population of test organisms inoculated on or in a suitable carrier material (see ISO 11138-1:2017, Annex B). Caution should be exercised to ensure that the carrier material selected is able to withstand sterilization processing without adversely affecting its performance characteristics and to minimize the loss of the inoculated test organisms during transport and handling.

5.3.2 The resistance characteristics of a test organism in suspension can be considerably changed upon deposition on or in carriers. Several factors can influence the resistance characteristics, such as the surface on to 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.

5.3.3 If an inoculated carrier is removed from the biological indicator primary packaging or additional packaging is placed over the primary packaging for cycle development, cycle validation studies, or for process challenge devices (PCDs) used for routine process monitoring, then it is the responsibility of the user to provide a rationale for this application. It should be recognized that the resistance of the

microorganism on the inoculated carrier could differ from the labelled resistance of the packaged biological indicator.

5.3.4 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.

5.3.5 The carrier material should be evaluated by the biological indicator manufacturer or the user to determine that the sterilizing agent for which the biological indicator is intended neither retains nor releases inhibitory substances (e.g. sterilizing agent residuals) to such an extent that the recovery of low numbers is inhibited (see ISO 11138-1:2017, 5.2).

5.4 Self-contained biological indicators

Self-contained biological indicators consist of either a) or b).

a) An ampoule containing 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 through a sterile barrier or a tortuous path.

   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. The biological indicator manufacturers' recommendations should be followed for incubation of self-contained biological indicators.

   NOTE 1 Due to the low volume and the possibility of evaporation of the growth medium, prolonged post-exposure incubation might not be possible.

   Chemical residuals resulting from processes such as ethylene oxide or vapour hydrogen peroxide can inhibit growth of surviving organisms. The biological indicator manufacturer's recommendations should be followed for proper handling (including aeration) of biological indicators prior to incubation (see 8.2.3).

b) A hermetically sealed ampoule containing a suspension of test organisms in growth medium.

   These are referred to as sealed-ampoule biological indicators. After exposure to the process, the sealed ampoule is incubated intact, and no aseptic transfer is required.

   NOTE 2 This type of indicator is sensitive only to exposure time and temperature and is primarily used to monitor moist heat sterilization of aqueous fluids.

   Self-contained biological indicators are generally larger than biological indicators that consist only of an inoculated carrier in a primary packaging, and might not fit into locations within the product that represent the process challenge locations (PCLs). If a biological indicator cannot be placed into a load without deforming it or otherwise potentially compromising its primary packaging, then a different biological indicator should be used. Also, the user should be aware that the claimed resistance characteristics can be dependent on the air-removal method employed in the sterilization cycle.

6 Selection of supplier

6.1 General

6.1.1 The user of biological indicators should, whenever possible, make purchase decisions ensuring that the biological indicators chosen meet standard specifications. The user should consider the particular sterilization process as the basis for the choice of biological indicator.

