BS EN ISO 11138-7:2019

BSI Standards Publication

Sterilization of health care products – Biological indicators

Part 7: Guidance for the selection, use and interpretation of results (ISO 11138-7:2019)

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National foreword

This British Standard is the UK implementation of EN ISO 11138-7:2019. It supersedes BS EN ISO 14161:2009, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee CH/198, Sterilization and Associated Equipment and Processes.

A list of organizations represented on this committee can be obtained on request to its secretary.

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This European Standard was approved by CEN on 4 February 2019.

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European foreword

This document (EN ISO 11138-7:2019) has been prepared by Technical Committee ISO/TC 198 "Sterilization of health care products" in collaboration with Technical Committee CEN/TC 102 “Sterilizers and associated equipment for processing of medical devices” the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2019, and conflicting national standards shall be withdrawn at the latest by October 2019.

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Foreword

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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.

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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.

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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]

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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]

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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.]

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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.

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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.

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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

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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

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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.

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6.1.2 When the user has a process that requires performance characteristics that differ from the label claim for the biological indicator, it is the responsibility of the user to verify that the biological indicator has the performance characteristics needed.

6.1.3 The user of biological indicators should have a system in place to provide assurance that the biological indicators obtained consistently meet the specified characteristics. Such assurance can be provided by one or more of the following:

a) information from the manufacturer covering the performance characteristics of the batch of biological indicators prepared;

NOTE Requirements for information supplied by manufacturers of biological indicators are provided by the ISO 11138 series.

b) a statement from the manufacturer that the biological indicators meet the agreed specifications between the supplier and the user;

c) if needed, various degrees of testing of each batch of biological indicators received by the user, to verify that the performance characteristics meet the agreed specifications between the supplier and the user.

6.1.4 When the user has established a high level of confidence in the supplier (see 6.1.3), the testing performed by the user can be minimal. At a minimum, the user should have a mechanism to ensure that a shipment of biological indicators contains all agreed-upon documentation, such as appropriate label information, packet inserts, storage and handling instructions, etc. There should be a mechanism to ensure that the BI supplier continues to maintain the expected quality standards, such as a BI supplier or BI manufacturer's declaration of conformity to standards. If the user has not established the supplier relationship required to be ensured of consistent biological indicator performance, additional testing could be necessary until an appropriate assurance can be established that the biological indicators meet the BI manufacturer's label claim and/or user requirements. (For information on documentation, see Annex D.)

6.1.5 Testing by the user, if deemed necessary, can consist of population assays and defined resistance tests such as *D* value or survival-kill time on samples from each new batch of biological indicators received (see also 8.6 and Clause 11). Testing should be conducted under exact conditions specified by the manufacturer. Provided that the biological indicator manufacturer produces the based upon detailed standard specifications, i.e. the ISO 11138 series, and the user uses the biological indicator as intended by the biological indicator manufacturer, testing of the resistance characteristics by the user is considered unnecessary.

The manufacturer's label claims for resistance, such as *D* values, *z* values (if any) and survival-kill results are determined using a resistometer (see ISO 18472).

6.2 Documentation

6.2.1 General

6.2.1.1 The labelling requirements for biological indicators are given in ISO 11138-1:2017, 4.3.

6.2.1.2 The labelling includes the information presented on the primary and secondary packaging of the biological indicator as well as any included package inserts providing additional information beyond that which can be printed on the packaging. The user might need or want evidence of conformity to product standards and/or quality system standards to include in appropriate documentation files.

6.2.1.3 When a statement of performance or conformity to a standard is provided by the manufacturer as a certificate, the user should have confirmation of the competence of the manufacturer as indicated by the second sentence of 6.2.2.1.

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6.2.1.4 If an independent (third party) test laboratory is used to confirm the performance characteristics of biological indicators, the test laboratory should be accredited for the specific test methods used (see ISO/IEC 17025 and ISO/IEC 17011).

6.2.1.5 The status of the manufacturer of the biological indicator with regard to conformity to the appropriate quality standard, such as ISO 13485 or other quality assurance programmes should be verified. If conformity to the appropriate standards can be demonstrated, an audit might not be necessary.

6.2.2 Manufacturer audit

6.2.2.1 If necessary, the user should confirm that a qualified auditing body has performed an audit of the biological indicator manufacturer. Alternatively, the user could perform the audit.

NOTE The auditing standard ISO 19011 gives guidance on requirements for the process of auditing, the qualification criteria for quality system auditors, and the management of audit programmes.

6.2.2.2 A qualified auditor should perform the audit, as part of the purchaser's quality system. If an audit of the biological indicator manufacturer is performed, the following should be considered:

a) test organism:

   1) strain selection and maintenance;

   2) conditions for culturing of test organisms, including growth medium and components, incubation temperature and period;

   3) harvesting, purity and cleanliness of the test organism;

   4) viable test organism count and biochemical characteristics of the test organism;

b) biological indicator:

   1) qualifying of components for use in the preparation of biological indicators, such as carrier material and primary packaging, and consideration of any potential toxic effects of these materials on the test organisms;

   2) population of the test organisms during manufacture of the biological indicators;

   3) consistency (e.g. growth promotion, pH, stability) and fill volume of any growth medium supplied with the biological indicator;

   4) resistance of the biological indicator, including the type of test equipment and its calibration, the recovery medium employed and incubation conditions;

   5) storage stability and continued resistance of the biological indicator until its expiration date;

c) quality control:

   1) label claims for the biological indicator;

   2) storage stability and continued conformity of the biological indicator to its label claims.

6.2.2.3 The manufacturer should be able to provide adequate documentation of the quality systems pertaining to the manufacturing of biological indicators and provide documentation of conformity of products to declared specifications.

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7 Biological indicators in process development

7.1 General

7.1.1 For additional information on process development, refer to the sterilization standards for those processes (e.g. ISO 11135, ISO 14937, ISO 17665-1 and ISO 20857).

7.1.2 When a biological indicator is used for process development, the appropriateness of the indicator should be determined.

7.1.3 Sterilization processes vary widely with regard to their operational characteristics and the type of products that are sterilized. While each application is unique, it can be acceptable to group similar products in the same product family for the purposes of the development, validation and routine monitoring of a sterilization process. Careful consideration should be given to those aspects of product design or packaging which can impart an additional challenge to the sterilization process. Biological indicators can be used to determine those locations on the product which represent a rigorous challenge to the process, and likewise to establish the extent to which different types of product are related with regard to the challenge presented to the sterilization process. This could lead to the selection of a particular product configuration for further analysis.

7.1.4 Biological indicators should be used in combination with appropriate physical and/or chemical measurements of the process parameters in order to demonstrate the efficacy of a sterilization process.

7.1.5 General advice on the number of biological indicators per sterilizer volume cannot be given, as this depends on the reproducibility of the cycles as well as the potential for differences in the process parameters throughout the load during sterilization. However, other sterilization standards could provide guidance on the recommended number of biological indicators to be used, e.g. ISO 11135. The correct number of biological indicators to use can be determined from data collected from the use of biological indicators and/or bioburden studies, as well as from documentation on the distribution of the sterilizing agent throughout the load.

7.1.6 Implementation of the combined biological indicator/bioburden method requires consideration of many of the same factors noted in 5.4 and 7.2, with regard to the placement of the biological indicator in those locations within the product and load which present a rigorous challenge to the sterilization process. The method is only applicable when data are sufficient to be subjected to valid statistical analysis and there is a high level of confidence that the bioburden data are representative of "worst-case" conditions. There are many causes of variation, such as raw materials, process control, and seasonal variations. Consideration should be given to the presence and nature of the bioburden distribution in the product. As the distribution of bioburden within the product can vary significantly, it is important to determine how this distribution can affect the challenge presented by the product to the sterilization process and thereby affect the choice of biological indicator.

Determination of the product bioburden and its species and resistance distribution requires consideration of the factors discussed in the ISO 11737 series. The method selected should be validated against the requirements of those standards and the statistical confidence of the bioburden estimate be established.

7.2 Overkill approach

7.2.1 This method is often referred to as the "half-cycle method" or "overkill sterilization method" and is discussed in ISO 14937. This method is based on the following assumptions:

a) that the biological indicator (reference microorganism) provides a greater challenge than the bioburden;

b) that the full sterilization process achieves an SAL of at least 10^{-6} (see ISO 11135 and ISO 14937);

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c) that, at half cycle, the user can typically demonstrate at least a 6-log reduction (see Figure A.1).

NOTE The ISO 11138 series permits resistance characteristics other than the minimum required for monitoring purposes.

7.2.2 These overkill criteria can be addressed by placing biological indicators or inoculated carriers with, for example, a population of 10^6 test organisms meeting minimum resistance requirements at the PCL(s) within product throughout the load. These locations within the load should have been previously demonstrated to present a challenge to the sterilization process and correlate with the "most difficult-to-sterilize" locations, such that choosing these locations will secure the appropriate log reduction for the entire product load. At least a 6-log reduction in the population of organisms should be demonstrated within one-half the normal holding time or dose of the sterilization process to be validated when all of the biological indicators are statistically equivalent samples. The percentage of biological indicators showing growth can be correlated to the log reduction achieved [see Note to 11.3.1 c)]. If testing under half-cycle conditions demonstrates a reduction in the population of test organisms in excess of six logs, it is possible that no growth of the test organism could result, depending on the sample size. As illustrated in Figure A.1, there is a 1 % probability of positive growth with a spore-log-reduction (SLR) of eight logs at the upper end of the half-cycle window.

7.2.3 Placement of the biological indicator either within the product or within the load is likely to alter its apparent resistance characteristics in comparison to the resistance noted on the labelling of the biological indicator. This can require adjusting the half-cycle exposure period to compensate for the additional resistance caused by the placement of the biological indicator in the product or load. Similar adjustments could be needed when test organism suspensions are used to prepare inoculated product (see 5.2).

7.2.4 Suitable physical measurements should be used to establish the process challenge locations throughout the load that can aid in determining placement locations for the biological indicators. A sufficient number of probes should be prepared with biological indicators placed at the previously determined location within the product.

NOTE For moist heat sterilization, the *z* value of the biological indicator can be different from the *z* value of 10 °C, which is normally assumed for process lethality based on temperature measurements. This can lead to discrepancies between the integrated process lethality determined by use of biological indicators and the lethality determined by direct temperature measurements.

7.3 Combined biological indicator and bioburden method

7.3.1 The combined biological indicator and bioburden method requires the population and microorganism resistance of the product bioburden to be known. This method has the advantage of permitting a reduction in cycle exposure period and minimizing exposure of the product to the sterilizing agent and is discussed in ISO 14937.

7.3.2 The combined biological indicator/bioburden methodology requires selecting process conditions that deliver a process lethality sufficient to inactivate the bioburden to the labelled product sterility assurance level (SAL). The number of replicate cycles required to demonstrate the appropriate efficacy will depend on the confidence in accuracy and degree of bioburden inactivation repeatability determined in bioburden evaluations. Figure A.1 shows the general relationship between the inactivation of a biological indicator and the inactivation of product bioburden. Since the bioburden is typically less resistant to the sterilizing agent than is the biological indicator, the desired SAL can normally be achieved with less treatment than would be recommended when using the overkill method. The extent of the required treatment is dependent upon the relationship between the biological indicator and bioburden relative to population and resistance to the sterilizing agent.

7.3.3 For this procedure, the appropriate biological indicator with a test organism population of less than 10^6 CFU (colony-forming units) per carrier can be employed, as long as the desired SAL can be demonstrated. The variation in bioburden should be considered when determining the minimum

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biological indicator population that should be used to validate a sterilization process. The population of the biological indicator should not be less than 10^3 test organisms per carrier (see Figure A.1).

7.3.4 Procedures for the estimation of the product bioburden are discussed in ISO 11737-1. Due to bioburden variation, it could be necessary to characterize bioburden and bioburden resistance on a routine basis.

Studies of the resistance of the total bioburden population could be required in order to ensure that the challenge provided is less than that of the biological indicator. One possible method to determine this would be to run sub-lethal sterilization cycles to determine that the bioburden population does not survive as great an exposure as does the biological indicator.

7.3.5 The fundamental criteria of the combined biological indicator/bioburden methodology requires selecting processing conditions that reduce the population of the bioburden to 10^0. An additional safety factor consistent with the labelling of the product should be applied to the holding period (see Figure A.1). The number of replicate cycles for which this should be demonstrated during sterilization cycle development depends on the accuracy and confidence limits of the bioburden estimates.

7.3.6 Microorganism strains whose resistance is greater than the overall resistance of the product bioburden may be employed as biological indicators if their sterilization kinetics satisfy the criteria for a biological indicator (kill curve log-linear within a coefficient of determination of 0,8). However, these indicator organisms might be less resistant to the process than those specified in the ISO 11138 series (see Figure A.2).

7.3.7 If the resistance of the bioburden is greater than that of commercially available biological indicators, resistant strains isolated from the bioburden should be considered for inclusion in process development studies (see ISO 11135 and ISO 17665-1). Alternatively, the holding period or dose should be increased by a factor determined by the relative resistance of the bioburden and the biological indicator or a biological indicator with a higher population creating a challenge greater than or equal to the resistant strains may be used.

7.4 Bioburden method

7.4.1 Reference should be made to the ISO 11737 series for the appropriate microbiological methods to estimate the bioburden. Some bioburden microorganisms can have greater resistance than the biological indicators described in the ISO 11138 series. Bioburden microorganisms with higher resistance could be used as a model biological indicator (see 7.3). The method is discussed in ISO 14937.

7.4.2 Information on validation and routine monitoring and the applicability of absolute bioburden methods is given in the relevant International Standards for the particular sterilization process. Detailed specifications for an absolute bioburden method, without any reference to biological indicators, are given in the ISO 11737 series.

8 Biological indicators in sterilization validation

8.1 General

When biological indicators are used in the validation process, one should also consider the type(s) of biological indicators that can be used in the routine monitoring. Different biological indicators can provide varying degrees of challenge to the sterilization process (see Figure A.2). If different biological indicators are used for validation and routine monitoring, both should be included in the validation studies so that the resistance relationship of the two can be established and documented.

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Validation of a sterilization process requires documentation that the process is capable of consistently producing a product that meets its predetermined specifications (see ISO 11135, ISO 14937, ISO 17665-1 and ISO 20857).

8.2 Placement and handling of biological indicators

8.2.1 The number of biological indicators in products and/or product loads should be documented.

8.2.2 The user should document the placement of the chosen biological indicators in the sterilizer chamber, within the product load or a PCD (see Annex B). Other considerations to be addressed in biological indicator placement within the product load are loading patterns, load density and geometry, PCL(s), placement of physical and/or chemical sensors or probes, potential stratification of physical elements, the effect of packaging, etc.

8.2.3 The biological indicators should be removed from the sterilizer load as soon as possible after the process, without compromising the safety of personnel. They should be tested within a specified time interval that has been established for that product and process. The time intervals between preparation of the indicators and their use in the sterilization process, and between the end of the process and the culturing of the indicators should be justified in order to demonstrate that these intervals have no effect on the results of the biological testing of the sterilizer or sterilization process. These justified time intervals should not be exceeded. If the biological indicators are handled in a manner other than those stated by the manufacturer, the procedures should be validated to determine if they affect the performance of the biological indicator. Any established time intervals should be followed.

8.2.4 Applicable requirements for worker safety should be observed when removing the biological indicators from the sterilizer.

8.3 Sterilizer qualification

8.3.1 IQ and OQ are performed to obtain and document evidence that the sterilizer, its services and ancillary equipment have been provided and installed in accordance with its specifications, and that the sterilizer functions within predetermined limits when operated in accordance with instructions (see ISO 11135, ISO 17665-1 and ISO 20857).

Biological indicators can be used in OQ/PQ, for example, to establish evidence of uniformity of distribution of sterilizing agents.

8.3.2 Manufacturers of sterilizers might have performed type tests using biological indicators for specific types of load (see Annex B).

8.4 Performance qualification

Following completion of sterilizer qualification (see 8.3), performance qualification (PQ) testing is conducted to document the reproducibility and the efficacy of the sterilization process, including its ability to produce product meeting its predetermined quality specifications. Validation establishes process conformity with predetermined specifications; relevant International Standards such as ISO 11135, ISO 14937, ISO 17665-1 and ISO 20857 can apply. Different biological indicators can provide varying degrees of challenge to the sterilization process (see Figure A.2). Correlation between the biological indicators used for cycle development and validation and routine monitoring should be established and documented.

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8.5 Review and approval of validation

Upon successful completion of qualifications, a review of the validation documentation, including biological indicator performance, is necessary prior to beginning manufacture, in order to verify that the process conforms to requirements.

8.6 Requalification

8.6.1 When performing requalification, the same resistance characteristics, number of biological indicators, their placement in product load, etc., should be used. If a new biological indicator is being qualified for the process, it is important to establish and document a correlation between the new biological indicator and the previous biological indicator.

8.6.2 When using moist heat or ethylene oxide sterilization processes, ISO 17665-1 or ISO 11135 should be used.

8.6.3 A minimum frequency for evaluating the resistance characteristics of a biological indicator batch should be established. Considerations leading to different intervals for requalification of the biological indicator system could include seasonal changes, product and material changes as well as equipment changes, etc. If the resistance characteristics of the biological indicator change outside the predetermined limits, requalification should be performed. If the recovery medium is changed, the new growth medium should be correlated to the previous one used and the choice of new growth medium should be validated (see also 12.4).

NOTE For biological indicators purchased from a qualified supplier, resistance characteristics can be expected to remain within the expected tolerance (see ISO 11138-1) throughout the labelled shelf-life if stored as recommended on labelling.

9 Biological indicators in routine monitoring

9.1 General

9.1.1 Biological indicators provide a method for demonstrating microbial lethality in a sterilization process; however, fractional (e.g. half cycles), might need to be conducted during validation in order to quantify the lethality. For well developed, validated processes parametric release may be possible (e.g. ethylene oxide, see ISO 11135; or dry heat, see ISO 20857), however local regulatory requirements can also require the use of biological indicators as part of process monitoring and batch release. For processes where the process variables cannot be reliably measured and therefore parametric release of batches is not possible, biological indicators provide the best available alternative for demonstrating microbial lethality in the sterilization process. See ISO 11135 for ethylene oxide and ISO 20857 for dry heat.

9.1.2 The type of biological indicator and its placement in the product or product load should be consistent with product load locations that have been determined during the sterilization process development or the validation. If the microbiological challenge system used for routine monitoring of the sterilization process differs from that used in the validation of the process, the relationship between the system for validation and for routine monitoring should be documented.

9.1.3 The specified time intervals between preparation of the indicators and sterilization, and between the end of the process and the culturing of the indicators should be justified in order to demonstrate that these intervals have no effect on the performance of the biological indicator (see 8.2.3).

9.1.4 Biological indicators for routine monitoring that are used in the combined biological indicator/bioburden method (7.3) will not comply with all parts of the ISO 11138 series if the population and/or resistance is below the minimum requirements of the respective part (see ISO 11138-1:2017, 6.1.3).

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