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Sterilization of healthcare products — Microbiological methods— Guidance on conducting bioburden determinations and tests of sterility for biologics and tissue-based products

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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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).

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

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

The sources and types of some microorganisms, as well as the test methods used to evaluate biologics and tissue-based products, can be unique relative to other health care products, such as plastic and metal medical devices. This document provides guidance to address issues that are applicable to the microbiological testing of biologics and tissue-based products, where this testing constitutes bioburden testing or a test of sterility performed in relation to product sterilization. Except where otherwise indicated in this document, the requirements in ISO 11737-1:2018 and ISO 11737-2:2019 apply.

Sterilization of healthcare products — Microbiological methods— Guidance on conducting bioburden determinations and tests of sterility for biologics and tissue-based products

1 Scope

1.1 Inclusions

1.1.1 This document provides guidance for bioburden testing and tests of sterility for biologics and tissue-based products, where this testing is in relation to product sterilization.

NOTE This document is intended to be used in conjunction with ISO 11737-1 and ISO 11737-2.

1.1.2 Guidance in this document can be applicable to biologics and tissue-based products that are not sterile but are microbiologically controlled.

1.2 Exclusions

1.2.1 This document does not include guidance for validation requirements for testing, eliminating and/or inactivating viruses and prions or sterilization of tissue-based products.

NOTE Guidance on inactivating viruses and prions can be found in ISO 22442-3.

1.2.2 This document does not include guidance for containment or biosafety issues for biologics and tissue-based products.

1.2.3 This document does not include guidance for testing biologics and tissue-based products for specific infectious agents as listed in relevant national or international guidance (e.g. viruses/protozoa/parasites, intracellular microorganisms or mycoplasma screening).

1.2.4 This document does not include guidance for the acceptance criteria for biologics and tissue-based products during procurement or tissue to be processed and/or released for use.

1.2.5 This document does not include guidance for the testing associated with procurement and screening of biologics and tissue-based products.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 11737-1:2018, *Sterilization of health care products — Microbiological methods — Part 1: Determination of a population of microorganisms on products*

ISO 11737-2:2019, *Sterilization of health care products — Microbiological methods — Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 11737-1:2018, ISO 11737-2:2019 and the following 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
biologics
product that is synthesized from living organisms, or their products, and used as a diagnostic, preventive or therapeutic agent

3.2
companion tissue
tissue from the same donor(s) that is not intended to be used for transplantation

Note 1 to entry: For the purposes of this document, companion tissue is expected to be processed in the same manner as tissue that is used for transplantation. Companion tissue is representative of tissue intended for transplantation but is only used for evaluation and/or testing purposes.

3.3
donor identification
unique identifier assigned to all *biologics* (3.1)/tissue and *companion tissue* (3.2) that originates from the same donor

3.4
method suitability test
bacteriostasis/fungistasis (B/F) test
technical operation performed to detect the presence of substances that inhibit microbial multiplication

Note 1 to entry: This testing is also referred to as “method verification.”

[SOURCE: ISO 11139:2018, 3.20, modified — Note 1 to entry has been added]

3.5
processing
<*biologics* (3.1) and *tissue-based product* (3.7)> any activity performed in the preparation, manipulation, preservation for storage and packaging of a biological or tissue-based product

3.6
product family
group or subgroup of product characterized by similar attributes determined to be equivalent for evaluation and processing purposes

[SOURCE: ISO 11139:2018, 3.218]

3.7
tissue-based product
product consisting of organization of cells, cells and extra-cellular constituents, or extra- cellular constituents

Note 1 to entry: This can include tissues from tissue banks or material of animal origin.

4 Definition and maintenance of product families

4.1 Product families can be organized for a multitude of purposes. The purpose for which the family is being organized will dictate the criteria for that family. Care should be taken in organizing families to ensure that appropriate and relevant criteria are in place.

4.2 In cases where a product family representative is tested instead of each type of health care product, an appropriate rationale should be written to ensure that results for the product family representative are representative of the whole family. The applicable standards, i.e. ISO 11137-2, ISO 11135, etc. provide guidance specific to establishment of a product family. Some of that guidance can be applicable to establishment of a product family for other testing purposes. For other sterilization modalities, refer to the applicable standard for additional guidance. Additionally, the following items can be considered:

a) the purpose for which the family is being established (e.g. for sterilization purposes, or for bioburden testing purposes);

b) processing procedures that are applied to the biologic/tissue;

c) storage conditions that are applied to the various tissue types or sizes;

d) tissue collection methods.

4.3 For biologics or tissue-based product that is sterilized, the final determination of the product family representative is based on recommendations per the relevant sterilization process standard (e.g. ISO 11137-2 for radiation, ISO 11135 for EO, etc.).

4.4 In considering the relative size of biologic/tissue products, a larger size might not necessarily correspond to a higher product bioburden if processing of the product is the same. If equivalence can be demonstrated within a family of products, a rationale should be provided. If equivalence cannot be demonstrated, either a larger size should be tested, or an SIP employed (see 5.3).

5 Selection and testing of product for bioburden and tests of sterility

5.1 General

The results of raw material pre-disinfection cultures of a biologic/tissue are typically utilized to determine the suitability of that biologic/tissue for further processing (e.g. collection, cleaning processes, disinfection processes, washing, soaking, etc.), or its supplier, or for the defined monitoring program and can also be used for trending/monitoring purposes.

In performing bioburden applicable to a sterilization process, such as dose- establishment or for routine bioburden monitoring, the relevant bioburden is that present on the product immediately prior to sterilization. If product is subjected to cleaning, rinsing and disinfection steps, the bioburden is usually low and comparable to the bioburden (numbers and types) of other healthcare products. This is due to:

a) the reduction of the bioburden through the cleaning processes, disinfection processes, washing, soaking, ultrasonication and/or centrifugation or other processes;

NOTE 1 If these processes are not appropriately controlled and monitored, they could introduce additional bioburden to the product.

b) the contribution of microorganisms from the environment, contact surfaces and handling during processing or manufacturing steps.

NOTE 2 Refer to ISO 11737-1:2018 for guidance on bioburden characterization. ISO 14160 provides guidance on characterization and evaluation for pre-sterilization bioburden for liquid chemical sterilization used on animal tissues.

NOTE 3 As described in ISO 11737-1:2018, it is not necessary in all cases to fully characterize product bioburden. The extent of characterization performed is expected to be based on the purpose for which the testing is being carried out.

NOTE 4 When evaluating bioburden to support a microbicidal process validation (i.e. disinfection, sterilization), the resistance of the species to the relevant microbicidal process is more relevant than its pathogenicity. A pathogenic microorganism could have a low resistance to the disinfectant/sterilant, while a non-pathogenic microorganism could be highly resistant.

5.2 Nature of product

Processing of biologics/tissues should be controlled to maintain low or consistent levels of bioburden. The unique source of these biologics/tissues, compared to other health care products made of synthetic materials, creates the potential for a microflora different than that found on plastics or metal devices. However, due to the processing, antimicrobial treatment and chemicals often applied to biologics/tissues, the bioburden after controlled and proven processing tends to be relatively consistent and low. When a biologic/tissue is pooled during processing it typically results in a relatively consistent bioburden within the pool. See also ISO 11737-1:2018.

5.3 Sample Item Portion (SIP)

In many cases a limited amount of biologic/tissue product is available for testing, therefore, it is reasonable to utilize product that is not clinically suitable or is non- conforming product, but which is handled in the same manner and undergoes the entire manufacturing process. For example, to maintain an SIP of 1,0, a biologic/tissue can be used that is not considered clinically suitable for use but is microbiologically representative of the biologic/tissue and process.

In general, if an SIP < 1,0 is used, a rationale applying the principles of ISO 11737-1:2018 should be used.

If an SIP <1,0 is used, qualification of the SIP could be required. Use of a product that is smaller than the largest product produced can also be addressed through establishment of product families and substantiation of an equivalent product approach. For radiation sterilization, guidance is provided in ISO 11137-2.

NOTE If it has been demonstrated that product within a product family is considered equivalent because of the disinfection process and rationale, and if smaller size pieces or quantities of a biologic/tissue in that family are used for dose establishment, all sizes are considered an SIP=1.

5.4 Sampling conditions

5.4.1 General

The conditions the test sample has been exposed to should represent typical shipping, storage and processing conditions, including any refrigeration, frozen conditions or lyophilization. Shipping, storage and sampling conditions should be designed to minimize conditions which are conducive to microbial growth on the biologic/tissue.

5.4.2 Considerations for human tissue donor batches in sterilization

For medical devices, the term batch means a quantity of identical devices manufactured under similar conditions. Some sterilization validations (e.g. radiation) require that a specific number of products be tested from each batch. Human tissue-based products and some biologics are typically processed in batches based on a single donor ("processing batch"). Processing batch sizes often vary in the number of products per batch. Products in a processing batch might not be multiples of the same product type. For example, a human tissue batch is often a mixture of tissues from multiple sites within the donor that are further processed together, and the number of products per batch can depend on the donor and might be small. Refer to Kowalski.[11] Due to this distinction in definition and constituents of a batch for biologics/tissues, some allowance, with documented rationale, should be made regarding sample sizes and batches when following standards initially written for other types of health care products.

5.4.3 Use of multiple batches

Biologics/tissues from different processing batches and/or donor identifications may be combined for testing purposes in order to achieve the required number of samples for the particular bioburden or sterilization method, based on the rationale for the sampling. For example, it might be necessary to use five test samples from each of six batches rather than 10 from each of the three batches in order to obtain a sample size of 30. It is the number of test samples that is critical, not the number of batches from which they originate as long as a minimum of 3 batches are represented. A batch may be given a unique identifier apart from the processing batch and/or donor identification, as long as traceability to the original donor samples comprising the batch is maintained.

NOTE For qualification of biologics/tissues in radiation dose setting, the batch sampling concept from ISO 11137-2 can be modified.

5.4.4 Considerations for packaging

Typically, a bioburden determination or a test of sterility is performed on product after its removal from its packaging system. It is common to omit the packaging system from these determinations. Depending on the intent for sterility, internal packaging components, such as a tray or product insert, might need to be tested based upon factors such as whether:

a) the component is intended to be sterile;

b) the package is an integral part of the product; or

c) specific evaluation is required.

When packaging is tested, it might be preferred that it be tested separately from product, depending on the circumstances.

5.5 Microbiological testing

5.5.1 Bioburden test considerations for biologics/tissues

5.5.1.1 General

Establishing and maintaining a sterilization dose or bioburden-based sterilization process is dependent upon an estimated determination of bioburden. The requirements, guidelines, and processes that are in place for biologic/tissue products usually result in finished products that are relatively consistent and low in bioburden if requirements and guidelines for microbial control are appropriately followed. However, there are some characteristics that are unique to biologic/tissue types compared to other health care products. For sterilization validations that utilize an overkill approach (e.g. typically using a biological indicator and 12-log reduction), the sterilization process is not directly dependent on the product bioburden count. In some cases, the bioburden count is used for trending and demonstration of process control.

Characteristics of biologics/tissues that can affect the determination of bioburden include:

a) Each human tissue donor or animal tissue batch is a separate case, therefore bioburden diversity can vary. The bioburden might contain microbial species that are not commonly associated with typical medical device materials, such as synthetic materials or metal alloys. Based on an understanding of the biologic/tissue type and processes involved, an assessment should be performed of the predominant types of microorganisms present at a stage in the process that is appropriate to the purpose for the assessment. For example, in the bioburden assessment for a bioburden-based sterilization process, the appropriate stage is usually immediately prior to sterilization rather than upon receipt (which is prior to cleaning and disinfection). The information obtained in this assessment should be used to develop appropriate test methods. For example, with some tissue types it could be appropriate to test for anaerobic microorganisms, increase incubation time or use specialized media, where this practice might not be common for other health care products.