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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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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).

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).

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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.*

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.

b) Some tissues can include complex surface characteristics. The location and adherence of microorganisms on and/or in certain complex tissues could make them difficult to remove. Thus, the bioburden recovery efficiency should be evaluated and used as appropriate for bioburden determination.

ISO 11737-1:2018 is primarily written in the context of conventional medical devices, but includes methods that are also applicable to most biologics/tissues, including shaking, ultrasonication, stomaching, blending, culturing, membrane filtration with rinsing for neutralization, and recovery evaluation. The aspects in 5.5.1.2 to 5.5.1.10 should be considered when performing bioburden tests on biologics/tissues.

If bioburden samples are pooled for testing, reference ISO 11737-1:2018 for additional information.

5.5.1.2 Extraction fluid

Generally, the extraction fluids used with conventional medical devices are also appropriate to use with biologic/tissue products. Residual processing chemicals and antibiotics can be difficult to completely remove from some biologics/tissues and could leach into extraction fluids during testing. Thus, if inhibitory substances are present, it might be necessary to use a neutralizer in the extraction fluid, rinsing, or a neutralizing step to the testing process to prevent inhibition of microbial growth. Most extraction fluids can be filtered, which can help to eliminate inhibitory factors from the tests.

Guidance on general bioburden testing eluents and diluents is provided in ISO 11737-1:2018. Guidance on method suitability to demonstrate the absence/presence of inhibitory substances can be found in 5.5.3.1. Additional guidance can be found in Annex B of ISO 11737-1:2018, which describes methods to screen for release of substances affecting bioburden determinations.

5.5.1.3 Extraction methods

Bioburden on biologics/tissues tends to be influenced by the environment the biologic/tissue exterior is exposed to, and thus the bioburden is primarily found externally. Based on an understanding of the biologic/tissue processing, if the bioburden is expected to reside externally, traditional extraction methods are appropriate. These methods can include mechanical shaking, manual shaking, ultrasonication or stomaching. If it is expected that bioburden might also be located inside the biologic/tissue, other extraction methods could be indicated. These methods can include blending, stomaching, grinding/milling, maceration or enzymatic digestion. The effect of such treatment on product bioburden should be evaluated.

5.5.1.4 Membrane filter selection

Membrane filtration may be used to facilitate removal of inhibitory substances in the extract or rinse solution(s). If inhibition is observed, cellulose-based filters commonly used for bioburden testing are prone to trap antibiotics and/or chemicals inside the filter, even after multiple rinses. Therefore, certain filter types that are less apt to retain residual antibiotics and/or chemicals during filtration should be used for biologics/tissues. Polycarbonate, nylon and hydrophobic-edge filters could be better suited for filtration of fluids that contain inhibitory substances. Wetting the filter with sterile fluid prior to filtration and adequately rinsing the filter with sterile fluid after filtration can also be helpful in reducing inhibitory substances on membrane filters. Even with the use of these filters and methods, additional neutralization steps might still be necessary. Guidance on method suitability to demonstrate the absence/presence of inhibitory substances can be found in 5.5.3.1. Additional guidance can be found in Annex B of ISO 11737-1:2018, which describes methods to screen for release of substances affecting bioburden determinations.

There can be cases where a membrane filter with a nominal pore size less than 0,45 µm is required, for example, to minimize loss of contaminants that can pass through a 0,45 µm filter.

5.5.1.5 Filtration considerations

Some biologic/tissue extracts might not filter well due to particulates, lipids and/or fats that sometimes elute from the biologic/tissue during extraction. In these situations, smaller volumes can be filtered through multiple filters, or direct culture methods can be used to test the extract solution, such as pour plates, spread plates and the most probable number (MPN) test. A large pore pre-filter can be used to reduce the quantity of materials in solution that could obstruct filters. However, use of a pre-filter can also trap microorganisms. Thus, an evaluation should be performed to account for microorganisms that could be trapped by the pre-filter.

5.5.1.6 Most Probable Number (MPN)

MPN testing is an option provided in ISO 11737-1:2018 and can be a good option for some biologic/tissue products (see B.3.3 of ISO 11737-1:2018). This is because most biologics/tissues from a donor or batch are processed together in the same disinfecting/rinsing solutions, assisting in creating a more evenly distributed bioburden. Also the MPN method can be desirable because bioburden can be difficult to extract from some biologics/tissues, or because a low limit of detection for the bioburden test is required.

When performing an MPN bioburden test on extraction fluid or product, if all test samples are positive for growth a smaller portion should be tested.

For example, an MPN bioburden test is performed on whole pieces of soft tissue which cannot be filtered due to high amounts of lipids being present on the surface (SIP of 1,0). In this test all pieces of tissue are positive for growth. These results are unsuitable to enumerate bioburden using MPN. An additional MPN test is performed on additional pieces of soft tissue, but in this case each piece is cut in half and only one half of each piece is tested (SIP of 0,5). In this test, a fraction of the pieces are positive for growth. This data are suitable to enumerate bioburden using MPN because of the fraction negative result.

5.5.1.6.1 When an SIP less than 1,0 is tested for radiation verification experiments, ISO 11137-2 requires that at least 85 % of SIPs tested are positive for growth. However, this requirement does not apply when SIPs are used in an MPN bioburden test, and when fraction negative results are obtained (i.e. not all samples are either positive or negative for growth).

5.5.1.7 Media for incubation

Standard media formulations can be used with some biologics/tissues and will result in reliable data with no growth inhibition. However, for biologics/tissues that carry inhibitory substances, the media formulations used for bioburden testing can require neutralizers or use of standard media with neutralizing rinses to overcome the inhibition.

Another consideration is the type of media to use, based on the species of microorganisms expected to be present on or in the biologics/tissues after processing and packaging. Many tissue types originate from an anaerobic environment, thus testing for anaerobic microorganisms should be assessed when establishing the test method. This assessment can include initial screening testing or a review of the types of microorganisms commonly obtained in recovery cultures or those expected to be present due to the type of biologic/tissue or processes.

If the bioburden procedure involves the use of enrichment media or other non-standard media, consideration should be given to whether similar approaches should be used in the test of sterility. The microorganisms referenced in pharmacopeias should be used to demonstrate that the non-standard media will also detect microorganisms in addition to the specific ones of concern. Demonstration of the appropriateness of media and neutralizing/rinsing steps should be accomplished by performing a method suitability test (see 5.5.3.1).

5.5.1.8 Culture conditions

Testing for only aerobic or facultative bacteria and fungi might not always be sufficient when testing biologics/tissues. An evaluation of the opportunity for obligate anaerobes to be present on such

material is recommended. If it is not known whether obligate anaerobes might be present, they can be tested for initially and, based on data that they are not present, it can be determined that routine testing for anaerobes is not necessary. Microorganisms that grow in an anaerobic bacterial test can be facultative anaerobes, which means that they can also grow in the aerobic bacterial test.

The duration of incubation and the temperature(s) used for testing of other health care products are usually applicable to tissue-based products. Based on an understanding of the microorganisms that are present or expected to be present on the biologic/tissue, it can be necessary to consider alternative incubation times and temperatures. Alternative incubation conditions should be validated or justified to demonstrate the ability to recover types of microorganisms of concern.

ISO 14160 provides guidance for selection of appropriate incubation conditions for enumeration and the isolation of microorganisms that can be associated with materials of animal origin.

5.5.1.9 Enumeration and characterization

Often during bioburden testing, debris from the biologics/tissues or fats and oils are removed by the extraction fluid. If residual debris is present, when tested via membrane filtration or spread/pour plates, this material can appear to be a bacterial or fungal colony. Thus, care should be taken during enumeration after incubation to ensure that what is being counted are truly microbial colonies.

As described in ISO 11737-1:2018, the degree of characterization necessary is dependent on the nature of the product, diversity and/or level of the detected population, and the use of the data (e.g. the sterilization microbiological qualification approach).

5.5.1.10 Interpretation of data

Confirmation testing such as sub-culturing might be needed when the presence of microbial growth is unclear due to substances appearing as microbial colonies.

If it is determined that there is a need to understand what portion of the product bioburden consists of spores, care should be taken when determining final results because sporeformer growth can result from aerobic spores, potentially generating falsely elevated results, because in most cases aerobic spores will grow in the aerobic bacterial test.

If it is determined that there is a need to perform tests for anaerobes, care should be taken when determining final results because growth on the anaerobic plates can be facultative anaerobic growth, potentially generating falsely elevated results. The anaerobic count should only be added to the aerobic count if it is known that they are obligate anaerobes.

5.5.2 Test of sterility considerations for biologics/tissues

5.5.2.1 General

This section provides guidance for tests of sterility on biologic/tissue products that have been exposed to a fraction of the specified sterilization process when the sterilization process standard calls for a test of sterility. These tests are intended to be performed when validating a sterilization process. For specific sterilization modalities, refer to the applicable standard for additional guidance.

NOTE See ISO 11737-2:2019 for more information related to tests of sterility, as well as information on tests for sterility not being recommended for routine lot/batch release for terminally sterilized products.

5.5.2.2 Performance of test of sterility

Products in a test of sterility should be tested in a manner that exposes or allows outgrowth into media from all product surfaces where microorganisms can reside by ensuring that the growth medium comes in contact with all surfaces. For guidance on testing by elution, see 5.5.2.4. If it is necessary that a test of sterility for a biologic/tissue will require cutting, grinding, or maceration, the appropriateness of such manipulation should be addressed before it is implemented. For example, it could be appropriate to cut

down the side of a vein if it appears that the growth medium does not have access to all locations in the interior of the vein.

5.5.2.3 Culture conditions

A single growth medium may be used on the basis that it will be optimal for culturing aerobic and facultative microorganisms that can survive exposure to the sterilization process. Soybean Casein Digest Medium (SCDM) as a single growth medium at 30 ± 2 °C for 14 days to culture aerobic and facultative microorganisms is commonly employed, as this temperature range is sufficient to successfully culture most microorganisms expected to be found on health care products. Currently no data are available to indicate that incubation parameters other than those indicated are necessary for general recovery. For more information see Reference [7]. Alternative conditions can be appropriate, based on the types of microorganisms present on the tissue (e.g. 20-25 °C and/or 30-35 °C).

When a growth medium other than SCDM is used in the test of sterility, the need for different incubation conditions should be considered. Although anaerobic growth media (e.g. thioglycollate broth) is not commonly used in tests of sterility for qualification of sterilization, it might be appropriate for testing some biologics/tissues due to the possible presence of obligate anaerobes.

5.5.2.3.1 A choice of culture conditions shall be made if:

a) the particular international standard for validation and routine control of the sterilization process does not stipulate the growth medium to be used;

b) the use of a single set of culture conditions is not appropriate because of the types of microorganisms expected to be present on the product (e.g. the presence of anaerobes or other fastidious microorganisms).

5.5.2.3.2 Factors to be considered in choosing culture conditions in these instances should include the following:

— the nature of the product;

— the nature of manufacturing process(es);

— the sources of potential microbial contamination;

— the types of microorganisms likely to be encountered or of concern.

5.5.2.4 Membrane filtration of eluate

Direct immersion of the biologic/tissue is the preferred method for the test of sterility; however, there can be cases where this is not possible. An alternative method is to test an eluate (rinsate) of the product sample by membrane filtration or direct immersion of the eluate/rinsate. ISO 11737-2:2019 describes issues that have to be addressed to consider use of elution for a test of sterility.

5.5.2.5 Examination of growth medium

After incubation, the growth medium is examined for turbidity as evidence of microbial growth. Biologic/tissue products or leachables from these products could generate turbidity not resulting from microbial growth. Turbidity that is questionable as being microbial growth should be verified for growth by either:

a) transferring portions (each not less than 1 ml) of the medium to fresh containers of the same medium, and incubating the sub-cultured containers for at least 4 days (as described in Reference [20]); or

b) sub-culturing the medium using commonly accepted microbiological practices (e.g. streaking for isolation).

Any microorganisms that grow in a test of sterility should be characterized or identified, where possible, to the genus and species level.

5.5.3 Verification of microbiological methods

5.5.3.1 Method suitability

Processed biologics/tissues could contain residual antibiotics or processing chemicals that can cause inhibition during a microbiological test. These inhibitory factors should be eliminated, neutralized or reduced as much as possible during testing to assure that false negative results are not obtained.

Guidance on method suitability tests can be found in ISO 11737-1:2018, ISO 11737-2:2019, ISO 14160 and various pharmacopeias.

For bioburden testing, the method suitability test from ISO 11737-1:2018 is often used as a guideline. For performance of a test of sterility or a bioburden test by MPN, the method to be used for testing should be subject to verification to demonstrate that inhibition has been addressed. If method suitability results indicate the presence of inhibition, the media volumes for testing can be increased in an effort to dilute the inhibitory factors, and/or neutralizers can be added to the media. Method suitability is needed to demonstrate effectiveness of any neutralization steps.

Also, the test method can be altered to reduce inhibitory effects, e.g. the sample may be divided into smaller portions and tested in multiple containers, or membrane filtration can be employed. For membrane filtration, single or multiple rinses of approved fluid (see Reference [20]) can be used to reduce the inhibitory factors. In addition, various neutralizing agents can be added to the media or rinses when filtering.

5.5.3.2 Bioburden test verification (recovery efficiency test)

Recovery efficiency could be a required procedure when performing bioburden testing, depending on the use of the data. See ISO 11737-1:2018 for two common recovery efficiency methods. Often, processed tissue will have low numbers of microorganisms, which can make a repetitive recovery method less suitable than an inoculated recovery method. An MPN test of product does not require a recovery efficiency test since an extraction of the product is not taking place. A method suitability test is performed to validate an MPN test.

5.5.3.3 Method suitability for test of sterility (bacteriostasis/fungistasis test)

Growth of inoculated microorganisms provides evidence to demonstrate that there are no inhibitory factors in the sample and medium combination that could prevent multiplication of microorganisms. This is called a method suitability test or sterility test validation which has historically been referred to as a bacteriostasis/fungistasis (B/F) test. See also ISO 11737-2:2019.

The method suitability of the test of sterility or MPN is accomplished by inoculating the test medium containing the product sample with less than 100 CFU of the challenge microorganism(s). The test medium and incubation conditions should be the same as that which will be used in the test of sterility or MPN as applicable. The test of sterility method suitability should be performed on samples that have been subjected to the sterilization process that is being evaluated. This test need not be repeated on a routine basis unless there is a change to the product or test method.

For pharmaceuticals, the United States Pharmecopeia <71> calls for specific microorganisms to be used in the procedure; however, for biologics/tissues, these or alternative/additional microorganisms may be used. European Pharmacopeia Chapter 2.6.27 and British Pharmecopeia Appendix XVI E include information on microbiological control of cellular products. These chapters list additional microorganisms that should be considered for inclusion during test method verification.

For animal tissue, ISO 14160 provides guidance for neutralization validation. Test microorganisms referenced in various Pharmacopoeias may be used.

