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Sterilization of health care products — Microbiological methods —

Part 1: Determination of a population of microorganisms on products

Stérilisation des produits de santé — Méthodes microbiologiques —

Partie 1: Détermination d'une population de microorganismes sur des produits

Reference number
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Foreword

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

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

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

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

For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

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

This third edition cancels and replaces the second edition (ISO 11737-1:2006), which has been technically revised. It also incorporates the Technical Corrigendum ISO 11737-1:2006/Cor.1:2007.

The main changes compared to the previous edition are as follows:

— the term “bioburden spikes” has been introduced as a normal and consistent part of the bioburden, and examples of data have been provided;

— clarification has been added that package testing is not typically done except when it is an integral part of the product;

— more information has been provided on the most probable number (MPN) technique and its applications;

— details have been provided on ways to improve limit of detection (LOD) and correct use of the data;

— some discussion has been deleted of statistical methods for the evaluation of bioburden data where information was not typical or not required;

— a table has been added with criteria for selection of a bioburden recovery efficiency approach, the use of the correction factor (CF) has been explained, and the bioburden recovery efficiency value of < 50 % mentioned for technique modifications has been eliminated;

— more information has been provided on the application and performance of a bioburden method suitability test;

— a section has been added to detail rules for direct plate counts, estimated counts and counts beyond the ideal range;

— a table has been added to clarify where typical responsibilities reside for the manufacturer or the laboratory;

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— the focus on a risk-based approach has been increased, including the purpose for which bioburden data will be used.

A list of all parts in the ISO 11737 series can be found on the ISO website.

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Introduction

A sterile health care product is one that is free of viable microorganisms. International Standards that specify requirements for the validation and routine control of sterilization processes require, when it is necessary to supply a sterile health care product, that adventitious microbiological contamination of a health care product prior to sterilization be minimized. Such products are non-sterile. The purpose of sterilization is to inactivate the microbiological contaminants and thereby transform the non-sterile products into sterile ones.

The kinetics of inactivation of a pure culture of microorganisms by physical and/or chemical agents used to sterilize health care products can generally best be described by an exponential relationship between the numbers of microorganisms surviving and the extent of treatment with the sterilizing agent. Inevitably, this means there is always a finite probability that a microorganism can survive regardless of the extent of treatment applied. For a given treatment, the probability of survival is determined by the number and resistance of microorganisms and by the environment in which the microorganisms exist during treatment. It follows that the sterility of any one product in a population subjected to sterilization processing cannot be guaranteed and the sterility of a processed population is defined in terms of the probability of there being a viable microorganism present on a product item.

Generic requirements of the quality management system for design and development, production, installation and servicing are given in ISO 9001 and particular requirements for quality management systems for medical device production are given in ISO 13485. The standards for quality management systems recognize that, for certain processes used in manufacturing, the effectiveness of the process cannot be fully verified by subsequent inspection and testing of the product. Sterilization is an example of such a process. For this reason, sterilization processes are validated for use, the performance of the sterilization process is monitored routinely and the equipment is maintained.

International Standards specifying procedures for the validation and routine control of the processes used for the sterilization of health care products have been prepared (see, for example, ISO 14937, ISO 11135, the ISO 11137 series, the ISO 17665 series and ISO 14160). However, it is important to be aware that exposure to a properly validated and accurately controlled sterilization process is not the only factor associated with the provision of assurance that the product is sterile and, in this respect, suitable for its intended use. Furthermore, for the effective validation and routine control of a sterilization process, it is important to be aware of the microbiological challenge that is presented in the process, in terms of number, characteristics and properties of microorganisms.

The term “bioburden” is used to describe the population of viable microorganisms present on or in a product and/or a sterile barrier system. A knowledge of bioburden can be used in a number of situations as part of the following:

— validation and requalification of sterilization processes;

— routine monitoring for control of manufacturing processes;

— monitoring of raw materials, components or packaging;

— assessment of the efficiency of cleaning processes;

— an overall environmental monitoring programme.

Bioburden is the sum of the microbial contributions from a number of sources, including raw materials, manufacturing of components, assembly processes, manufacturing environment, assembly/manufacturing aids (e.g. compressed gases, water, lubricants), cleaning processes and packaging of finished products. To control bioburden, attention should be given to the microbiological status of these sources.

It is not possible to enumerate bioburden exactly and, in practice, a determination of bioburden is made using a defined method. Definition of a single method for use in determining bioburden in all situations is not practicable because of the wide variety of designs and materials of construction of health care products. Nor is it possible to define a single technique to be used in all situations for the removal of

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microorganisms in preparation for enumeration. Furthermore, the selection of culture conditions for enumeration of microorganisms will be influenced by the types of microorganism likely to be present on or in health care products.

This document specifies the requirements to be met for the determination of bioburden. In addition, it gives guidance in the annexes to provide explanations and methods that are deemed suitable to conform with the requirements. Methods other than those given in the guidance may be used, if they are effective in achieving conformity with the requirements of this document.

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Sterilization of health care products — Microbiological methods —

Part 1: Determination of a population of microorganisms on products

1 Scope

This document specifies requirements and provides guidance on the enumeration and microbial characterization of the population of viable microorganisms on or in a health care product, component, raw material or package.

NOTE 1 The nature and extent of microbial characterization is dependent on the intended use of bioburden data.

NOTE 2 See Annex A for guidance on Clauses 1 to 9.

This document does not apply to the enumeration or identification of viral, prion or protozoan contaminants. This includes the removal and detection of the causative agents of spongiform encephalopathies, such as scrapie, bovine spongiform encephalopathy and Creutzfeldt-Jakob disease.

NOTE 3 Guidance on inactivating viruses and prions can be found in ISO 22442-3, ICH Q5A(R1) and ISO 13022.

This document does not apply to the microbiological monitoring of the environment in which health care products are manufactured.

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 10012, *Measurement management systems — Requirements for measurement processes and measuring equipment*

ISO 13485, *Medical devices — Quality management systems — Requirements for regulatory purposes*

ISO 15189, *Medical laboratories — Requirements for quality and competence*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

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:

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

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

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3.1
batch
defined quantity of a *product* (3.16) intended or purported to be uniform in character and quality, which has been produced during a defined cycle of manufacture

[SOURCE: ISO 11139:—[1], 3.21]

3.2
bioburden
population of viable microorganisms on or in a *product* (3.16) and/or *sterile barrier system* (3.22)

[SOURCE: ISO 11139:—, 3.23]

3.3
bioburden correction factor
numerical value applied to a viable count to compensate for incomplete removal of microorganisms from a *product* (3.16) and/or failure to culture microorganisms

[SOURCE: ISO 11139:—, 3.24]

3.4
bioburden estimate
value *established* (3.10) by applying a *bioburden correction factor* (3.3) to a *bioburden* (3.2) count

[SOURCE: ISO 11139:—, 3.25]

3.5
bioburden method suitability
assessment of the test method to demonstrate its ability to allow microbial growth

[SOURCE: ISO 11139:—, 3.168, modified — "bioburden" has been added to the term.]

3.6
bioburden spike
individual *bioburden* (3.2) value that is significantly greater than other bioburden values in a set

[SOURCE: ISO 11139:—, 3.26]

3.7
correction
action to eliminate a detected nonconformity

Note 1 to entry: A correction can be made in conjunction with a *corrective action* (3.8).

[SOURCE: ISO 9000:2015, 3.12.3, modified — The Note 1 to entry has been revised and the Note 2 to entry has been deleted.]

3.8
corrective action
situation action to eliminate the cause of a nonconformity and to prevent recurrence

Note 1 to entry: There can be more than one cause for a nonconformity.

Note 2 to entry: Corrective action is taken to prevent recurrence whereas *preventive action* (3.15) is taken to prevent occurrence.

Note 3 to entry: There is a distinction between *correction* (3.7) and corrective action.

[SOURCE: ISO 9000:2015, 3.12.2, modified — "situation" has been added to the definition and the Note 3 to entry has been replaced.]

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1) Under preparation. Stage at the time of publication: ISO/DIS 11139:2017.

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3.9
culture condition
combination of growth media and manner of incubation used to promote germination, growth, and/or multiplication of microorganisms

Note 1 to entry: The manner of incubation can include the temperature, time, and any other conditions specified for incubation.

[SOURCE: ISO 11139:—, 3.71]

3.10
establish
determine by theoretical evaluation and confirm by experimentation

[SOURCE: ISO 11139:—, 3.107]

3.11
facultative microorganism
microorganism capable of both aerobic and anaerobic metabolism

[SOURCE: ISO 11139:—, 3.114]

3.12
health care product
medical device, including *in vitro* diagnostic medical device, or medicinal *product* (3.16), including biopharmaceutical

[SOURCE: ISO 11139:—, 3.132]

3.13
microbial characterization
process by which microorganisms are grouped into categories

Note 1 to entry: Categories can be broadly based, for example, on the use of selective media, colony or cellular morphology, staining properties or other characteristics.

[SOURCE: ISO 11139:—, 3.170]

3.14
obligate anaerobe
organism that lives and grows in the absence of molecular oxygen

[SOURCE: ISO 11139:—, 3.186]

3.15
preventive action
action to eliminate the cause of a potential nonconformity or other potential undesirable situation

Note 1 to entry: There can be more than one cause for a potential nonconformity.

Note 2 to entry: Preventive action is taken to prevent occurrence whereas *corrective action* (3.8) is taken to prevent recurrence.

[SOURCE: ISO 9000:2015, 3.12.1]

3.16
product
tangible result of a process

EXAMPLE Raw material(s), intermediate(s), sub-assembly(ies), *health care product(s)* (3.12).

[SOURCE: ISO 11139:—, 3.219]

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3.17
recovery efficiency
measure of the ability of a specified technique to remove, collect and/or culture microorganisms from a *product* (3.16)

[SOURCE: ISO 11139:—, 3.228]

3.18
requalification
repetition of part or all of *validation* (3.23) for the purpose of confirming the continued acceptability of a specified process

[SOURCE: ISO 11139:—, 3.235]

3.19
sample item portion
SIP
defined part of a *health care product* (3.12) that is tested

[SOURCE: ISO 11139:—, 3.244]

3.20
specify
stipulate in detail within an approved document

[SOURCE: ISO 11139:—, 3.263]

3.21
sterile
free from viable microorganisms

[SOURCE: ISO 11139:—, 3.275]

3.22
sterile barrier system
minimum package that minimizes the risk of ingress of microorganisms and allows aseptic presentation of the *sterile* (3.21) *product* (3.16) at the point of use

[SOURCE: ISO 11139:—, 3.276]

3.23
validation
confirmation process, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: The objective evidence needed for a validation is the result of a test or other form of determination such as performing alternative calculations or reviewing documents.

Note 2 to entry: The word “validated” is used to designate the corresponding status.

Note 3 to entry: The use conditions for validation can be real or simulated.

[SOURCE: ISO 9000:2015, 3.8.13, modified — “process” has been added to the definition.]

4 General requirements

4.1 Documentation

4.1.1 Procedures for the determination of bioburden shall be specified.

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4.1.2 Documents and records required by this document shall be reviewed and approved by designated personnel (see 4.2.1). Documents and records shall be controlled in accordance with ISO 13485, ISO 15189 or ISO/IEC 17025.

4.1.3 Records retained shall include all original observations, calculations, derived data and final reports. The records shall include the identity of all personnel involved in sampling, preparation and testing.

4.1.4 Calculations and data transfers shall be subject to appropriate checks.

4.2 Management responsibility

4.2.1 The responsibility and authority for implementing and performing the procedures described in this document shall be specified. Responsibility shall be assigned to competent personnel in accordance with ISO 13485, ISO 15189 or ISO/IEC 17025.

4.2.2 If the requirements of this document are undertaken by organizations with separate quality management systems, the responsibilities and authority of each party shall be specified.

NOTE See Annex D for additional information.

4.2.3 All items of equipment required for the correct performance of the specified tests and measurements shall be available.

4.3 Product realization

4.3.1 Procedures for purchasing shall be specified. These procedures shall conform with ISO 13485, ISO 15189 or ISO/IEC 17025.

4.3.2 A documented system conforming with ISO 13485, ISO 15189, ISO/IEC 17025 or ISO 10012 shall be specified for the calibration of all equipment, including instrumentation for test purposes, used in meeting the requirements of this document.

4.3.3 Methods shall be specified for the preparation and sterilization of materials used in the determination of bioburden, including appropriate quality tests.

4.4 Measurement, analysis and improvement

4.4.1 For the purpose of bioburden test methods and results, measurement uncertainty, precision and bias typically do not apply and therefore this type of data analysis may not be necessary, except in evaluating the overall competency of the laboratory.

4.4.2 For control of nonconforming products, procedures for the investigation of out-of-specification results and for correction, corrective action and preventive action shall be specified. These procedures shall conform with ISO 13485, ISO 15189 or ISO/IEC 17025.

5 Selection of products

5.1 General

5.1.1 The procedures for the selection and handling of products for the determination of bioburden shall ensure that the selected product is representative of routine production, including packaging materials and processes.

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5.1.2 If product(s) are grouped in a product family for the purpose of the determination of bioburden, the rationale for inclusion of a product within a product family shall be recorded. The rationale shall include criteria to ensure that bioburden determined for a product selected from the product family is representative for the whole product family.

5.1.3 Consideration shall be given to the timing of the determination of bioburden relative to manufacturing because bioburden can change with the passage of time.

5.2 Sample item portion (SIP)

5.2.1 Either the entire product (SIP = 1,0) or a portion of the product (SIP < 1,0) may be used for the determination of bioburden.

5.2.2 If an SIP < 1,0 is used, the SIP shall be of sufficient size to adequately represent the bioburden of the entire product. The determination of portions selected shall be based on whether the bioburden is evenly distributed or not, as described in 5.2.3 to 5.2.5.

5.2.3 When the bioburden distribution is known, the following applies:

a) if the bioburden is evenly distributed on and/or in the item, the SIP may be selected from any portion of the item;

b) if the bioburden is not evenly distributed, the SIP shall include either

   1) portions of the product selected that proportionally represent each of the materials from which the product is made, or

   2) the portion of the product that contains the most severe microbial challenge (numbers and/or types) to the sterilization process.

When selecting the portion that contains the most severe microbial challenge, the relationship of the bioburden of the SIP tested to the entire product bioburden should be established.

5.2.4 If the bioburden distribution is not known, the SIP shall consist of portions of the product selected that proportionally represent each of the materials from which the product is made.

5.2.5 The SIP can be calculated on the basis of dimensional characteristics, such as length, mass, volume or surface area (see Table A.1 for examples).

NOTE Some standards specifying requirements for validation and routine control of the sterilization process stipulate criteria for the adequacy of the SIP, e.g. the ISO 11137 series.

6 Methods of determination and microbial characterization of bioburden

6.1 Determination of bioburden

6.1.1 Selection of an appropriate method

The method shall be appropriate to the purpose for which the data are to be used. The method/s shall comprise techniques for the following:

a) neutralization of inhibitory substances, if needed;

b) removal of microorganisms, if appropriate;

c) culturing of microorganisms;

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d) enumeration of microorganisms.

6.1.2 Neutralization of inhibitory substances

If the physical or chemical nature of the product is such that substances can be released that adversely affect the detection of the product bioburden, then a system shall be used to neutralize, remove or, if this is not possible, minimize the effect of any such released substance. The effectiveness of such a system shall be demonstrated.

NOTE Annex B describes techniques that can be used to assess the release of microbicidal or microbiostatic substances.

6.1.3 Removal of microorganisms

6.1.3.1 For an identified product where the removal of viable microorganisms is part of the method, the efficiency of the removal shall be considered and the outcomes of this consideration recorded (see 4.1.3). Consideration shall, at least, be given to the following:

a) ability of the technique to remove microorganisms;

b) possible type(s) of microorganism and their location(s) on the product;

c) effect(s) of the removal technique on the viability of microorganisms;

d) the physical or chemical nature of the product under test.

6.1.3.2 For an identified product where the removal of viable microorganisms is not part of the method (e.g. direct culture of a product), the efficiency of the enumeration of microorganisms shall be considered and the outcomes of this consideration recorded (see 4.1.3). Consideration shall, at least, be given to the following:

a) possible type(s) of microorganism and their location(s) on the product;

b) the physical or chemical nature of the product under test.

6.1.4 Culturing of microorganisms

Culture conditions shall be selected after consideration of the types of microorganisms likely to be present and the physical or chemical nature of the product to be tested. The results of this consideration and the rationale for the decisions reached shall be recorded (see 4.1.3).

6.1.5 Enumeration of microorganisms

The technique for enumeration shall be selected after consideration of the types of microorganisms likely to be present. The results of this consideration and the rationale for the decisions reached shall be recorded (see 4.1.3).

6.2 Microbial characterization of bioburden

6.2.1 Appropriate techniques for microbial characterization of bioburden shall be selected.

NOTE Microbial characterization is necessary to detect a change to the product bioburden that can affect some aspects of the use of bioburden data (e.g. establishing a sterilization process). Furthermore, knowledge of the types of microorganisms can be helpful for identifying sources of contamination.

6.2.2 Bioburden shall be characterized using one or more of the following technique(s):

a) colony morphology;

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b) cell morphology;

c) differential staining;

d) culture using selective and/or differential conditions;

e) biochemical properties;

f) genotypic analysis, e.g. pattern or fingerprint-based techniques or sequence-based techniques;

g) proteomic methods, e.g. mass spectrometry.

7 Validation of the method for determining bioburden

7.1 General

The method(s) for determining bioburden shall be validated and documented.

NOTE See A.7.1 for information on validation and the use of classic microbiological methods.

7.2 Validation

Validation shall consist of the following:

a) assessment of test method suitability to demonstrate lack of inhibition of growth in the test;

   NOTE 1 The absence of inhibition of growth can be supported by data from bioburden recovery efficiency testing, if an inoculated product was used.

b) assessment of the adequacy of the technique for the removal of microorganisms from a product if the removal is part of the method (i.e. bioburden recovery efficiency), if appropriate for the purpose for which the data are being generated;

   NOTE 2 Annex C provides information on the validation of bioburden recovery efficiency.

c) assessment of the adequacy of the technique for the enumeration of microorganisms, including culture conditions and microbiological counting techniques;

d) assessment of the suitability of the technique(s) of microbial characterization.

8 Routine determination of bioburden and interpretation of data

8.1 General

Routine determination of bioburden shall be performed by employing documented sampling plan(s) that specify sample size and sampling frequency.

8.2 Limits of detection and plate counting

The determination of bioburden shall be performed using the method(s) specified for a product or a product family (see 5.1.2). The method selected shall take into account factors that will affect the results, such as the limits of detection and plate counting.

8.3 Microbial characterization

Microbial characterization of bioburden shall be performed to a degree dependent on the purpose for which the data derived from the determination of bioburden are to be used (see 6.2).

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8.4 Bioburden data for extent of treatment

If bioburden data are to be used to establish the extent of treatment of a sterilization process (i.e. bioburden-based method), any requirements applicable to the use of bioburden data, specified in the appropriate standard for the development, validation and routine control of the sterilization process, shall be met.

8.5 Bioburden spikes

If bioburden data demonstrate a test result that is significantly greater than other values (bioburden spike), these data shall be evaluated for the impact as appropriate depending on the purpose for the data.

8.6 Bioburden levels

Acceptable levels for bioburden on or in a product or group of products shall be specified. If these levels are exceeded, action shall be taken (see 4.4.2). Acceptable levels shall be reviewed and revised as necessary.

8.7 Data analysis

Data derived from the determination of bioburden obtained over a period of time shall be used to identify trends.

8.8 Statistical methods

If used, the application of statistical methods to define sample size, sampling frequency and/or acceptable levels shall conform with ISO 13485.

9 Maintenance of the method for determining bioburden

9.1 Changes to the product and/or manufacturing process

Changes to the product and/or manufacturing process shall be reviewed to determine whether they are likely to alter bioburden with consideration to the purpose for which bioburden data are to be used. The results of the review shall be recorded (see 4.1.2). If there is potential for alteration of bioburden, specific determinations of bioburden shall be performed to evaluate the extent and nature of any effect of the change.

9.2 Changes to the method for determining bioburden

Any change to a routine method of bioburden determination shall be assessed. This assessment shall include evaluation of the effect of the change on the outcome of determination. The results of the assessment shall be recorded (see 4.1.3).

NOTE The assessment of the change can indicate that the previous validation and bioburden recovery efficiency are still applicable.

9.3 Requalification of the method for determining bioburden

The original validation data (see 7.2) and any subsequent requalification data shall be reviewed at specified intervals in accordance with a documented procedure. The outcome of the review and any requalification undertaken shall be recorded (see 4.1.3).

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Annex A
(informative)

Guidance on the determination of a population of microorganisms on products

NOTE For ease of reference, the numbering in this annex corresponds to that used in the main body of this document.

A.1 Related to the Scope

This annex gives guidance on the implementation of the requirements specified in this document. The guidance given is not intended to be exhaustive, but to highlight important aspects to which attention should be given.

Methods other than those given in this annex may be used, but these alternative methods should be demonstrated as being effective in achieving conformity with the requirements of this document.

This annex is not intended as a checklist for assessing conformity with the requirements of this document.

A.2 Related to the Normative references

No guidance offered.

A.3 Related to the Terms and definitions

No guidance offered.

A.4 Quality management system elements

NOTE It is not a requirement of this document to have a full quality management system. However, the minimum elements of a quality management system that are necessary to control the determination of bioburden as used in the validation and monitoring of health care products to be sterilized are normatively referenced at appropriate places in the text (see, in particular, Clause 4). Attention is drawn to the standards for quality management systems (see ISO 13485) that control all stages of production or reprocessing of health care products.

A.4.1 Documentation

In ISO 13485, the requirements in the documentation section relate to the generation and control of documentation (including specifications and procedures) and records.

Computers can be used in laboratories for direct and indirect collection, processing and/or storage of data. Both the hardware and software used for such applications should be controlled.

The computer system in use should be identified, both in terms of hardware and software, and any changes in either of these aspects should be documented and subject to appropriate approval.

If calculations are performed by electronic data processing techniques, the software (e.g. spreadsheet calculations) should be validated prior to use, and records of this validation should be retained.

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For software, there should be documentation describing the following:

a) applications software run on the computer system;

b) operations software;

c) data packages in use.

All software should be validated before being put into service.

If computer software is developed in-house, suitable procedures should be developed to ensure the following:

— documentation on development, including the source code, is retained;

— records of acceptance testing are retained;

— modifications to programs are documented;

— changes in equipment are documented and formally tested before being put into use.

These controls should also be applied to any modification or customizing of commercial software packages.

There should be procedures to detect or prevent unauthorized changes to software programs.

Software programs that organize, tabulate and/or subject data to statistical or other mathematical procedures, or which otherwise manipulate or analyse the electronically stored data, should permit retrieval of original data entries. Special procedures for archiving computer data are likely to be required and these procedures should be documented.

Requirements for the control of documents and records are specified in ISO 13485, ISO 15189 or ISO/IEC 17025.

Requirements for technical records are specified in ISO/IEC 17025.

See also ISO/IEC 90003 for guidance on the application of quality management systems for computer software.

A.4.2 Management responsibility

In ISO 13485, the requirements in the management responsibility section relate to management commitment, customer focus, quality policy, planning, responsibility, authority, communication and management review.

Data obtained from performing bioburden determinations should be reliable. It is important that the determinations are performed under controlled conditions. Therefore, the laboratory facilities used for the determinations, whether on the site of the manufacturer of the health care product or located at a remote location, should be managed and operated in accordance with a documented quality system.

The determination of bioburden can involve separate parties, each of whom is responsible for certain elements of the method or procedure. (See Annex D for guidance on typical responsibilities.) This document requires that the party accepting particular responsibilities be defined and that this definition of responsibilities be documented. This definition of authority and responsibility is documented within the quality management system(s) of the identified parties. The party accepting responsibilities for defined elements is required to assign these elements to competent personnel, with competence demonstrated through appropriate training and qualification.

If bioburden determinations are performed in a laboratory under the direct management of the manufacturer of the health care product, the operation of the laboratory resides within the manufacturer's quality management system. If an external laboratory is used, all tests should be conducted in accordance with recognized current/valid best laboratory practices (e.g. ISO 15189,

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ISO/IEC 17025), where applicable, and the data should be evaluated by competent, informed professionals.

Any laboratory should be committed to providing a quality service and this commitment should be documented as a quality policy. The lines of authority and responsibility within the laboratory organization should be formally established and documented. An individual should be nominated to be responsible for the establishment of the laboratory quality system and should have the authority to ensure that the system is implemented.

The operation of the laboratory should be subject to regular internal audits. The results of the audit should be documented and reviewed by the laboratory management (see, for example, ISO 15189 or ISO/IEC 17025).

Requirements for responsibility, authority and human resources are specified in ISO 13485.

Requirements for the provision of resources are specified in ISO 13485.

Requirements for equipment are specified in ISO 15189 and ISO/IEC 17025.

A.4.3 Product realization

In ISO 13485, the requirements in the product realization section relate to the product lifecycle, from the determination of customer requirements, design and development, purchasing, control of production, and calibration of monitoring and measuring devices.

There should be a system for identifying the maintenance requirements for each piece of laboratory equipment. Equipment that does not require calibration should be clearly identified.

Any equipment, or parts thereof, that come into contact with a product, eluent, culture media, etc., during testing should be sterile. All microbiological media and eluents used to remove microorganisms from the product should be prepared in a manner that ensures their sterility.

Appropriate quality tests for culture media should include growth promotion tests. Generally, growth promotion tests are performed on each batch using an inoculum of low numbers [not more than 100 colony-forming units (CFUs)] of selected microorganisms. Growth promotion tests are described in some Pharmacopoeias [(e.g. US Pharmacopoeia (USP), European Pharmacopoeia (EP)] that detail suitable microorganisms. Other recognized quantitative and semi-quantitative methods for media quality control are also acceptable.

Requirements for purchasing are specified in ISO 13485. In particular, it should be noted that the requirements in ISO 13485 for the verification of purchased products apply to all products and services received from outside the organization.

Requirements for the calibration of monitoring and measuring devices are specified in ISO 13485.

Requirements for equipment and for measurement traceability are specified in ISO/IEC 17025.

A.4.4 Measurement, analysis and improvement

A.4.4.1 Bioburden test results do not generally fit a mathematical distribution model. Therefore, measurement uncertainty, precision and bias may not be necessary, except for evaluating the overall competency of the laboratory. For bioburden test methods, the measurement of uncertainty, precision and bias are taken into account by the determination of the bioburden recovery efficiency.

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A.4.4.2 The requirements in the measurements, analysis and improvement section of ISO 13485 relate to in-process monitoring, control of nonconforming products, analysis of data and improvement (including corrective and preventive actions).

All bioburden results that exceed the specified level and/or indicate an adverse trend should be investigated. The initial phase of the investigation should involve assessing if the results are a true finding or are in error. The following can contribute to an error and should be addressed:

— inappropriate samples (e.g. non-representative, non-homogeneous, rejected materials);

— inappropriate sampling materials (e.g. swabs, containers, packages);

— unsuitable conditions of transport/handling/storage;

— inappropriate test materials (e.g. storage, pipettes, filtration apparatus);

— incorrect handling or test method(s);

— inappropriate culture media or diluents;

— inappropriate laboratory environment;

— inappropriate incubation environment;

— errors of calculation or transcription;

— deviation to test method (e.g. dilution error, filtration error, aseptic technique error).

If the results are due to a sampling or laboratory error, the bioburden result that exceeds the specified level should be verified by the performance of another determination employing new samples from the same batch of the product, if possible. If the product supports microbial growth and would result in invalid data, or if the same batch is no longer available, a new batch should be used.

If the original result is confirmed as a true finding, the following, at least, should be considered in the second phase of the investigation:

a) the implication of the result in relation to the purpose for which the data will be used (e.g. the effectiveness of the sterilization process);

b) the need to increase the sample size and/or frequency;

c) an assessment of the manufacturing process that addresses the following:

    1) raw materials/components (e.g. vendors, changes);

    2) cleaning/lubrication/manufacturing liquid;

    3) transport/holding containers;

    4) work surfaces;

    5) personnel attire/hygiene/practices;

    6) handling/assembly;

    7) environmental conditions and monitoring results (including seasonal factors, if any);

    8) packaging materials and procedures;

    9) storage conditions;

d) microbial characterization of microorganism(s) recovered, including:

    1) potential sources;

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2) comparison with previous isolates.

Based on the results of the investigation, specific corrective action can be required. If corrective action is required, the effectiveness should be demonstrated.

Procedures for corrective action are specified in ISO 13485, ISO 15189 and ISO/IEC 17025.

A.5 Selection of products

A.5.1 General

A.5.1.1 Procedures for selecting and handling samples of a product should be documented. They should be conducted to avoid the introduction of inadvertent contamination and significant alterations to the numbers and types of microorganisms in the sample. Sampling techniques should be consistent and should allow for event-based and time-based comparisons of bioburden.

In choosing samples of a product for the determination of bioburden, there are several possibilities:

a) take an actual product (at random or at a specified frequency);

b) manufacture a product specifically for bioburden testing using the routine manufacturing procedures;

c) take a product that is not suitable for sale, which can be scrapped or otherwise rejected.

The choice can depend on a number of factors, but the first prerequisite is that the product selected should possess bioburden representative of that of the actual product. If the decision is made to utilize a rejected product, that product should have undergone all the essential stages of production, including possible cleaning and packaging processes.

When sampling for the determination of bioburden, a product should be contained in its usual packaging. Typically, it is sufficient to perform a bioburden determination on a product after its removal from its packaging system and to omit the packaging system from the determination. Depending upon the sterile label claim, internal packaging components, such as a tray or product insert, may need to be tested based upon factors such as

— what is intended to be sterile,

— when the package is an integral part of the product, or

— for specific evaluation.

A.5.1.2 The use of bioburden data (e.g. control of raw materials, acceptance of incoming components, evaluation of process steps, qualification of a sterilization process) should be taken into account when establishing product families for bioburden determination. The following should be considered when establishing product families for bioburden determination:

a) nature and source of raw materials;

b) nature and source of components;

c) complexity of manufacturing process, i.e. degree of handling, number of process steps;

d) types of manufacturing processes used;

e) manufacturing and/or assembly environment;

f) product design and size;

g) manufacturing equipment;

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h) manufacturing location.

In addition, the numbers and types of microorganisms can influence the selection of a bioburden test method for the product family. For each product family, a master product or representative product(s) should be selected for the routine determination of bioburden. The selection of the master product should be based on a documented rationale.

If the products within a family are considered equivalent then a representative product can be selected for the determination of bioburden. The selected product can be monitored routinely or the other members of the group can be chosen on a rotational basis. If a selected product is monitored routinely, the continued equivalence of other products in the family should be periodically monitored or a rationale provided.

A.5.1.3 If data from bioburden determinations are to be used to establish or maintain a sterilization process, the period of time that elapses between the selection of product samples and the determination of bioburden should be representative of the time period between the completion of the last manufacturing step and sterilization of the product.

A.5.2 Sample item portion (SIP)

A.5.2.1 Whenever practicable, the determination of bioburden should utilize the whole product, although this might not be feasible if the product cannot be accommodated in available laboratory testing vessels. In this case an SIP is used. Consideration should be made of the distribution of bioburden across the whole of the product. If the distribution is expected to be uneven across the product, a determination of the area of the product most heavily contaminated should be identified. This area should be included in the SIP selected.

A.5.2.2 As large a portion of the product as possible should be used for the SIP. The SIP should be representative so that the bioburden of the whole product can be determined. Careful selection of the SIP of the product is necessary when large products, such as surgical gowns or external drainage kits, are tested.

A.5.2.3 Consideration should be given to aspects of manufacturing that contribute to the distribution of microorganisms on products.

A.5.2.4 Examples of an SIP that can be selected from the device with a more severe challenge to the sterilization process are tubing sets with connections, stopcocks, etc.

A.5.2.5 Examples of products for which various bases for SIP calculation are employed are given in Table A.1.

When preparing or assembling an SIP, care should be taken during manipulations of products. If portions are to be separated from products, this should be done under clean conditions in a controlled environment (e.g. inside a laminar flow cabinet) in order to avoid adding contamination.

Table A.1 — Examples of SIP calculation

Basis for SIP	Product
Surface area	Implants (non-absorbable)
Mass	Powders Gowns Implants (absorbable)
Length	Tubing (consistent diameter)
Volume	Fluid in a container

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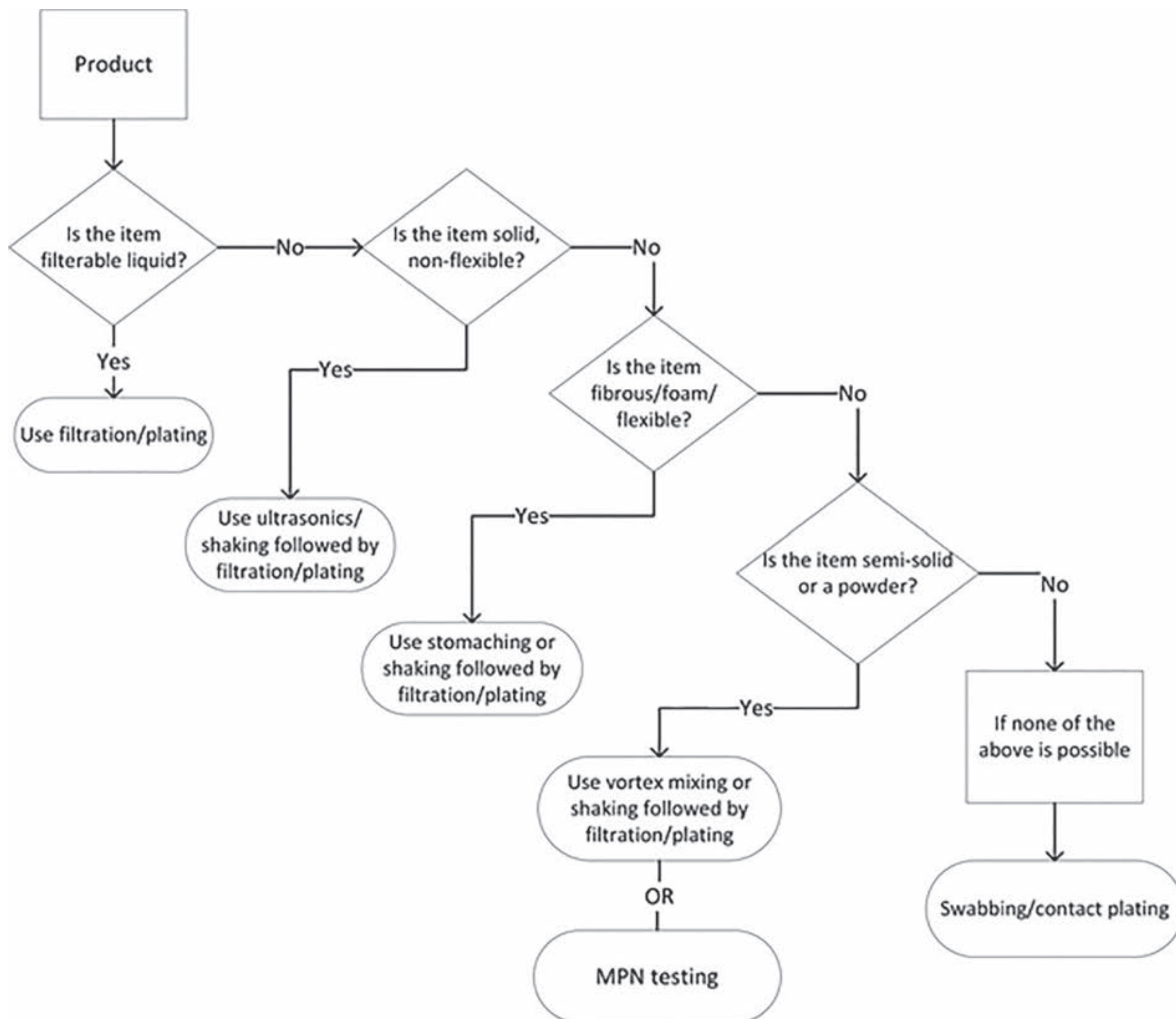
A.6 Methods of determination and microbial characterization of bioburden

A.6.1 Determination of bioburden

A.6.1.1 Selection of an appropriate method

Figure A.1 is a decision tree that has general application in the initial stages of the selection of a method of bioburden determination. This figure can apply to both culture and non-culture based methods.

For a product with high bioburden and for which a culture method is employed, ensure that a sufficient number of dilutions are carried out to obtain countable results and to prevent issues such as the masking of colonies or too numerous to count (TNTC) plates.



NOTE 1 This decision tree does not preclude the use of alternative, rapid microbiological methods to determine bioburden (e.g. autofluoresence, flow cytometry, direct epifluorescence, filter technique and solid phase cytometry).

NOTE 2 This decision tree does not encompass all types of products that can be tested or all types of testing that can be used.

Figure A.1 — Decision tree for selection of a method of bioburden determination

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For a product with very low bioburden, it might not be possible to recover detectable bioburden from individual product units even though a suitable bioburden test method with validated bioburden recovery efficiency is used. Caution should be exercised in respect to estimation of average bioburden where zero colonies are detected to avoid overestimation of the true product bioburden. The desired limit of detection for the bioburden test method should reflect the intended use of bioburden data, and, if necessary, the bioburden test method should be designed to minimize the limit of detection as much as reasonably practicable.

To optimize the bioburden determination methods for low bioburden products, it can be necessary to consider use of an alternative approach. Examples are given in a) to e).

a) Pooled sample approach: where multiple product units are combined into a single test. A bioburden recovery efficiency should be determined for this approach. The total recoverable CFUs for the pooled sample is divided by the number of pooled units to estimate the CFUs per unit. Pooling of units can permit estimation of a low number of CFUs per unit; however, it provides no information about bioburden distribution or variability on individual units that comprise the pooled sample. Pooling can be applicable in cases of consistent numbers of CFUs per unit.

   It is important to be aware that pooling can reduce the ability to detect an inadvertent change within the manufacturing process, depending on the method of pooling.

b) Most probable number (MPN) approach: see B.3.3.

c) Combining and eliminating tests approach for groups of microorganisms: for many types of products it is not necessary to split the extract into portions for separate tests, such as aerobes, anaerobes, spores and fungi. If evaluation has shown that testing for anaerobes is not indicated, then this test can be eliminated in the future. Additionally, if aerobic spores are detected in the aerobic bacterial count and fungal counts are not high, then it is possible to combine the aerobic bacterial, bacterial spores and fungal tests into a single test. For example, filtration of the entire volume of extraction fluid through a single filter that is placed on a suitable general purpose culture medium, which is then incubated at two different temperatures (e.g. 30 °C to 35 °C and 20 °C to 25 °C). Other examples include the use of a single incubation at (30 ± 2) °C, or incubation at other temperature ranges suitable for detecting a specific microbial population. Elimination of dilution factors in this way (provided the elimination is justified) can minimize the potential to overestimate the average bioburden.

d) One half limit of detection approach: this assists in calculating a bioburden average when “less than” values are present in the bioburden results. This approach provides a lower bioburden result when a smaller percentage of results are at 0 CFU per plate (for more information refer to Reference [24]).

e) Poisson-based substitution for “less than” values approach: this provides a means to determine an estimate of the average bioburden.

   Bioburden is typically not distributed on products throughout the manufacturing process in a fashion such that it can be statistically analysed using a Poisson distribution. Applications of the use of Poisson distribution to bioburden should be carefully considered in relationship to the intended use of the information. (For more information refer to References [24] and [20].)

The selection of a method for determining bioburden should consider the possible occurrence of biofilm on or in the product. Biofilm can form on or in the product when in contact with liquids unless appropriate bioburden control measures are taken. Health care products incorporating tissue have a potential for biofilm occurrence.

A.6.1.2 Neutralization of inhibitory substances

See Annex B.

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A.6.1.3 Removal of microorganisms

See Annex B.

A.6.1.4 Culturing of microorganisms

The nature of raw materials, the method of manufacture and the conditions under which the product manufacture occurs, are factors that influence product bioburden and should be considered when choosing the culture media and incubation conditions. Unless fastidious microorganisms are likely to be present, general purpose, non-selective culture media and incubation conditions are appropriate. The recommendations of the laboratory, with the input of the manufacturer, for the use of standard bioburden culture conditions can suffice as the consideration and rationale.

When selecting culture media and incubation conditions, the following, at least, should be considered:

a) no single combination of medium and incubation conditions can support the growth of all microorganisms; the choice of conditions should minimize the potential to overestimate the average bioburden due to counting the same microorganism on different media;

b) validation exercises can require the use of a wider range of culture media and conditions of incubation than those used routinely;

c) likely microbial contamination sources and the types of the microorganisms that can be encountered, bearing in mind that some contamination sources can vary seasonally.

   Health care products manufactured from synthetic material are unlikely to be contaminated with obligate anaerobes. Health care products manufactured from tissue or other natural materials can be at risk of contamination with obligate anaerobes.

Examples of culture media and incubation conditions are given in Table A.2.

It should be noted that all non-selective anaerobic culture methods can support the growth of facultative anaerobic microorganisms.

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Table A.2 — Examples of culture media and incubation conditions[a]

Types of microorganism	Solid media	Liquid media	Incubation conditions[b]
Facultative, non-fastidious, aerobic bacteria[d]	Soybean casein digest agar (Tryptone soya agar) Nutrient agar Blood agar base Glucose tryptone agar (plate count agar)	Soybean casein digest broth (Tryptone soya broth) Nutrient broth	30 °C to 35 °C for 3 d to 7 d
Yeasts and moulds	Sabouraud dextrose agar Malt extract agar Rose Bengal agar Chloramphenicol agar Soybean casein digest agar (Tryptone soya agar) Potato dextrose agar Glucose tryptone agar (plate count agar)	Sabouraud dextrose broth Malt extract broth Soybean casein digest broth (Tryptone soya broth)	20 °C to 25 °C for 5 d to 7 d
Anaerobic bacteria	Reinforced clostridial agar [c] Schaedler agar [c] Blood agar[c] Fastidious anaerobe agar[c] Soybean casein digest agar (Tryptone soya agar)[c] Columbia agar[c] Wilkens-Chalgren agar[c]	Robertson's cooked meat broth Fluid thioglycollate broth	30 °C to 35 °C for 3 d to 7 d

[a] This list is not exhaustive.

[b] The incubation conditions listed indicate conditions that are commonly used for the types of microorganism listed.

[c] Cultured under anaerobic conditions. Performance can be enhanced if the culture media are pre-reduced.

[d] Some culture media used for facultative, non-fastidious, aerobic bacteria are able to support the growth of yeasts and moulds.

A.6.1.5 Enumeration of microorganisms

The laboratory may specify the technique for enumeration, which will suffice as the consideration and rationale. See also B.6.

A.6.2 Microbial characterization of bioburden

A.6.2.1 The degree of characterization necessary is dependent on the nature of the product, diversity of the detected population, and the use of the data (e.g. sterilization qualification).

A.6.2.2 A wide range of methods can be used to characterize microorganisms comprising the bioburden on or in a health care product. Typical microbial characterization methods for bioburden include colony

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morphology, cellular morphology, staining properties, selective culturing and microbial identification. Details regarding these methods are as follows.

a) Colony morphology is simple to record when the colony count is obtained. Describing the colony morphology is somewhat subjective and includes colour, shape, size, texture, margin, elevation and other physically observable characteristics of the colony. This information alone is not conducive to trending (see A.8). It can usually be used to distinguish between bacterial and mould isolates and to initially determine if the colonies on a plate are likely to be the same microorganism. Further characterization in order to identify sources of contamination requires more specific methods.

b) Cellular morphology and staining techniques, such as a wet mount and Gram stain, are often used to characterize microorganisms. The benefits of these methods are that they require minimal equipment and time, and can provide valuable information regarding the general characteristics of the microorganisms. Characterization of fungi (i.e. mould and yeast) via a physical description and a wet mount can be sufficient for the majority of isolates.

c) Selective culturing and differential media can be used to inhibit the growth of particular microorganisms, select for certain microorganisms, or assist in differentiating some microorganisms from others (e.g. colour of the colony on specific media) which can be useful in characterizing the microorganism.

d) Microbial identification can be performed using phenotypic or genotypic methods, or a combination of both. Classical phenotypic tests, such as colony and cell morphology, Gram and spore stain reactions, ability to grow aerobically or anaerobically, and simple biochemical reactions (e.g. catalase, oxidase, indole), usually provide some indication of the group or genus to which a bacterium belongs. More complex biochemical and serological tests, or genotypic or proteomic methods can identify a bacterium to genus, species or strain level. A similar approach can be taken with yeast and mould. A combination of morphological and physiological properties can be used to establish genera, with biochemical assimilations used to differentiate species.

Table A.3 provides information on common bioburden characterization methods.

Table A.3 — Attributes of common bioburden characterization methods

Method	Example	Specificity
Colony morphology	Form, elevation, margin, size, colour	Low
Cell morphology	Shape (rod, cocci, yeast) Size, aggregation (clusters, chains) Anatomy (fungal structures)	Low to moderate
Staining properties	Differential stains (Gram reaction, spore staining, acid fastness) Mycological stains	Low to moderate
Selective culturing and differential media	Heat shock, incubation parameters, selective culture media	Moderate to high
Genus/species identification	Genetic and biochemical ID techniques and systems	High

A.7 Validation of the method for determining bioburden

A.7.1 General

In general, classical microbiological methods present a challenge to the user in the validation of the determination of bioburden. It is not usually necessary to validate classical microbiological methods or methods described in national and international standards and pharmacopoeias. These methods should only need to be verified for their accuracy and reliability under their unique conditions of use. Such actions are usually sufficient to confirm the validity of the determination of bioburden.

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In the validation of bioburden test methods there are two aspects to consider. The first is the ability to neutralize inhibitory factors in the test system to allow microorganisms to replicate (bioburden method suitability) and the second is the ability to remove and culture microorganisms from a product (bioburden recovery efficiency).

When methods for determining bioburden include removal of the microorganisms from a product, it is the efficiency of the removal process that is of greatest concern. Validation of the removal and culturing process is called bioburden recovery efficiency (see Annex C for details).

A.7.2 Validation

A.7.2.1 Bioburden method suitability

Bioburden method suitability testing is used to demonstrate that the product does not prevent the growth or detection of microorganisms. The product can contain substances that are inhibitory to microorganisms in bioburden test conditions.

Dilution or suitable inactivation/neutralization methods should be used in testing products that contain antimicrobial substances.

Inhibitory effects of substances eluted from the product should be investigated in preliminary experiments, to evaluate whether the product contains substances that can cause inhibition to microorganism growth in bioburden test conditions. A documented rationale may be acceptable if the device comprises materials that are known or have been demonstrated to be inert.

Bioburden method suitability should be considered

a) when there are new or modified products, and

b) whenever there is a change in the conditions of the test (e.g. incubation conditions, extraction media).

The application of methods with given suitability for microbicidal or microbiostatic substances (e.g. membrane filtration with validated membrane rinsing procedure) to products might not require a product specific bioburden method suitability test.

A.7.2.2 Bioburden recovery efficiency

There are essentially two traditional approaches available for validation of the efficiency of the removal of a microorganism from health care products (see C.1.4). These approaches are

— repetitive recovery: the repetitive treatment of a product sample followed by quantitative assessment of the extent of recovery, or

— inoculated product: a product inoculated with known levels of microorganism(s), followed by quantitative assessment of the extent of recovery.

The first of these approaches has the advantage of utilizing the naturally occurring microorganisms but usually needs a moderate to high initial bioburden. If this is the case, then the first approach can be preferred based on the product and/or configuration. The second approach creates a model system for testing purposes but raises questions as to how it compares to recovering natural microorganisms. For additional information see Table C.1.

More non-traditional products (e.g. complex or complicated products containing powders, liquids, antimicrobial agents, multiple components) can require a combination of methods to assess bioburden recovery efficiency. Refer to Annex C for additional information.

For a liquid product that is filtered, or when the MPN method is used, determination of a bioburden recovery efficiency and calculation of a bioburden correction factor are not necessary. However, test method suitability for enumeration should still be assessed.

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A.7.2.3 Enumeration and culture conditions

For further guidance on enumeration, see B.6.

The culture conditions (i.e. media and incubation conditions), selected for use in determination of bioburden cannot be expected to detect all potential microorganisms. In practice, therefore, it is likely that bioburden will be underestimated. Nevertheless, a decision on appropriate culture conditions will have to be made.

One approach to the assessment of culture conditions consists of selecting the culture conditions based on a knowledge of the manufacturing process, environment, materials and the microorganisms expected to be present. If specific product characteristics indicate that additional assessment is needed, the microorganisms enumerated under typical culture conditions are compared to those detected by alternative culture conditions. If this approach indicates that a low proportion of the bioburden is being detected in the typical culture conditions, the alternative culture conditions should be reconsidered to optimize the determination. This is of particular concern for health care products where antimicrobials can affect microbial growth.

When selecting techniques for use in the microbial characterization of microorganisms, consider the following:

— risk to the manufactured product considering the mode of sterilization qualification;

— previously available data;

— the purpose for generating the data;

— the nature of the manufacturing process (e.g. water involved, manual, automated) and the product.

A.8 Routine determination of bioburden and interpretation of data

A.8.1 General

In order to demonstrate that effective control of microbiological quality has been implemented and maintained, a programme of monitoring the product and/or components should be developed.

It is common practice to use a sample size of between three to ten items for routine monitoring of bioburden levels.

Where bioburden data are used to satisfy the requirements of another International Standard (e.g. the ISO 11137 series), sample size and test frequency can already be predefined by that standard, which would supersede the sample size recommended here.

A rational choice of sample size primarily depends upon two factors.

a) The change in bioburden to be detected.

   This will depend upon the consequences associated with a change (either increase or decrease) in bioburden level and how the bioburden information is being applied. For early detection of a small change in the mean bioburden level, a large sample size can be needed.

b) The variation in estimates of the number of viable microorganisms present on individual items.

   The degree of this variability will determine the sample size necessary to detect a given change. Small item-to-item variation in such estimates will require a smaller sample size to detect a change than that required for large item-to-item variation.

Larger sample sizes can provide increased confidence in detecting significant changes.

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It should be recognized that the manner in which bioburden data are used can influence the desired level of confidence in detecting a change of a given magnitude. A rational choice of the magnitude of change to be detected and the probability of achieving that detection should be made.

A rational choice for the frequency of monitoring should be made, taking into account a variety of factors including the following:

— the availability of historical data;

— the purpose for generating the data;

— the nature of the manufacturing process;

— the production frequency for the product;

— the criticality of detecting bioburden changes in a timely fashion;

— seasonal and environmental variations.

Sampling can be performed at a frequency based on time (e.g. monthly, quarterly), or on production volume (e.g. alternate batches). However, in order to establish baseline levels, it is common practice to determine bioburden at a higher frequency during the initial production of a new product and for this frequency to be reduced as a knowledge of bioburden develops.

The frequency of determinations of bioburden should allow detection of changes in bioburden, for example, due to seasonal variations, manufacturing changes or changes in materials.

A.8.2 Limits of detection and plate counting

A.8.2.1 Limits of detection

Limits of detection (LOD) for bioburden test methods should be taken into account in determining the bioburden value. For microbiology reporting, when a portion of the extract is tested for bioburden, and zero colonies are recovered, the results are typically reported as less than “X” where “1/X” is representative of the fraction of the portion tested. For example, if a product is extracted in 400 ml and ¼ of the extract is filtered, results of zero colonies will be reported as less than 4 colony forming units (i.e. < 4 CFU). Therefore, the LOD for this example is 4. In microbiological reporting, a result of < 4 CFU means that it is possible that the entire extract contains either 0, 1, 2 or 3 CFUs, but microbiological reporting rules require that it be reported as < 4 CFU.

Individual bioburden results are reported in whole numbers because the number is representative of a colony forming unit. Averages or other mathematical calculations using bioburden data are typically reported to one decimal place.

LOD can be improved by the following:

a) modification to the test method (e.g. filtering a larger portion of the extract);

b) pooling multiple samples;

c) utilizing another test method, such as MPN.

A.8.2.2 Plate counting

In published standard microbiological methods for other industry applications, it is recommended to select plates that contain acceptable ranges (e.g. less than 200 CFU, 25 CFU to 250 CFU, or 30 CFU to 300 CFU). This is applicable when there are multiple dilutions performed and therefore from which to choose. However, with bioburden testing this is not always the case because

— many products have a low bioburden that would exhibit a plate count less than e.g. 30 CFU, and

— multiple dilutions are not always necessary when counts are low.

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For these situations, it is appropriate to record and utilize counts less than e.g. 30 CFU.

Plate counts can be determined in three different ways:

a) a direct count of the CFU;

b) an estimated count;

c) counts beyond countable or estimated ranges.

A direct count tabulates all the colonies directly using any method that facilitates accurate counts (tally counter, marking plates, etc.).

An estimated count can be performed when colonies on a portion of a plate can be counted and multiplied to represent the remaining portions of the plate.

Estimated counts are one way to obtain a count when spreading colonies are present. This technique is typically applied when the spreading colonies do not obscure other colonies (due to size or opaqueness).

Counts that are beyond the countable or estimated range can be semi-quantitated if that value can be approximated based on the presence of discernible colonies. However, if this cannot be done, a result of too numerous to count (TNTC) should be assigned. It is an acceptable practice to omit TNTC results from the average for a group of samples. TNTC results should be investigated.

When duplicate plate counts, dilution factors or aliquots are used, the plate counts should be adjusted accordingly to obtain the count for a single product.

A.8.3 Microbial characterization

If, on microbial characterization, types of microorganisms are recovered that are not part of the normal bioburden, consideration should be given to assessing the relevance of the presence of these isolates.

A.8.4 Bioburden data for extent of treatment

No additional guidance.

A.8.5 Bioburden spikes

Bioburden data can demonstrate a value that is significantly greater (commonly called a bioburden spike) than other values within a set of values. This bioburden spike can occur in one of two situations:

a) the value is not a normal and consistent part of the bioburden distribution;

b) the value can be a normal and consistent part of the bioburden distribution.

It can be determined that the bioburden spike is not a normal and consistent part of the bioburden distribution through an investigation into the manufacturing practices, microbiological testing, and handling of samples. Refer to this subclause to determine how to handle this situation.

It can be determined that the bioburden spike is a normal and consistent part of the bioburden distribution by reviewing historical data. Historical data can demonstrate a periodic occurrence of a greater value that is within expectations making it a consistent part of the bioburden. If these data are comprised of microorganisms typically found on the product, this makes it a normal part of the bioburden. These spikes should be included when determining the extent of treatment of a sterilization process. For example, a bioburden spike can occur due to raw materials that are not consistent, or manufacturing processes that involve excessive handling.

In the example given in Table A.4, there are 3 batches (batches 2, 5, 6) in 10 batches that contain individual values that are significantly greater than the batch average (in this example, five or more times the batch average). It was determined that these high values are a normal and consistent part of the bioburden. Consequently, the high values and/or the batch averages for those batches that contain

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high values might need to be taken into consideration when establishing the overall average bioburden for determining the extent of treatment for a sterilization process.

Table A.4 — Example of bioburden data containing bioburden spikes

Batch #	Item #										Average (CFU/ device)
	1	2	3	4	5	6	7	8	9	10	
1	4	20	12	12	4	32	28	4	4	8	12,8
2	12	32	20	**458**	88	120	40	44	36	60	91,0
3	36	44	52	88	36	48	344	96	180	128	105,2
4	30	4	8	4	12	24	24	20	28	4	15,8
5	36	52	48	36	**920**	4	36	72	4	36	124,4
6	36	32	12	36	36	36	**386**	72	88	36	77,0
7	40	20	52	44	36	4	36	44	52	308	63,6
8	24	20	12	16	4	24	36	80	24	8	24,8
9	8	40	20	48	12	8	4	20	28	44	23,2
10	40	104	8	16	28	24	44	8	4	8	28,4
											56,6

A.8.6 Bioburden levels

A predetermined course of action should be taken when specified levels are exceeded. If corrective actions lead to changes to the process that affect the bioburden, new data should be obtained and new levels established for the product.

The specified levels used for bioburden are commonly based upon historical data for a product and the purpose for which the data are to be used. Prior to the collection of historical data, if it is desired to establish temporary levels, then these can be set after evaluating the first three or more batches of a given product. Historical data from similar products, manufacturing processes and/or manufacturing environments may also be used when setting temporary levels for new product lines. For some product sources, significant seasonal variations in bioburden can be expected. Seasonal humidity and/or temperature levels/changes can also alter the types and numbers of microorganisms in the bioburden. Based upon successive test results, bioburden data should be re-evaluated after a period of time to verify whether the original levels are appropriate.

Historic bioburden data are used to establish bioburden levels that are commonly defined as alert levels and action levels. Establishment of these levels should take into consideration the approach used based on the intent of the use of the information. For example, levels can be used to evaluate raw material suppliers, qualify or demonstrate the continued effectiveness of the sterilization process or assess the efficacy of environmental control in a manufacturing process.

Along with the establishment of the levels, establishment of actions to be taken, if the level is exceeded, are to be considered. These actions should be based on the knowledge that bioburden consists of living microorganisms and bioburden testing determines the product bioburden that has been deposited in and/or on a product in a variety of ways. It is not expected that these microbiological data are precise. Rather it is common that a substantial range is present in microbiological data for bioburden. It is also not expected or necessary that microbiological data for bioburden fit any statistical distribution.

One common method for determining bioburden alert and action levels is through the use of standard deviations. In this instance, the standard deviation calculation is used to understand the dispersion of the data and it is less critical whether the bioburden data fit a particular statistical distribution.

Data identified as unusually high or low, or as atypical of the trend, should be investigated. Atypical data, with identified cause (e.g. laboratory error, occasional high values found in the manufacturing process), can be omitted from calculations in setting the levels for bioburden monitoring. When bioburden data

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are analysed for use in a quality-related decision, individual test outcomes, such as “no growth” or “too numerous to count” (TNTC), are included in the analysis.

A.8.7 Data analysis

Graphical representation of data collected over time can be useful in distinguishing actual trends from sampling variability. Graphical representation can also indicate that a significant change in the microbiological population has occurred even though the bioburden values reside within the pre-set levels.

Before statistical calculations can be performed on data derived from bioburden determinations, especially where many observations are recorded, it can be necessary to manipulate the data in such a way that the significant features are revealed. This can be done in a qualitative manner by grouping the measurements to form frequency tables and charts. Upon completion, the data can be examined for trends.

There are a number of techniques for trending that can be applied to bioburden. These trending techniques can be, but are not limited to, trending of bioburden averages or bioburden estimates, Shewhart control charts (ISO 7870-2), control based on range (BOR), or cumulative sum charts (ISO 7870-4). Each of these different techniques can be used to establish a possible shift from the usual random spread of results and to highlight deviations.

In some instances, it can be appropriate to utilize more than one of these techniques to determine whether or not action is to be taken based upon the available data set or whether additional data are required.

A.8.8 Statistical methods

ISO 13485 requires the planning and implementation of appropriate methods of measurement and analysis, including selecting suitable statistical methods. The examination of data derived from determinations of bioburden for a wide range of products illustrates the variability of such data. Determinations from a group will vary within the group of items, and, therefore, analyses of data generally use means. Clearly, these means can take high, intermediate or low values, and mean values will vary over time. Furthermore, the types of microorganism that comprise the bioburden can also vary.

A commonly observed characteristic of the frequency distributions of data derived from determinations of bioburden is that distributions are skewed and can demonstrate significant tailing. For low or intermediate data, the modal value is zero. In these circumstances, the bioburden is generally low but there can be occasional high values, even though the control measures are effectively applied.

A.9 Maintenance of the method for determining bioburden

A.9.1 Changes to the product and/or manufacturing process

Changes to the product and/or manufacturing process should also be reviewed for any potential effects to the efficacy of the method for determining bioburden. The results of the review should be recorded (see 4.1.2). In some cases, it can be necessary to change and/or requalify the method of bioburden determination.

A.9.2 Changes to the method for determining bioburden

No guidance offered.

A.9.3 Requalification of the method for determining bioburden

No guidance offered.

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Annex B
(informative)

Guidance on methods to determine bioburden

B.1 General

B.1.1 Bioburden determinations can be employed in a variety of situations. The individual responsible for the conduct of such determinations should take into account to what extent method development and validation needs to be performed. In addition, the particular circumstances under which the determinations are made should be considered, e.g. deciding sampling rates, the method to be used, nature of the culture media and relevant incubation conditions.

B.1.2 The sequence of key steps of the process for determining bioburden is illustrated in Figure B.1. The individual responsible for the conduct of such determinations should use knowledge of the raw materials, components, manufacturing environment, production processes and the nature of the product to select appropriate techniques for the various steps. For proper method development and validation it is possible that a combination of different methods might need to be employed initially in order to establish the method(s) most suitable for routine use.

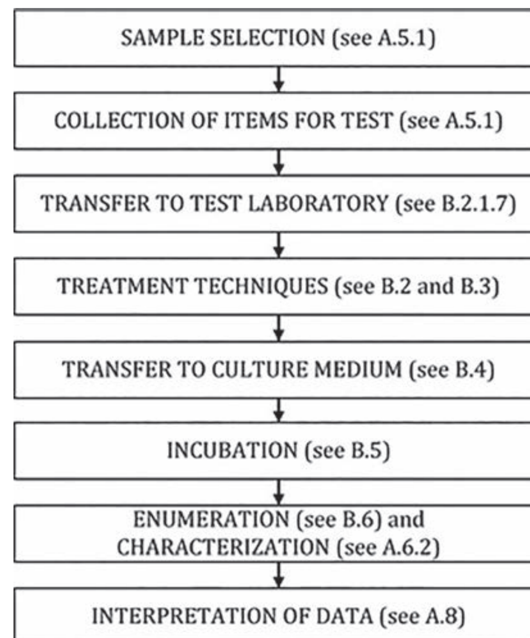

Figure B.1 — Sequence of key steps of the process for determining bioburden

B.2 Methods where removal of microorganisms by elution is used

B.2.1 General

B.2.1.1 Several methods described in this annex can be combined to increase the number of microorganisms found and reduce variability.

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B.2.1.2 The degree of adhesion of microorganisms to surfaces varies with the nature of the surface, the microorganisms involved and other materials present (e.g. lubricants). The origin of the contamination will also influence the degree of adhesion. To remove microorganisms, treatments used can consist of rinsing together with some form of physical force or direct surface sampling. A surfactant can be used to enhance recovery but it should be recognized that surfactants at high concentrations can be inhibitory to the growth of microorganisms.

B.2.1.3 For materials in contact with non-sterile fluids, microorganisms can occur as a biofilm unless appropriate bioburden control measures are taken. A biofilm is a structure in which microorganisms are encapsulated in a matrix that adheres strongly to surfaces. Microorganisms in biofilms can exhibit increased resistance to sterilization processes. Biofilms can initiate quickly and can develop to a much greater extent on health care products incorporating tissue or on used devices. In such instances, consideration should be given to the potential for biofilm formation and it should not be assumed that the treatments outlined in B.2.2 would be appropriate for liberating microorganisms completely from a biofilm. An indication that a biofilm is present can be obtained during validation of the removal technique if repeated high microbial counts are recorded during repetitive recovery. A high level of endotoxin can also be an indication of biofilm.

B.2.1.4 Any treatment used during bioburden determination should be reproducible and should avoid conditions that are likely to affect the viability of microorganisms, such as excessive cavitation, shear forces, temperature rise or osmotic shock.

B.2.1.5 Some treatments are easier to control than others. The variables and ways of controlling them should be considered when selecting a treatment and devising suitable conditions of treatment. For example, for a given treatment, the time can be extended or the nature of mechanical agitation modified to increase the removal of microorganisms.

B.2.1.6 Certain methods of treatment can disaggregate the product under test (e.g. disintegration, processing in a stomacher and vortexing). The presence of disaggregated material can render enumeration of microorganisms difficult. Additional treatment, for example to separate the disaggregated material from the eluent, can be necessary. Care should be taken to ensure that the counts obtained are representative. Certain types of microorganisms are more prone to aggregation/reaggregation than others based primarily on their relative hydrophobicity.

B.2.1.7 Every effort should be made to transfer items for testing to the laboratory as quickly as possible. If delay in transfer is unavoidable, the conditions under which the items are stored should be selected to minimize changes in the microbial population. The maximum storage time should be specified. Desiccation can be the cause of significant decreases in numbers of microorganisms and should be considered in the selection of storage conditions and storage times.

B.2.2 Removal techniques

B.2.2.1 Processing in a stomacher

B.2.2.1.1 The test item and a known volume of eluent are enclosed in a sterile stomacher bag. Reciprocating paddles operate on the bag, forcing the eluent through and around the item.

B.2.2.1.2 The time of treatment should be defined.

B.2.2.1.3 This method is particularly suitable for soft, fibrous and/or absorbent materials but is also unsuitable for any materials that would puncture the bag (e.g. devices containing needles or rigid items).

B.2.2.1.4 This method can yield a suspension having a low concentration of microorganisms if a relatively large volume of eluent is used. If practicable, the eluent should be filtered.

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B.2.2.2 Ultrasonication

B.2.2.2.1 The test item is immersed in a known volume of eluent within a suitable vessel. Either the vessel and contents are treated in an ultrasonic bath or an ultrasonic probe is immersed in the contained eluent. Microorganisms can also be inactivated by ultrasonication, especially with more energy transfer, and inactivation is more likely with probe use than in an ultrasonic bath. The sonication method should be assessed in accordance with B.9.

B.2.2.2.2 The nominal frequency of sonication and duration of treatment should be defined. Furthermore, the position(s) in which items are placed in an ultrasonic bath should be defined. Consideration should be given to limiting the number of items to be processed concurrently as some of the sonication power can be reduced through shielding.

B.2.2.2.3 The technique is particularly suitable for solid impermeable items and for products with complex shapes. It can be destructive to some health care products, particularly those containing electronic components, such as implantable pulse generators.

B.2.2.2.4 The sonication energy and duration of sonication should not cause disruption and death of microorganisms or cause the eluent to overheat.

B.2.2.3 Shaking (mechanical or manual)

B.2.2.3.1 The test item is immersed in a known volume of eluent within a suitable vessel and shaken on a mechanical shaker (e.g. reciprocating, orbital or wrist action) for a defined time/number of cycles in order to assist the removal of microorganisms. Manual shaking can be used but its effectiveness can vary depending on the operator.

B.2.2.3.2 The time and frequency of shaking should be defined.

B.2.2.3.3 Glass beads of a defined size can be added to increase surface abrasion and thereby bioburden recovery efficiency. The size of added glass beads, together with the time and frequency of shaking, should not be such as to cause overheating and/or possible damage to the microorganisms.

NOTE The addition of glass beads will increase the surface area to which microorganisms can adhere.

B.2.2.4 Vortex mixing

B.2.2.4.1 The test item is immersed in a known volume of eluent in a closed container that is placed on the rotating pad of the vortex mixer so that a vortex is created. The vortex produced will depend upon the pressure applied manually. Variations in the vortex can cause variable removal.

B.2.2.4.2 The container to be used, the time of mixing and the speed at which the mixer is set should be defined.

B.2.2.4.3 The method is quick and simple to perform but is mainly suitable for small items in small containers. Variations in removal should be assessed among different individuals operating the vortex mixer.

B.2.2.5 Flushing

B.2.2.5.1 The eluent is passed through the internal lumen of the test item. Liquid flow can be induced by gravity or pumping. Alternatively, the product can be filled with the eluent, clamped and agitated.

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B.2.2.5.2 The time of contact between the device and eluent, the rate of flushing and the volume of fluid should be defined.

B.2.2.5.3 Device configurations and lumen sizes can limit the physical forces necessary to remove microorganisms completely from internal surfaces.

B.2.2.6 Blending (disintegration)

B.2.2.6.1 The test item is immersed in a known volume of eluent within a suitable vessel. The item is blended or chopped for a specified time.

B.2.2.6.2 The specified time depends on the item and the blender, but should not be extended such as to cause overheating of the eluent and possible damage to the microorganisms.

B.2.2.6.3 This technique provides a way of dividing an item into small enough parts so that the microorganisms can be enumerated by a plating technique.

B.2.2.7 Swabbing

B.2.2.7.1 Swabs consist of absorbent material which is usually mounted on some form of stick or handle. The sampling material can be soluble or insoluble.

B.2.2.7.2 The normal method of use is to moisten the swab with eluent and wipe a pre-determined surface area of the item. The bioburden recovery efficiency can be improved in some circumstances by first moistening the surface and then swabbing with a dry swab. The swab is transferred to diluent and agitated to remove microorganisms from the swab. Alternatively, in the case of soluble swabs, the swab is dissolved in diluent.

B.2.2.7.3 Swabs are a useful method of sampling irregularly shaped or relatively inaccessible areas. They are also useful when a large area is to be sampled.

B.2.2.7.4 This technique is particularly prone to errors due to variation in the way the swab is manipulated. Furthermore, it is unlikely that all microorganisms on the surface will be collected by the swab. Some of the microorganisms that are collected can become trapped in the matrix of the swab itself. Because of these issues, recovery using this method is generally low.

B.2.2.7.5 There should be no microbicidal or microbiostatic agents present in the swab.

B.2.3 Eluents, diluents and transport media

B.2.3.1 During bioburden determination, eluents can be used to remove microorganisms from the product. Transport media can be used to transfer removed microorganisms for enumeration and diluents used to obtain suspensions containing microorganisms in countable numbers.

B.2.3.2 The nature of the eluents and diluents can have a marked influence on the overall efficiency of the method used. In selecting a diluent or eluent, consideration should be given to its composition (e.g. constituents and their concentrations, osmolarity and pH). Ideally, the composition should be such that proliferation or inactivation of microorganisms does not occur; however, it might not be possible to ascertain this for all potential contaminants.

B.2.3.3 When a liquid is used for removal of microorganisms from solid surfaces, the incorporation of a mild surfactant can be considered, see Table B1.

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B.2.3.4 Eluents and diluents commonly used include those given in Table B.1.

Table B.1 — Examples of eluents and diluents

Solution	Concentration in water	Applications
Buffered sodium chloride-peptone solution	0,067 M phosphate 0,43 % sodium chloride 0,1 % peptone	General
Calgon Ringer	1/4 strength	Dissolution of calcium alginate swabs
Peptone water	0,1 % to 1,0 %	General
Phosphate buffered saline	0,02 M phosphate 0,9 % sodium chloride	General
Ringer	1/4 strength	General
Sodium chloride	0,25 % to 0,9 %	General
Thiosulphate Ringer	1/4 strength	Neutralization of residual chlorine
Water	N/A	Dilution of aqueous samples. Preparation of isotonic solutions of soluble materials prior to counting
NOTE This list is not exhaustive. A surfactant, such as polysorbate 80, may be added to eluents and diluents. A concentration of between 0,1 % and 1 % is generally used, depending upon the specific application. The appropriate concentration to be used with any particular treatment needs careful selection because foaming can occur.		

B.3 Methods where removal of microorganisms by elution is not used

B.3.1 Contact plating

B.3.1.1 Contact plates or slides are means by which solidified culture medium can be applied to a surface with the intention that viable microorganisms will adhere to the surface of the medium. The plate or slide can then be incubated to produce colonies that are enumerated.

B.3.1.2 Such systems have the advantage of being easy to use. Results are directly related to the area in contact with the solidified culture medium.

B.3.1.3 The natural clumping of cells on surfaces, spreading of colonies at the agar interfaces, drying out of the agar and possibility of anaerobic locations are potential disadvantages.

B.3.1.4 This method should be used only when other methods are not applicable because the efficiency is generally low. Contact plates and slides are generally only useful on flat or at least regular surfaces.

B.3.2 Agar overlaying

B.3.2.1 Agar overlaying involves coating the surfaces of a product with a molten agar medium and allowing it to solidify, followed by incubation to produce visible colonies. This method is not commonly used but can be applicable when the bioburden is low and the product configuration suitable.

B.3.2.2 The natural clumping of cells on surfaces, spreading of colonies at the agar interfaces, drying out of the agar and possibility of anaerobic locations are potential disadvantages. Moreover, some microorganisms will not necessarily persist in a viable state following overlay at an unfavourable temperature, which can result in false-negative results or hinder correct evaluation.

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B.3.3 Most probable number (MPN) approach

B.3.3.1 The MPN method is a well-established and fully documented method for estimating the number of viable microorganisms in a product in which the microorganisms are randomly distributed. The method is particularly appropriate for a product having bioburden of a low mean number.

B.3.3.2 The method consists of taking replicate samples of a product (by volume or weight) which contain, on average, the same number of viable microorganisms in each sample/subsample (hence the requirement for randomness of distribution) and scoring each sample individually for the presence of viable microorganisms by means of transferring to liquid culture media and incubation. Generally, the same culture media and conditions referred to in ISO 11737-2, for seven days, is appropriate. A range of dilutions can be inoculated into nutrient medium such that a fraction of the inoculated media does not produce visible growth on subsequent incubation. From the frequency of the occurrence of positive tests within a set of replicates, an estimate is made of the number of viable microorganisms present in the sample or the bulk product from which the sample has been taken; the 95 % confidence limits about the estimate are relatively wide. The estimate and its confidence limits are derived from published MPN Tables,[26] which have been developed on the assumption that numbers of viable microorganisms present in replicate samples are distributed around a mean number in accordance with Poisson distribution. The FDA BAM Annex 2[27] includes a spreadsheet for calculation of MPN 95 % confidence levels.

B.3.3.3 The key requirement for the application of the MPN method is the random distribution of microbial population throughout the product under investigation. Accordingly, the MPN method can have value for the determination of bioburden for liquid health care products, viscous fluids, powders or in situations where the bioburden is being estimated in a liquid used as an eluent for a single product.

B.3.3.4 MPN methods are simple to perform, and the statistical basis for the method makes it more appropriate for general assessment rather than accurate determinations. The MPN calculation is expressed using Formula (B.1):

$$\mathrm{MPN} = \mathrm{Ln} \times 1 / \mathrm{SIP} \quad \text{(B.1)}$$

where Ln is the number tested/number negative for growth.

B.3.3.5 Results of MPN per product can be equated to results of CFU per product for bioburden enumeration and calculations.

B.3.3.6 If microbicidal or microbiostatic substances are present, the considerations outlined in B.8 will apply.

B.4 Transfer to culture medium

B.4.1 General

B.4.1.1 Treatment will usually produce a suspension of microorganisms. Enumeration of the viable microorganisms in the suspension can be undertaken using one of the techniques described below.

B.4.1.2 Prior to transfer to culture medium, additional treatment can be necessary in order to disrupt aggregates of microorganisms and thereby reduce underestimation. In some cases, the technique used to remove the microorganisms from the item under test can also disrupt such aggregates.

B.4.1.3 The presence of microbicidal or microbiostatic substances will influence the choice of culture method. If microbicidal or microbiostatic substances are present in the eluent, these can be reduced to an ineffective concentration by dilution, removed by filtration or chemically inactivated.

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B.4.2 Membrane filtration

B.4.2.1 Filtration of an eluent, followed by incubation of the filter on an appropriate growth medium to give visible colonies, is an effective means of enumerating viable microorganisms. A filter of appropriate nominal pore size not greater than 0,45µ is generally adequate to capture microorganisms; however, consideration should be given to the use of a smaller pore size if it is expected that the microorganisms present on or in the product warrant this.

B.4.2.2 A vacuum, or in some instances pressure, source is usually required. Care should be exercised in order to avoid excessive backpressures, which can cause distortion of or damage to the membrane filter.

B.4.2.3 Membrane filtration of eluents containing particulates, such as remnants of fibrous products, can be difficult, as the particulates can block the filter.

B.4.2.4 For incubation, the membrane filter can be placed either on an agar surface or on an absorbent pad saturated with liquid nutrient medium. Colonies produced on the surface of the membrane filter are counted and isolated for microbial characterization.

B.4.2.5 Membrane filtration is particularly useful for suspensions of low concentrations of microorganisms.

B.4.2.6 Filtration is also useful when the liquid substrate is suspected of containing microbicidal or microbiostatic substances, as the microorganisms are removed from the eluent and can be washed on the membrane filter prior to incubation. Some types of membrane can absorb or release substances that can inhibit the growth of microorganisms, so it is important that only membrane filters suitable for enumeration of microorganisms be used. The membrane filter and the eluent should be compatible.

B.4.3 Pour plating

B.4.3.1 With a pour plate technique, separate aliquots of a suspension are mixed with molten agar medium at a temperature of approximately 45 °C; the mixture is then allowed to solidify in the petri dish. Plates are incubated and the colonies are counted.

B.4.3.2 Pour plating does not separate microorganisms from the eluent. If microbicidal or microbiostatic substances are present, the considerations outlined in B.8 will apply.

B.4.3.3 The amount of eluent that can be pour-plated is limited. Therefore, this method might not have the desired sensitivity for suspensions with low concentrations of microorganisms.

B.4.3.4 It is desired to keep the agar temperature as low as possible to avoid damage to microorganisms, because even 45 °C can inactivate some environmental microorganisms. Therefore, pour plating has limitations in the types of microorganisms that can be detected, though modifications using carboxy methyl cellulose as a setting agent can be possible in specialized cases.

B.4.4 Spread plating

B.4.4.1 With a spread plate technique, an aliquot of suspension is spread on the surface of a culture medium using a spreading device.

B.4.4.2 The aliquot of suspension that has been spread on the surface of the medium should be absorbed so that discrete colonies can develop; the need for absorption governs the volume of the aliquot that can be processed using one plate.

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B.4.4.3 If microbicidal or microbiostatic substances are present, the considerations outlined in B.8 will apply.

B.4.4.4 The amount of eluent that can be spread-plated is limited. Therefore, this method might not have the desired sensitivity for suspensions with low concentrations of microorganisms.

B.4.5 Spiral plating

B.4.5.1 The spiral plating technique uses automated equipment, which deposits an aliquot of a suspension on the surface of solid medium. The suspension is spread at a decreasing rate in a spiral track from the centre of the culture plate to the periphery. After suitable incubation, the count of viable microorganisms in the original suspension is established using a particular counting grid and counting technique when either total plate or sector counts are the basis for calculations.

B.4.5.2 The spiral plating technique has been shown to give reproducible results that correlate very well with those using conventional serial dilution and surface spreading techniques. Due to the design of the apparatus and the use of capillary tubing and small volumes, spiral plating primarily lends itself to inoculating suspensions that are well dispersed, free from aggregates of material and contain a high concentration of microorganisms

B.4.5.3 If microbicidal or microbiostatic substances are present, the considerations outlined in B.8 will apply.

B.5 Incubation (culture media and incubation conditions)

B.5.1 Examples of some culture media and incubation conditions are given in Table A.2. This list is not all inclusive, and determination of the type(s) of bioburden microorganisms present on products, including by molecular means, can trigger the inclusion or exclusion of these or many other media for microbial culture.

B.5.2 It should be noted that all non-selective, anaerobic culture methods can permit the growth of facultative anaerobic microorganisms. However, the range of such microorganisms can vary considerably with different culture media and incubation conditions.

B.6 Enumeration (counting colonies)

B.6.1 In an enumeration technique using colony counts, procedures should be established to address various situations, such as

a) detecting small colonies (e.g. using a stereo microscope),

b) counting and reporting unusual colonies (e.g. spreaders),

c) enumerating and reporting crowded plates [e.g. obscured colonies or too numerous to count (TNTC) plates], and

d) reporting counts from serial dilutions.

B.6.2 In the enumeration technique using colony counts, consideration should be given to the number of colonies produced on a plate. This number should be such that each viable microorganism is able to express itself as a visible colony without being affected adversely by its near neighbours.

B.6.3 Standard plate counting practice normally specifies a lower limit for the number of colonies on a plate. This limit is based on the availability of multiple dilutions from which to choose. Multiple dilutions do not necessarily apply to bioburden determinations for health care products where the bioburden is low.

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B.6.4 When counting plates the variability in results between technical personnel should be assessed. For an example of a reference to acceptable variability between technicians, see Standard Methods 9215 Heterotrophic Plate Count.

B.6.5 The presence of fibres can prevent the formation of discrete colonies and thereby make enumeration difficult.

B.6.6 The use of an agar layer poured carefully over the surface of the test plate can provide a test result that is easier to enumerate after incubation, if spreading microorganisms are present.

B.6.7 For automated enumeration methods, validation of the system should be performed in accordance with ISO/IEC 17025.

B.6.8 If multiple test conditions are used (e.g. aerobic count from one plate and fungal count from another plate), and there are no colonies recovered, the LOD values are cumulative. For example, if the aerobe count is < 2 CFU and the fungal count is < 2 CFU, then the total count is < 4 CFU.

B.7 Other techniques for detecting microorganisms

Techniques other than colony counts can be used for determining bioburden. These include the measurement of metabolic activity (e.g. impediometry or epifluorescence). Such methods are termed “indirect” because, to have a meaning relative to the numbers of viable microorganisms as defined previously, they have to be calibrated against colony counts. Alternative techniques should be of adequate sensitivity to detect low levels of microorganisms. Normally, the lower limit of numbers detected exceeds 100 CFU.

NOTE Some rapid microbiological methods (e.g. bioluminescence, enzymatic, cytometry) can provide detailed information as to the range and relative numbers of microorganisms present in bioburden and allow assessment of the variability that can occur. They can also provide bioburden information more rapidly than direct culturing.

B.8 Screening for the release of substances affecting bioburden determinations

B.8.1 Screening is aimed at investigating the effects on viability of potentially fragile microorganisms of substances that can be released from the product into a suspending fluid. It is an example of an approach that may be used to assess a technique for conformity with 6.1.2.

B.8.2 Products are selected and each should be subjected to the technique for removal of microorganisms to be used routinely. If the removal technique uses an eluent, B.8.3 can apply, whereas, if the product is introduced directly into medium, B.8.4 can be more appropriate.

B.8.3 The eluent should not inhibit the growth of microorganisms removed from the product.

B.8.4 If the product is to be introduced directly into the growth medium (for example, as in an MPN estimation; see B.3.3), the method suitability test described in pharmacopoeias can be used. In this test, the product is introduced into the medium together with low numbers of microorganisms and incubated under the same conditions as proposed for routine bioburden determination. The number of microorganisms used should be approximately 50 to 100. See B.8.5 for the assessment of results. After a defined period, the medium is examined for visible growth.

If a health care product incorporates an antimicrobial substance that can be released slowly into the medium, then it is appropriate to challenge the product-medium combination with a low number of microorganisms at the end of the incubation period.

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B.8.5 If the number of microorganisms inoculated and the number recovered differ appreciably, or no growth of the microorganisms is observed in a suitability test, the technique for bioburden determination should be reconsidered. It can be necessary to introduce a dilution, neutralization or filtration stage to reduce, inactivate or remove the inhibitory substance(s).

If the effects of the eluent need to be assessed, known numbers of microorganisms can be inoculated into both the eluent and a control solution for a time approximating that proposed for routine bioburden determination. The recovered microorganisms from the eluent are counted at the end of this treatment and compared to the counts from the control solution.

B.9 Screening for the adverse effects of physical stress

Physical forces can be used to remove microorganisms from the product (see B.2.2). The effects of these forces on the bioburden determination should be taken into consideration. If the effects of physical forces need to be assessed, known low numbers (not more than 100 CFU) should be exposed to the physical forces to be used in the absence of the device. Enumeration of the microorganisms gives a measure of the effects of the physical forces.

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Annex C
(informative)

Validation of bioburden recovery efficiency

C.1 General

C.1.1 Before validation

Before starting validation, the removal technique should be justified and defined for each product, or parts thereof, or product group. The documented rationale should be included for the product, sample size, choice of recovery technique, etc.

C.1.2 Grouping of products for purposes of bioburden recovery efficiency

Products, or parts thereof, that are similar can be grouped together as a product group and a representative product chosen for the bioburden recovery efficiency validation. Evaluation criteria for inclusion can include similar types of raw materials, design and size, manufacturing processes, manufacturing environment, manufacturing personnel, and manufacturing location. The results of the bioburden recovery efficiency validation can then be applied to all products in the group for future testing.

C.1.3 Sample size

C.1.3.1 The number of products, or parts thereof, for which the bioburden recovery efficiency is to be determined, should be selected.

C.1.3.2 Common approaches are to utilize three to ten products for recovery validation testing. The sample size should be based primarily on the purpose for which the testing is being performed (e.g. in support of substantiation of a radiation sterilization dose, or an overkill sterilization cycle). When reviewing bioburden recovery efficiency results, a review of the consistency of results, or lack thereof, can indicate that a different extraction method should be applied. Alternatively, a larger sample size can provide a more accurate determination of bioburden recovery efficiency.

C.1.4 Guidance on selection of bioburden recovery efficiency approach

C.1.4.1 Bioburden recovery efficiencies are performed to establish a bioburden correction factor that can be applied to bioburden data to account for microorganisms that remain on the product after the removal technique and/or that are undetected by the culture conditions used. Bioburden data that have been adjusted by inclusion of a bioburden correction factor are understood to more accurately represent the true bioburden count; this is called a bioburden estimate. A bioburden recovery efficiency test can also be used to compare bioburden test methods.

C.1.4.2 The primary determining factor in the selection of a bioburden recovery efficiency approach (i.e. repetitive recovery versus inoculated product) is the level of naturally occurring product bioburden. Generally, the repetitive recovery method is best for products with a higher product bioburden and the inoculated product method is best for products with a lower product bioburden. Bioburden recovery efficiency results and the corresponding bioburden correction factor can differ based on bioburden extraction parameters (e.g. number and type of extractions for repetitive recovery, or use of an inoculated product versus repetitive recovery). Therefore, it is important to consider the reason why bioburden data are being collected and the purpose of the bioburden recovery efficiency determination.

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C.1.4.3 Table C.1 summarizes typical product and method characteristics that should be considered when selecting an appropriate bioburden recovery efficiency approach.

Table C.1 — General considerations for selecting a bioburden recovery efficiency approach

	Recovery methods	
	Repetitive recovery	**Inoculated product**
Principle	Repeated application of a specified technique on an individual sample.	Inoculation of a product with a specified level of a *Bacillus* spore suspension. Other bacteria can also be appropriate, depending on a variety of factors.
Product characteristics	Products with moderate to high (e.g. 100 CFU to 1 000s CFU), or high (e.g. > 1 000 CFU) bioburden. Usually includes the following product types: — products with multiple materials or surfaces; — products with bonded or woven matrices (e.g. fabrics, roll stock, foams); — multiple component products assembled together for use; — products with adhesives/glue; — devices of animal origin, especially when obtained from slaughterhouse materials.	Products with low (e.g. < 100 CFU) or very low (e.g. < 10 CFU) bioburden. Usually includes the following product types: — products that dissolve or disintegrate readily (e.g. soluble materials); — simple plastic devices (e.g. injection moulded with minimal handling); — products with very few components; — products with antimicrobial properties; — products that are cleaned.
Relevance to actual bioburden	Representative of properties and types of natural bioburden.	Less representative of properties and types of natural bioburden. Spores can often be easier to remove than many other bacteria, especially when manually deposited rather than sporulated in situ.
Consistency of results	Less consistent results expected between replicate samples because of variable natural bioburden.	More consistent results expected between replicate samples.
Approximate test time	3 days to 7 days (depending on natural bioburden).	2 days to 5 days (depending on microorganism used).
Test complexity	Can be more labour intensive.	Can be less labour intensive.
Challenges with method	Stability of natural bioburden, inconsistent/variable bioburden.	Clumping during drying, encrustation, adhesion or non-adhesion of the suspension.

C.1.4.4 Complex products with different types of components (e.g. kits, powders) can require more than one type of bioburden recovery efficiency determination if the product is tested in separate containers and/or different parts are tested using different techniques. This can require the application of more than one bioburden correction factor for items tested using different methods.

C.1.4.5 If the bioburden is low, and if a larger sample size of tested products is desired, then multiple products can be tested together as a pooled sample. In this situation, the bioburden distribution on individual products is not observable. If products are intended to be routinely pooled for testing the bioburden recovery efficiency determination should be performed in the same manner. For example, if five products will be pooled during routine testing, the bioburden recovery efficiency determination should be performed with five pooled products.

Bioburden recovery efficiency results of a test of pooled products can be unique to the number of products pooled. If the number of products pooled changes then a new assessment of bioburden recovery efficiency should be considered.

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C.2 Validation using repetitive recovery

NOTE This approach uses the bioburden as it occurs naturally on the product for the validation process. Sometimes it is referred to as “exhaustive recovery”.

C.2.1 General

C.2.1.1 The underlying principle of this approach is that the method of bioburden determination should be repeated until there is a significant decrease seen in the number of microorganisms recovered. After each repetition, the eluent is totally recovered from the product or product portion and enumerated. Results accumulated from the consecutive recoveries are compared. It should be noted, however, that this method is not necessarily precise. The exact relationship between the number of microorganisms recovered and the actual number on the product cannot always be demonstrated.

The exact number of repetitions applied will depend upon a number of factors including the nature of the product, the microorganisms that comprise the bioburden and the initial contamination level. Preliminary experiments or experience with testing similar products can be used to establish the number of repetitions to be applied.

C.2.1.2 The number of colonies counted after initial application of the removal method is expressed as a fraction of the total number of colonies from all repetitions (i.e. bioburden recovery efficiency).

C.2.1.3 Using the aerobe count for repetitive recovery is and has been industry standard. The aerobe count typically constitutes the majority of microorganisms on a health care product, therefore, it is a valid representation of the recovery properties for other types of counts. The repetitive recovery test measures the efficiency of a test method to remove microorganisms based on how they are adhered to the product, so those dynamics will generally apply to all types of microorganisms.

C.2.2 Examples to illustrate calculation of a bioburden correction factor

C.2.2.1 In this example, a set of data for validation by repetitive treatment is shown in Table C.2. The data in this example relate to ten replicate health care products and include five treatments in the repetitive recovery tests.

C.2.2.2 From the data in Table C.2, the proportions removed can be calculated as shown in Table C.3.

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Table C.2 — Example of repetitive recovery data

Product item	Treatment/extraction (CFU)					Total of 5 treatments (CFU)	1st treatment removal
	1	2	3	4	5		
1	450	200	20	10	< 5	685	65,7 %
2	200	120	200	130	20	670	29,9 %
3	90	130	80	20	10	330	27,3 %
4	1 200	550	40	90	60	1 940	61,9 %
5	450	330	20	20	10	830	54,2 %
6	200	285	190	< 5	20	700	28,6 %
7	930	650	650	40	70	2 340	39,7 %
8	1 350	220	280	60	30	1940	69,6 %
9	120	40	50	< 5	5	220	54,5 %
10	480	150	240	60	20	950	50,5 %
Average recovery by first treatment			48,2 %		CF = 2,07 = 2,1		
Worst-case recovery value			27,3 %		CF = 3,66 = 3,7		
NOTE The counts shown in treatment columns 1 through 5 have been adjusted using a dilution factor. It is also acceptable to use unadjusted counts for calculating the recovery efficiency, in which case a count of zero would be acceptable.							

Table C.3 — Example of repetitive recovery data

Product item	1	2	3	4	5	6	7	8	9	10
Number recovered by first treatment	450	200	90	1 200	450	200	930	1350	120	480
Total number recovered	685	670	330	1 940	830	700	2 340	1 940	220	950
Recovery by first treatment	65,7 %	29,9 %	27,3 %	61,9 %	54,2 %	28,6 %	39,7 %	69,6 %	54,5 %	50,5 %
Average recovery by first treatment	48,2 %				Correction factor (CF) = 2,07 = 2,1					
Worst-case recovery value	27,3 %				Correction factor (CF) = 3,66 = 3,7					

C.2.2.3 Using average recovery by first treatment and appropriate rounding, the bioburden correction factor for bioburden recovery efficiency would be as shown by Formula (C.1):

$$\frac{100}{48,2} = 2,07 = 2,1 \qquad (C.1)$$

For some applications, it can be appropriate to use the lowest recovery percentage value in order to reflect the worst case. This decision can be dependent on the purpose for which the bioburden estimates are to be used. For the data presented in Table C.2, the worst case bioburden correction factor including appropriate rounding, would be as shown by Formula (C.2):

$$\frac{100}{27,3} = 3,66 = 3,7 \qquad (C.2)$$

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C.3 Product inoculation method

C.3.1 Validation using inoculated products

C.3.1.1 An artificial bioburden can be created by inoculating a known number of a selected microorganism onto the product in order to establish bioburden recovery efficiency. The microorganisms can be vegetative cells but the most common approach utilizes aerobic bacterial spores. The use of vegetative microorganisms is difficult in practice because loss of viability can occur on drying.

Microbial inoculation has limitations, such as encrustation, adhesion or non-adhesion of the suspension, and clumping and variation in the level of the inoculum. These limitations should be taken into account when inoculating products.

C.3.1.2 A suspension of the microorganisms with which the product is to be inoculated should be prepared and its viable count determined.

C.3.1.3 Preliminary experiments can be necessary to establish the appropriate dilution. Typically it is appropriate to deposit a known level of viable microorganisms on the product which will result in a countable range during the plate count step.

C.3.1.4 A number of products, or parts thereof, for which the bioburden recovery efficiency is to be determined should be selected. Consideration should be given on a case-by-case basis as to whether a sterile product is necessary. Each product is inoculated with a volume of the suspension of microorganisms and, if appropriate for the particular product, allowed to dry under laminar airflow conditions. The viable count of the inoculum is determined at the time of inoculation.

The suspension should be distributed on the product in such a way that the part from which it is most difficult to remove natural contamination is included. The various material types of the product should also be considered for inoculation.

Inoculation of products made of absorbent materials can be accomplished by immersion into a suspension of a selected microorganism. This method can produce an even distribution of microorganisms on the product.

C.3.1.5 The defined method of determination of bioburden is used to assess the number of inoculated microorganisms that are removed from the product.

C.3.1.6 The number of microorganisms removed is expressed as a fraction of the number inoculated on to the product. This fraction can be calculated for each product and used to establish a bioburden recovery efficiency.

C.3.2 Example to illustrate calculation of a bioburden correction factor using product inoculation

C.3.2.1 In this example, a set of data for validation by inoculated recovery is shown in Table C.4. These data relate to three replicate product items.

C.3.2.2 For validation, a product inoculation method was selected because preliminary experiments indicated that the bioburden was very low.

C.3.2.3 An aqueous suspension of *Bacillus atrophaeus* (formerly *Bacillus subtilis* var *niger*) was prepared and the viable count of the suspension was determined.

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C.3.2.4 A dilution of the suspension was prepared such that 0,1 ml aliquots contained an average of 100 spores. Each device was inoculated with 0,1 ml of this diluted suspension and allowed to dry under laminar airflow.

C.3.2.5 The inoculated products were subjected to the chosen removal technique and the mean number of spores removed was 76, with a range from 68 to 83.

Table C.4 — Sample data for validation by inoculated recovery

Average inoculum count CFU	**Sample**	**Recovered inoculum count** CFU	**Recovery efficiency** %
100	1	76	76,0
	2	83	83,0
	3	68	68,0
		AVG recovery	75,7

C.3.2.6 The bioburden correction factor for bioburden recovery efficiency, including appropriate rounding, would be as shown by Formula (C.3):

$$\frac{100}{75{,}7}=1{,}32=1{,}3 \quad \text{(C.3)}$$

In some applications, it can be decided to use the lowest value of the range of percentage removals in order to reflect the worst case. This decision will be influenced by the use to be made of the data. For the above data the worst case bioburden correction factor, including appropriate rounding, would be as shown by Formula (C.4):

$$\frac{100}{68}=1{,}47=1{,}5 \quad \text{(C.4)}$$

C.3.3 Example to illustrate comparisons of two bioburden recovery efficiency methods

C.3.3.1 In this example, two sets of data for recovery validation are shown in Table C.5. The company established internal acceptance criteria for bioburden recovery efficiency based on risk. These data relate to five products that were tested with one technique (initial test), where the average recovery was below the established criteria. Consequently an additional step was added to the existing technique to determine if the bioburden recovery efficiency was improved (second test).

Table C.5 — Comparison of bioburden recovery efficiency percentage for two recovery methods

Technique	**% Bioburden recovery efficiency**					**Average recovery**
	1	**2**	**3**	**4**	**5**	
Initial test 5 min mechanical shaking with USP Fluid D	37,3	25,2	50,2	33,7	29,5	35,2
Second test 5 min mechanical shaking with USP Fluid D + 2 min sonication of sample	60,2	64,7	72,1	68,2	54,5	63,9

C.3.3.2 After modifying the original technique, the bioburden recovery efficiency did improve and met the established criteria. The purpose for bioburden data and the accuracy needed will influence whether or not more consistent data are required or if the bioburden recovery efficiency should be higher in order to better estimate the recovered bioburden.

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C.4 Bioburden recovery efficiency for complex product testing

C.4.1 In the example shown in Table C.6 multiple recovery methods are needed to estimate the bioburden level of a complex product. This example shows how two different bioburden correction factors can be applied to the respective groups of bioburden data. In order to establish the bioburden level of this complex product, all three of the bioburden estimates should be added together.

C.4.2 When testing tissue and biological products, additional guidance can be obtained from AAMI TIR37. Products that intentionally break down over time (e.g. drug eluting or bio-absorbable products) should account for these alterations in development of the bioburden recovery efficiency test method.

Table C.6 — Complex product bioburden estimate determined by utilizing two bioburden correction factors and MPN result

Container	% Bioburden recovery efficiency Product portion #1 Tested using flushing method	% Bioburden recovery efficiency Product portion #2 Tested using mechanical shaking for five (5) minutes	% Bioburden recovery efficiency Product portion #3 Tested using MPN of a powder material
1	49,5	79,3	N/A
2	53,9	89,4	N/A
3	38,4	67,4	N/A
4	64,3	76,0	N/A
5	29,7	69,3	N/A
Average recovery efficiency	47,2	76,3	N/A
Corresponding correction factor	2,1	1,3	N/A[a]
Complex product practical application of bioburden recovery efficiencies and MPN results			
Bioburden recovery (in CFU)	5	100	80 including dilution factors
Bioburden estimate (in CFU)	2,1 × 5 = 10,5	1,3 × 100 = 130, 0	80
Total bioburden estimate for product (in CFU)	10,5 + 130,0 + 80 = 220,5		

[a] Application of dilution factor can be required.

C.5 Data analysis and application of bioburden correction factor

C.5.1 Due to the variability of design, materials, product configurations, manufacturing processes, etc., it is not required by this document that a particular bioburden recovery efficiency result be obtained. However, if bioburden recovery efficiency results fall below a target or desired value, another technique should be attempted (e.g. addition of another extraction method or lengthening the current extraction method) to determine if better results can be obtained.

Items that can be considered in determination of a desired bioburden recovery efficiency value for a health care product include the following:

a) sterilization validation approach (e.g. overkill versus bioburden based);

b) use of bioburden data (e.g. support sterilization validation approach, raw material screening, trending);

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c) the type of product or material being testing (e.g. plastics and metals versus absorbent materials);

d) robustness of recovery method used (e.g. ultrasonication, shaking, or a combination of both).

Based on these concepts, a low bioburden recovery efficiency (e.g. 20 % for an absorbent or complex product) can be considered acceptable. Consideration for use of the lowest recovery percentage value to reflect the most conservative worst case estimate can be appropriate, as described in C.2.2.3 and C.3.2.6. Also, it should be noted there are times that determination of bioburden recovery efficiency is not necessary (e.g. component or raw material screening, or if the product is a liquid in which the entire contents are being filtered).

In microbiological test methods it is expected to obtain more variability than is typically observed in more predictable physical science test methods (e.g. chemistry or physics). This greater variability is largely due to the fact that microorganisms are viable and the number of microorganisms can change over time depending on conditions. Other factors that also influence bioburden recovery efficiency can include clumping of microorganisms, consistency of microorganisms depositing on the product surface, product's surface characteristics (e.g. coatings with specific silicone material, high porous surface areas), incubation conditions, and/ or inherent limitations in the ability to detect or measure the microorganisms.

Nevertheless, unexpectedly low or widely distributed bioburden recovery efficiency might not be appropriate depending on the criticality and purpose of the bioburden data, and, if this is the case, further improvement of the removal technique (e.g. enhanced by disassembling, more intensive mechanical shaking, active rinsing of cavities, prolongation of rinsing time, modification of eluent) should be investigated. An example where criticality and purpose of the bioburden data can warrant more effort and resources to obtain better recovery results is when the bioburden data are used to establish a "bioburden-based" sterilization process (e.g. radiation sterilization, and, in particular, dose substantiation methods that require low bioburden counts). An example where criticality and purpose of the bioburden data might not warrant more effort and resources to obtain better recovery results can be for the application of bioburden component screening.

C.5.2 In calculating a bioburden recovery efficiency it is not necessary to apply the limit of detection (e.g. a "less than" value) to zero CFU values.

C.5.3 When reviewing bioburden recovery efficiency results it is appropriate to round all values to one decimal place.

C.5.4 The bioburden correction factor is applied to bioburden data by multiplying the bioburden average by the correction factor. When the bioburden correction factor has been applied the resulting value is termed bioburden estimate. In some applications, it can be decided to apply the lowest bioburden recovery efficiency value of the range obtained to determine the bioburden correction factor to reflect the worst case. This decision will be influenced by the use to be made of the data.

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Annex D
(informative)

Typical assignment of responsibilities

The manufacturer and laboratory should have an agreement that assigns responsibilities (4.2.2) for the completion of the requirements as defined in this document. Ultimately, the manufacturer is responsible to ensure that the requirements are met. This annex gives information on typical assignments. The requirements given in Table D.1 are abbreviated. See the specific clause for details regarding each requirement.

Table D.1 — Typical assignment of responsibilities

Clause	Requirements of this document	Typical responsibility	
		Manufacturer	Laboratory
Quality management system elements			
4.1.1	Specification of procedures	R	R
4.1.2	Review and approval of documents and records	R	R
4.1.2	Control of documents and records	R	R
4.1.3	Content of records	N/A	R
4.1.3	Identity of personnel	N/A	R
4.1.4	Check of calculations and data transfer	N/A	R
4.2.1	Implement and perform procedures	N/A	R
4.2.2	Assignment of responsibilities	R	R
4.2.3	Equipment availability	N/A	R
4.3.1	Procedures of purchasing	N/A	R
4.3.2	Equipment calibration	N/A	R
4.3.3	Preparation and sterilization of materials	N/A	R
4.4.1	Uncertainty measurements	N/A	N/A
4.4.2	Investigation of results, corrective and preventive actions	R	I
Selection of product			
5.1.1	Selection and sampling of products	R	I
5.1.2	Rationale for product family	R	I
5.1.3	Timing of performance of sampling	R	I
5.2	Sample item portion (SIP)	R	I
Methods of determination and microbial characterization of bioburden			
6.1.1	Method selection	R	R
6.1.2	Minimization of inhibiting effects	I	R
6.1.3	Efficiency of bioburden removal	I	R
6.1.4	Selection of culture conditions	I	R

Key

R = responsibility

I = this can involve providing assistance or information

N/A = not generally applicable

NOTE The general capability of the test method is shown and documented as part of the fundamental method validation of the laboratory. Product specific aspects of validation are documented as part of product specific reporting.

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Table D.1 *(continued)*

Clause	Requirements of this document	Typical responsibility	
		Manufacturer	Laboratory
6.1.5	Selection of the enumeration techniques	N/A	R
6.2.1	Selection of techniques for microbial characterization	R	R
Validation of the method for determining bioburden			
7.2 a)	Test method suitability	R	R
7.2 b)	Technique for removal	R	R
7.2 c)	Adequacy for enumeration	N/A	R
7.2 d)	Microbial characterization	N/A	R
Routine determination of bioburden and interpretation of data			
8.1	Sampling plan	R	I
8.2	Selection of test methods	R	R
8.3	Degree of microbial characterization	R	I
8.4	Consideration of applicable standards and requirements	R	I
8.5	Handling of spikes	R	I
8.6	Specification of acceptable levels	R	N/A
8.7	Trending	R	N/A
8.8	Application of statistical methods	R	N/A
Maintenance of the method for determining bioburden			
9.1	Consideration of manufacturing/process changes	R	I
9.2	Change of test methods	I	R
9.3	Review of method validation data	R	R

Key

R = responsibility

I = this can involve providing assistance or information

N/A = not generally applicable

NOTE The general capability of the test method is shown and documented as part of the fundamental method validation of the laboratory. Product specific aspects of validation are documented as part of product specific reporting.

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