List of Titles

American National Standard

ANSI/AAMI/ISO 11737-1:1995

Sterilization of medical devices—Microbiological methods—Part 1: Estimation of the population of microorganisms on product

AAMI Association for the Advancement of Medical Instrumentation

List of Titles

Association for the Advancement of Medical Instrumentation
1110 N. Glebe Rd., Suite 220
Arlington, VA 22201-4795

Copyright and Permissions

11737-1 Estimation of Population of Microorganisms on Products

American National Standard

ANSI/AAMI/ISO 11737-1—1995

Sterilization of medical devices—Microbiological methods—Part 1: Estimation of population of microorganisms on products

Approved 24 July 1995 by
Association for the Advancement of Medical Instrumentation

Approved 11 September 1995 by
American National Standards Institute, Inc.

Abstract:

This standard specifies general criteria to be applied in the estimation of the population of viable microorganisms on a medical device or component, raw material or package thereof. This estimation consists of both enumeration and characterization of the population.

Association for the Advancement of Medical Instrumentation

The adoption of this International Standard as an American National Standard was approved by the AAMI Microbiological Methods Working Group, under the auspices of the AAMI Sterilization Standards Committee. Committee approval of the recommended practice does not necessarily imply that all committee, sub-committee, and working group members voted for its approval.

The **AAMI Sterilization Standards Committee** has the following members:

Cochairs:
Carl W. Bruch, PhD
Virginia C. Chamberlain, PhD

Members:
Carl W. Bruch, PhD, Consultant, Hudson, WI
Virginia C. Chamberlain, PhD, Center for Devices and Radiological Health, Food and Drug Administration
Neal E. Danielson, HCA Wesley Medical Center, Wichita, KS
Judith C. Dowler, Medical Devices Bureau, Health Canada, Ottawa, ON
Frank B. Engley, Jr., PhD, Univ. Missouri School of Medicine, Columbia, MO
Collette P. Keyser, RN, Alexandria, VA (Colonel, U.S. Army, Retired)
Robert F. Morrissey, PhD, Johnson & Johnson
Barry F.J. Page, Consultant, Garner, NC
Marimargaret Reichert, RN, MA, Reichert Consulting, Olmsted Falls, OH
Janet K. Schultz, RN, AMSCO International
James Whitbourne, Sterilization Technical Services
James L. Whitby, MA, MB, FRCP, Univ. Of Western Ontario, London, ON

The **AAMI Microbiological Methods Working Group** has the following members:

Cochairs:
Trabue D. Bryans
Harry L. Shaffer

Members:
Krisann Anderson, St. Jude Medical, St. Paul, MN
Edward Arscott, North American Science Associates, Inc.

Joy Barnitz, Bausch & Lomb, Inc.
Anne F. Booth, MS, Booth & Associates, Barrington, IL
Trabue D. Bryans, Practical Consulting and Training, Kennesaw, GA
Virginia C. Chamberlain, PhD, Center for Devices and Radiological Health, Food and Drug Administration
Gary N. Cranston, Consulting & Technical Services
Douglas D. Davie, Sterilization Validation Services
Joyce M. Hansen, Baxter Healthcare Corporation
Deborah Havlik, Griffith Micro Science, Inc.
Craig M. Herring, Johnson & Johnson
Mary S. Mayo, C. R. Bard, Inc.
John Nygard, Advanced Bio-Control Technologies
Gordon S. Oxborrow, 3M
Robert R. Reich, Pharmaceutical Systems, Inc.
Zenius V. Seliokas, Davis & Geck Company
Harry L. Shaffer, Titan Corporation
Robert Tomaselli, Convatec, Bristol-Myers Squibb
Thelma Wilcott, Becton Dickinson & Company

Alternates: Richard H. Bean, Bristol-Myers Squibb
Daniel J. Burgess, Pharmaceutical Systems, Inc.

NOTE—Participation by federal agency representatives in the development of this standard does not constitute endorsement by the federal government or any of its agencies.

Background of ANSI/AAMI adoption of ISO 11737-1:1995

Sterilization of medical devices — Microbiological methods — Part 1: Estimation of population of microorganisms on products

The International Organization for Standardization (ISO) is a worldwide federation of national standards bodies. The United States is one of the ISO members that took an active role in the development of this standard.

ISO 11737-1 was developed by ISO Technical Committee (TC) 198, *Sterilization of health care products* to fill a need for an international standard for the estimation of the population of microorganisms on medical devices. TC 198 approved the standard in 1995, and it was published by ISO in 1995.

United States participation in ISO/TC 198 is organized through the U.S. Technical Advisory Group for ISO/TC 198, administered by the Association for the Advancement of Medical Instrumentation (AAMI). AAMI also administers the Secretariat for TC 198 on behalf of the United States, which made a considerable contribution to this standard and holds the convenership of the responsible international working group.

AAMI encourages its committees to harmonize their work with international standards as much as possible. The proposal to adopt ISO 11737-1 in the United States was made by the AAMI Microbiological Methods Working Group. At the time part 1 was published in the United States, the working group was reviewing ISO/DIS 11737-2, *Sterilization of medical devices—Microbiological methods—Part 2: Tests of sterility performed in the validation of a sterilization process*, for adoption as a new American National Standard. Part 2 specifies the general criteria for tests of sterility on medical devices that have been exposed to a treatment with the sterilizing agent, which is a fraction of the specified sterilization process, and upon finalization will supersede AAMI TIR 8, *Microbiological methods for gamma irradiation sterilization of medical devices.*

AAMI (and ANSI) have adopted other ISO standards, some of which are referenced in this document. See the next page for a list of ISO standards adopted by AAMI, the corresponding U.S. designation, and the level of equivalency with the ISO standard.

The concepts incorporated in this standard should not be considered inflexible or static. In addition, AAMI and ANSI procedures require that standards be reviewed and, if necessary, revised every 5 years, and this standard, like any other, will be updated periodically to assimilate progressive technological developments as new data come to light.

Suggestions for improving this standard are invited. Comments and suggested revisions should be sent to Standards Department, AAMI, 3330 Washington Boulevard, Suite 400, Arlington, VA 22201.

NOTE—Beginning with the ISO foreword on page vii, this American National Standard is identical to ISO 11737-1:1995.

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 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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75% of the member bodies casting a vote.

International Standard ISO 11737-1 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products,* and is based on three European Standard drafts prepared by Working Group 5 of CEN Technical Committee 204, *Sterilization of medical devices.*

ISO 11737 currently consists of the following parts, under the general title *Sterilization of medical devices—Microbiological methods:*

— *Part 1: Estimation of the population of microorganisms on products*

— *Part 2: Tests of sterility performed in the validation of a sterilization process*

Additional parts will be published later.

Annexes A, B, and C of this part of ISO 11737 are for information only.

Introduction

A sterile product item is one which is free of viable microorganisms. The International Standards for sterilization of health care products require, when it is necessary to supply a sterile product item, that adventitious microbiological contamination of a health care product from all sources is minimized by all practical means. Even so, product items produced under standard manufacturing conditions in accordance with the requirements for quality systems for health care products may, prior to sterilization, have microorganisms on them, albeit in low numbers. Such product items are nonsterile. The purpose of sterilization processing is to inactivate the microbiological contaminants and thereby transform the nonsterile items into sterile ones.

The inactivation of a pure culture of microorganisms by physical and/or chemical agents used to sterilize health care products often approximates to an exponential relationship; inevitably this means that there is always a finite probability that a microorganism may 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 organisms exist during treatment. It follows that the sterility of any one item in a population of items subjected to sterilization processing cannot be guaranteed and the sterility of the processed population of items has to be defined in terms of the probability of the existence of a nonsterile item in that population.

Requirements for the quality system for the design/development, production, installation and servicing of health care products are given in ISO 9001 and ISO 9002. The ISO 9000 series of International Standards designates certain processes used in manufacture as "special" if the results cannot be fully verified by subsequent inspection and testing of the product. Sterilization is an example of a special process because process efficiency cannot be verified by inspection and testing of the product. For this reason, sterilization processes have to be validated before use, the performance of each process monitored routinely and the equipment properly 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 ISO 11134, ISO 11135 and ISO 11137). 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. Indeed for the effective validation and routine control of a sterilization process, it is also important to be aware of the microbiological challenge which is presented to that process, in terms of number, identities and properties of microorganisms.

The presterilization microbiological contamination is the sum of contributions from a number of sources; therefore attention also has to be given to factors including the microbiological status of incoming raw materials and/or components and their subsequent storage, and to the control of the environment in which the product is manufactured, assembled and packaged.

The term "bioburden" is commonly used to describe the population of viable microorganisms present on a material or product. It is not possible to determine the exact bioburden and therefore, in practice, a viable count is determined using a defined technique. Validation exercises are performed to relate this viable count to a bioburden estimate on a material or product by the application of a correction factor.

The knowledge of the bioburden results from the investigation of microbiological contamination levels. Bioburden estimations are performed in a number of separate situations as part of the:

a) validation and revalidation of a sterilization process for which the extent of exposure to sterilizing conditions is to be directly related to the bioburden estimate;

b) validation and revalidation of a sterilization process for which the extent of exposure to sterilizing conditions is not to be directly related to the bioburden estimate, but for which a general knowledge of bioburden is required;

c) routine control of the manufacturing process for a sterile product for which sterilization validation was as stated in a) above;

d) routine control of the manufacturing process for a sterile product for which sterilization validation was as stated in b) above.

Bioburden estimations may also be employed as part of the quality system for the manufacture of health care products as an element of:

e) an overall environmental monitoring program;

f) the assessment of the efficiency of a cleaning process in removing microorganisms;

g) the process monitoring for products which are supplied nonsterile but for which the microbiological

cleanliness is specified;

h) the monitoring of raw materials, components or packaging.

The bioburden estimation of a medical device generally consists of four distinct stages:

— removal of microorganisms from the medical device;

— transfer of these isolated microorganisms to culture conditions;

— enumeration of the microorganisms with subsequent characterization;

— application of the correction factor(s) determined during bioburden recovery studies in order to calculate the bioburden estimate from the presterilization count.

It is not possible to define a single technique to be used for the removal of microorganisms in all situations because of the wide variety of materials for construction and design of health care products. Furthermore, the selection of conditions for enumeration will be influenced by the types of contaminant which may be anticipated.

This part of ISO 11737 therefore specifies the general criteria to be applied to the estimation of bioburden. The annexes of this part of ISO 11737 provide additional guidance (annex A) and methods which may be used for validating the technique (annex B).

Sterilization of medical devices— Microbiological methods—Part 1: Estimation of population of microorganisms on products

1 Scope

1.1 This part of ISO 11737 specifies general criteria to be applied in the estimation of the population of viable microorganisms on a medical device or component, raw material or package thereof. This estimation consists of both enumeration and characterization of the population.

NOTES

1 Prior to routine use, a technique for estimating the population of microorganisms on product is validated. The level to which identification is necessary during characterization depends on the use to be made of the data generated.

2 Annexes to this part of ISO 11737 provide guidance on selection of a technique and outline method(s) which may be used to validate the technique selected.

1.2 This part of ISO 11737 is not applicable to the enumeration or identification of viral contamination.

This part of ISO 11737 is not applicable to the microbiological monitoring of the environment in which medical devices are manufactured.

NOTE 3 Attention is drawn to the International Standards for quality systems (see ISO 9001 and ISO 9002) which control all stages of manufacture including the sterilization process. It is not a requirement of this part of ISO 11737 to have a complete quality system during manufacture, but certain elements of such a system are required and these are normatively referenced at appropriate places in the text.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 11737. At the time of publication, the edition indicated was valid. All standards are subject

to revision, and parties to agreements based on this part of ISO 11737 are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below.

Members of the IEC and ISO maintain registers of currently valid International Standards.

ISO 9001:1994, *Quality systems—Model for quality assurance in design, development, production, installation and servicing.*

3 Definitions

For the purposes of this part of ISO 11737, the following definitions apply.

3.1 bioburden: Population of viable microorganisms on a product and/or a package.

3.2 bioburden estimate: Value established for the number of microorganisms comprising the bioburden by applying to a viable count or presterilization count a factor compensating for the recovery efficiency.

3.3 characterization: General process in which microorganisms are grouped into broad categories.

NOTE 4 Categories may be based, for example, on colony or cellular morphology, staining properties or other characteristics.

3.4 correction factor: Numerical value applied to a viable count or presterilization count to compensate for the incomplete removal of microorganisms from product and thus produce a bioburden estimate.

3.5 culture conditions: Stated combination of conditions, including the growth medium with the period and temperature of incubation, used to promote growth and multiplication of microorganisms.

3.6 medical device: Any instrument, apparatus, appliance, material or other article, whether used alone or in combination, including the software necessary for its proper application, intended by the manufacturer to be used for human beings for the purposes of

— diagnosis, prevention, monitoring, treatment or alleviation of disease;

— diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap;

— investigation, replacement or modification of the anatomy or of a physiological process;

— control of conception;

and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means.

3.7 presterilization count: Viable count obtained prior to sterilization.

3.8 product: Generic term used to describe raw materials, intermediate products, subassemblies and finished medical devices.

3.9 recovery efficiency: Measure of the ability of a specified technique to remove microorganisms from product.

3.10 revalidation: Set of documented procedures to confirm an established validation.

3.11 sample item portion (SIP): Defined portion of a health care product unit that is tested.

3.12 validation: Documented procedure for obtaining, recording and interpreting the results needed to show that a process will consistently yield a product complying with predetermined specifications.

NOTE 5 In the context of estimating the bioburden, the "process" is the test methodology and the "product" is the test result. The validation of a technique for bioburden estimation consists of a series of investigations to determine the effectiveness and reproducibility of the test method.

3.13 viable count: Number of microorganisms estimated by growth of discrete colonies under the stated culture conditions.

NOTE 6 A discrete colony may not necessarily originate from a single viable microorganism.

4 General

4.1 Documentation

4.1.1 Documented procedures and instructions on the testing techniques to be employed and the use and operation of all relevant equipment shall be available. These procedures and instructions shall be approved on issue and shall be controlled as specified in ISO 9001.

4.1.2 The procedures and instructions required by this part of ISO 11737 shall be implemented effectively.

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

NOTE 7 If calculations are performed by electronic data processing techniques, the software should be validated prior to use and records of this validation should be retained.

4.1.4 Records of all original observations, calculations, derived data and final reports shall be retained as specified in ISO 9001. The records shall include the identity of all personnel involved in sampling, preparation and testing.

4.2 Personnel

4.2.1 Responsibility for bioburden estimation shall be assigned to specific personnel as specified in ISO 9001.

4.2.2 Training shall be performed in accordance with documented procedures. Records of the relevant qualifications, training and experience of technical personnel shall be maintained.

4.3 Equipment

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

4.3.2 All equipment requiring planned maintenance shall be maintained in accordance with documented procedures. Records of maintenance shall be retained.

4.3.3 An effective system shall be established, documented and maintained for the calibration of all equipment having measurement or control functions. This calibration system shall comply with ISO 9001.

4.4 Media and materials

Methods shall be established and documented for the preparation and sterilization of materials used in bioburden estimation, including appropriate quality tests.

NOTE 8 Appropriate quality tests should include growth promotion tests on batches of media/each batch of medium.

5 Selection of product units

5.1 Product unit selection

The procedures for selection and procurement of product for testing shall be established to ensure that the product is representative of routine production.

5.2 Sample item portion (SIP)

If a sample item portion (SIP) of less than one whole product unit is to be used, it shall be selected to

possess microorganisms representative of the whole product. If it has been demonstrated that the microorganisms are evenly distributed on the product, the portion shall be selected from any single location. In the absence of such a demonstration, the portion shall be made up of pieces of product from several locations.

NOTE 9 The standards specifying the requirement for validation and routine control of the sterilization process should stipulate the criteria for the adequacy of SIP.

6 Selection of technique

6.1 For an identified product, factors relevant to the efficiency of removal of viable microorganisms from product shall be considered and recorded, if such removal is part of the technique. Factors shall include:

a) ability to remove microbiological contamination;

b) possible type(s) of contaminating microorganisms and their locations on product;

c) effect(s) of the removal method on the viability of microbiological contamination;

d) the physical or chemical nature of product to be tested.

6.2 If the physical or chemical nature of product to be tested [see item d) of **6.1**] is such that substances may be released which would adversely affect either the number or the types of microorganisms detected, then a system to neutralize, remove or, if this is not possible, minimize the effect of any such released substance shall be used. The effectiveness of each system shall be demonstrated.

NOTE 10 Annex B describes methods which may be used to assess the release of microbicidal or microbiostatic substances.

6.3 Culture conditions shall be selected after consideration of the types of microorganisms expected to be present. The results of this consideration and the rationale for the decisions reached shall be documented.

6.4 The selected technique shall be validated as specified in clause 7.

7 Validation of technique

7.1 Each procedure for the validation of bioburden estimations shall be documented.

7.2 The validation procedures shall consist of the following steps:

a) assessment of the adequacy of the technique used to remove microorganisms from the product, if such removal is part of the technique;

b) assessment of the adequacy of the technique used to enumerate removed microorganisms, including microbiological counting techniques and culture conditions; and

c) establishment of the recovery efficiency of the method used in order that the correction factor can be calculated.

NOTE 11 Annex B describes methods which may be used in the validation of techniques for bioburden estimation.

7.3 Any change in a routine method shall be assessed. This assessment shall include:

a) evaluation of the change;

b) establishment of the recovery efficiency of the revised method.

NOTE 12 The assessment of the change may indicate that the previous validation and recovery efficiency are still applicable.

7.4 The validation and any subsequent revalidation data shall be reviewed periodically and the extent of revalidation determined and documented. Procedures for the review of validation and revalidation shall be documented and records of the revalidation shall be retained.

The revalidation report shall be signed by the same functions/organizations that prepared, reviewed and accepted the original validation report.

8 Use of technique

8.1 Presterilization counts shall be performed in accordance with documented sampling plan(s) with defined sampling frequency and sample size.

8.2 If contaminants that are not normally encountered are isolated while performing presterilization counts, they shall be characterized. The influence of such contaminants on the manufacturing process, including the effectiveness of the sterilization process, shall be considered and documented.

8.3 Acceptable limits for either presterilization counts or bioburden estimates shall be established on the basis of previous data and documented. If these limits are exceeded, corrective action shall be undertaken as specified in ISO 9001. Established limits shall be reviewed formally at defined intervals and revised if necessary.

8.4 If statistical methods are used to define sample size, sampling frequency and/or acceptance limits, they shall conform with ISO 9001.

8.5 When presterilization counts are to be used to determine the extent of treatment of a sterilization process (unless a requirement in a standard for the validation of the particular sterilization process specifies otherwise), then:

a) a correction factor, based on the recovery efficiency, as determined during validation (see 7.2), shall be applied to the presterilization count to calculate the bioburden estimate before the extent of treatment is determined; and

b) the resistance of the microorganisms comprising the population present on product shall be considered in determining the extent of treatment.

NOTE 13 In applying microbiological data to establishing a sterilizing dose for sterilization by irradiation (see annex B of ISO 11137:1995 and see ISO 13409) a presterilization count may be used to select the verification and sterilizing doses.

8.6 If bioburden estimates have been used to determine the extent of treatment of the sterilization process:

a) consideration shall be given to the effect on the assurance of sterility if the acceptable limits are exceeded; and

b) the characterization of contaminants that are not normally encountered shall include an estimation of the resistance of those contaminants to the sterilization process. The consequences of the presence on product of contaminants with high resistance to the sterilization process on the assurance of sterility shall be considered.

All these considerations shall be documented and included in the determination of corrective action. This corrective action shall be conducted in accordance with ISO 9001.

8.7 Changes to product and/or processes shall be reviewed formally to determine whether they are likely to result in a change in the bioburden (see also 8.3). The results of the review shall be documented. If a change in bioburden is envisaged, specific bioburden estimations shall be performed to evaluate the effects of the change.

Annex A
(informative)

Guidance on estimating population of microorganisms on product

A.1 Introduction

This annex contains guidance on the implementation of the requirements specified in this part of ISO 11737, and is aimed at providing better understanding of the requirements. 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 effective in achieving compliance with the requirements of this part of ISO 11737.

This annex is not intended as a checklist for assessing compliance with the requirements of this part of ISO 11737.

A.2 General

In order that the data obtained from performing bioburden estimations will be reliable and reproducible, it is important that the estimations are performed under controlled conditions. The laboratory facilities used for the estimations, whether on the site of the manufacturer of the medical device or located at a remote location, should therefore be managed and operated in accordance with a documented quality system.

If bioburden estimations are performed in a laboratory under the direct management of the manufacturer of the medical device, the operation of the laboratory should be within the manufacturer's quality system. If an external laboratory is used, it is recommended that such a laboratory be formally certified against an appropriate ISO document (e.g. ISO/IEC Guide 25).

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 have sufficient 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.

Further information on quality management is available in ISO 9004. ISO/IEC Guide 25 outlines requirements for a laboratory quality system. Particular requirements for quality systems for manufacture of medical devices are available in ISO 13485 and ISO 13488.

A.3 Equipment and materials

A.3.1 Electronic data-processing equipment

Computers may be used in laboratories for both 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.

For software, there should be documentation describing:

— applications software run on the computer system;

— operations software;

— data packages in use.

All software should be acceptance-tested before being put into service (see, for example ISO 9000-3).

If commercial software packages are purchased, these packages should have been prepared under a quality system as described in ISO 9000-3.

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

— 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 software program changes.

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

A.3.2 Laboratory equipment

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 comes into contact with product, eluent, media, etc. during testing should be sterile.

A.3.3 Microbiological media

All microbiological media and eluents used to remove microorganisms from product, should be prepared in a manner that ensures their sterility.

The ability of microbiological medium to support growth of microorganisms should be established. This is commonly achieved by performing a growth promotion test on each batch of medium using an inoculum of low numbers (between 10 and 100 colony-forming units) of selected microorganisms. Growth support tests are generally described in pharmacopoeial monographs, and these monographs detail which microorganisms may be suitable.

A.4 Selection of techniques

A.4.1 General

The sequence of key stages of the technique for the estimation of microbial contamination is illustrated as follows:

SAMPLE SELECTION

↓

COLLECTION OF ITEMS FOR TEST

↓

TRANSFER TO TEST LABORATORY

↓

TREATMENT (if required)

↓

TRANSFER TO CULTURE MEDIUM

↓

INCUBATION

↓

ENUMERATION and

↓

CHARACTERIZATION (if appropriate)

↓

INTERPRETATION OF DATA

The individual responsible for the conduct of such estimations should use the knowledge of the raw materials, components, manufacturing environment, production process and the nature of the product to select appropriate methods for each of these stages.

As indicated in the introduction to the body of this part of ISO 11737, bioburden estimations may be employed in a variety of situations. The responsible individual should take account of the particular situation in deciding the sampling rate, range of culture media and incubation conditions together with the extent of method development and validation. Documenting these considerations, and the rationales for decisions taken, assists in subsequent review of procedures.

If the bioburden estimate is to be used to directly establish the extent of exposure to sterilizing conditions, consideration should be given to including packaging materials within the program for estimation of bioburden.

Ideally, bioburden should be estimated for each product on a regular basis. However, given the wide variety of products manufactured, often in small batches, this is not always practicable, and in such circumstances, products may be grouped on the basis of product type and equivalence of manufacturing environment and processes, and raw materials used. The rationale for placing products into such groups should be documented and should ensure that the data are representative of all products in the group.

A.4.2 Elements of bioburden estimation

A.4.2.1 General

Methods of taking and handling samples should be chosen and performed to avoid the introduction of inadvertent contamination and significant alterations to the numbers and nature of microorganisms in the sample. Sampling systems should be consistent to allow comparisons to be made over a period of time.

Generally, microorganisms are transferred from the item being tested, or a representative portion thereof, to a culture medium by immersing, rinsing or dissolving in an eluent. The eluent may then be passed through a membrane filter which is itself placed on a culture medium or directly plated onto culture medium. Large indivisible items may be tested by methods used for surface sampling (see A.4.2.4.8, A.4.2.4.9 and A.4.2.4.10).

Recovery of microorganisms from product surfaces may be enhanced by the presence of surface-active agents in the eluent and by subjecting the product to a physical treatment while in the liquid. Commonly used eluents are discussed in A.4.2.5.

A.4.2.2 Sample selection

A.4.2.2.1 In choosing the sample of product for presterilization counts there are two possibilities:

a) take product at random prior to sterilization;

b) take product that is not suitable for sale which may be a scrap or otherwise rejected product.

The choice for such a sample may depend on many factors, but the first prerequisite is that the sample should reflect as closely as possible the product as it is presented for sterilization. If the decision is made to utilize rejected product, it should be selected so that the product has undergone all essential stages of production, including possible cleaning and packaging processes. Product taken as indicated in a) above represents the more desirable sample.

Different strategies may be employed when selecting of a sample for bioburden estimation for different purposes, such as validating cleaning or assessing production processes.

A.4.2.2.2 Whenever practicable, the bioburden estimation should utilize the whole product, although this may not be feasible because the product cannot be accommodated in available laboratory glassware. In the latter instance, as large a portion of the product as possible should be used, and the portion of product used should allow, at completion of the estimation, the bioburden on the whole product to be estimated. Therefore, careful selection of the portion of the product to be used is necessary when large products, such as surgical gowns or external drainage kits, are tested.

A.4.2.2.3 When sampling for estimating the bioburden, care should be taken that the products are contained in their standard packaging.

When portions of the product are taken for bioburden testing, care should be taken during manipulations of the product. If portions must be separated from the products, this should be done under clean conditions (e.g. inside a laminar flow cabinet) in order to avoid adding contamination.

A.4.2.3 Sampling frequency

The frequency of bioburden estimation should be established on the basis of a variety of factors, including:

a) data from previous bioburden estimations;

b) the use to be made of the bioburden estimates;

c) the manufacturing processes used;

d) batch sizes;

e) production frequency for the product;

f) the materials used;

g) variations in bioburden estimates.

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

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

A.4.2.4 Treatment

A.4.2.4.1 General

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 employed may consist of rinsing together with some form of physical force or direct surface sampling. A surfactant may be used to enhance recovery, but it should be recognized that surfactants at higher concentrations may be inhibitory to microorganisms (see also A.4.2.5).

With certain materials, some microorganisms may occur as a biofilm, i.e. a structure in which microorganisms are encapsulated in a matrix which adheres strongly to surfaces. Microorganisms in biofilms may exhibit increased resistance to sterilization processes. The formation of biofilms would not commonly be expected in the manufacture of medical devices, although in certain instances, for example with materials of animal origin, they may be formed. In such instances, consideration should be given to the potential for biofilm formation and it should not be assumed that the treatment methods outlined in A.4.2.4.2 to A.4.2.4.10 would be appropriate for liberating microorganisms completely from a biofilm. An indication that a biofilm is present may be obtained during validation of the removal technique if repeated high microbial counts are recorded during repetitive recovery (see A.5.2.1.1).

Any treatment used during bioburden estimation 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.

Some methods of treatment are easier to control than others. The variables in the method and means of controlling these variables should be considered when selecting a method and devising a suitable combination of variables. For example, for a given method the time may be extended or the nature of mechanical agitation modified to increase the removal of organisms.

Certain methods of treatment may disaggregate the product under test (e.g. disintegration, stomaching and vortexing). The presence of disaggregated material may make enumeration of microorganisms difficult. Additional treatment, for example to separate the disaggregated material from the eluent, may be necessary. Care should be taken to ensure that the counts obtained are representative.

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 prevent loss of microorganisms or 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.

A.4.2.4.2 Stomaching

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.

This method is particularly suitable for soft, fibrous and/or absorbent materials, but it would be unsuitable for any materials which would puncture the bag, for example devices containing needles or particularly large items.

The time of treatment should be specified.

This method may yield a suspension having a low concentration of microorganisms because of the relatively large volume of eluent employed. Techniques such as membrane filtration (see A.4.2.6.2) or pour plating (see A.4.2.6.3) may be required for subsequent enumeration.

A.4.2.4.3 Ultrasonicating

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.

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 may be blocked.

The method is particularly suitable for solid impermeable items and for products with complex shapes. It may be destructive to some medical devices, particularly those containing electronic components, such as implantable pulse generators.

The sonication energy and time of sonication should not be so great as to cause disruption and death of microorganisms or to overheat the eluent.

A.4.2.4.4 Shaking with or without glass beads

The test item is immersed in a known volume of eluent within a suitable vessel and shaken on a mechanical shaker (reciprocating, orbital or wrist action) in order to assist the removal of microorganisms. Manual shaking may be used, but its effectiveness may vary depending on the operator. Glass beads of a defined size may be added to increase surface abrasion and thereby 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.

It should be noted that the addition of glass beads will increase the surface area to which microorganisms may adhere.

The time and frequency of shaking should be specified.

A.4.2.4.5 Vortex mixing

The test item is immersed in a known volume of eluent in a closed container which is pressed on the rotating pad of the vortex mixer so that a vortex is created.

The container to be used, the time of mixing and the speed at which the mixer is set should be specified. The vortex produced will also depend upon the pressure applied manually, which may cause variability. The method is quick and simple to perform but is suitable only for small items with regular surfaces.

A.4.2.4.6 Flushing

The eluent is passed through the internal lumen of the test item. Liquid flow may be induced by gravity or

pumping. Alternatively, the product may be filled with the eluent, clamped and agitated.

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

A.4.2.4.7 Blending (disintegrating)

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, which 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. This method provides a way of dividing an item into parts small enough so that the microorganisms can be enumerated by an appropriate method.

A.4.2.4.8 Swabbing

Swabs consist of absorbent material usually mounted on some form of stick or handle. The sampling material may or may not be soluble.

The normal method of use is to moisten the swab with buffer or liquid medium and wipe a predetermined sampling surface area. The recovery efficiency may be improved in some circumstances by first moistening the surface and then swabbing with a dry swab. The swab is then transferred to a buffer solution or liquid medium and agitated to remove microorganisms from the swab. Alternatively, in the case of soluble swabs, the swab is dissolved in a buffer solution or liquid medium. The resulting suspension is analyzed by filtration, plate count or other appropriate means.

Swabbing is a useful method of sampling irregularly shaped or relatively inaccessible areas. It is also useful when a large area has to be sampled.

This method is particularly prone to errors, due to variation in the way the swab is manipulated. Furthermore, it is unlikely that all microorganisms on a sample will be collected by the swab. Some of the microorganisms which are collected may become trapped in the matrix of the swab itself and therefore not detected.

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

A.4.2.4.9 Agar overlaying

Coating the surfaces of a product with a molten agar culture medium (at a maximum temperature of 45°C) and incubation to produce visible colonies may be applicable when the bioburden is low and the product configuration suitable.

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.

A.4.2.4.10 Contact plating

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 which can be counted.

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

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.

A.4.2.5 Eluents, diluents and transport media

During bioburden estimation, eluents may be used to remove microorganisms from the product. Transport

media may be used to transfer removed microorganisms for enumeration and diluents may be employed to obtain suspensions containing microorganisms in countable numbers.

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). The composition should be such that proliferation or inactivation of microorganisms does not occur.

When a liquid is used for removal of microorganisms from solid surfaces, the incorporation of a surfactant may be considered.

Eluents and diluents commonly used include those listed in table A.1.

A.4.2.6 Transfer to culture medium

A.4.2.6.1 General

Treatment will usually produce a suspension of microorganisms in the eluent which can be examined for the presence of viable microorganisms by one of the methods described in A.4.2.6.2 to A.4.2.6.7.

Prior to transfer to culture medium, additional treatment may be necessary in order to disrupt aggregates of microorganisms and thereby reduce variation. In some cases, the method used to remove the microorganisms from the item under test may also disrupt such aggregates, but in some instances a separate treatment may be necessary.

If microbicidal or microbiostatic substances are present in the eluent, these may be reduced to an ineffective concentration by dilution, removed by filtration or chemically inactivated. The presence of microbicidal or microbiostatic substances, therefore, may influence the choice of enumeration method.

In adopting an enumeration method which uses colony counts, consideration should be given to the upper limit of the number of colonies produced on incubation. This limit will vary and should be such that each viable microorganisms is able to express itself as a visible colony without being affected adversely by its near neighbors. In addition, the presence of fibers may prevent the formation of discrete colonies and thereby make enumeration difficult.

Table A.1 — Examples of eluents and diluents

Solution	Concentration in water	Applications
Ringer	1/4 strength	General
Peptone water	0.1 % - 1.0 %	General
Buffered peptone water	0.067 M phosphate 0.43% sodium chloride 0.1 % peptone	General
Phosphate-buffered saline	0.02 M phosphate 0.9% sodium chloride	General
Sodium chloride	0.25 % - 0.9 %	General
Calgon Ringer	1/4 strength	Dissolution of calcium alginate swabs
Thiosulfate Ringer	1/4 strength	Neutralization of residual chlorine
Water		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 (Tween® 80) may be added to eluents and diluents. A concentration of between 0.01% and 0.1% is generally used, depending upon the specific application. The appropriate surfactant concentration to be used with any particular treatment needs careful selection because foaming may occur.

A.4.2.6.2 Membrane filtration

In general, membrane filtration, followed by incubation of the filter on appropriate growth medium to give visible colonies, is an effective means of assessing contamination. Membrane filters having appropriate pore sizes are capable of removing microorganisms from the eluent which is passed through them. A filter of pore size 0.45 μm is generally used to facilitate colony production. For incubation, the membrane filter may be placed either on an agar surface or on an absorbent pad soaked in nutrient medium. Colonies produced on the surface of the membrane filter can be counted and isolated for characterization.

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

Filtration is 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. However, some types of membrane may absorb or release substances which could inhibit the growth of microorganisms, so it is important that only membrane filters suitable for enumeration of microorganisms are used. The membrane filter and the eluent should be compatible.

A vacuum, or in some instances pressure source is usually required. Care should be exercised in order to avoid excessive back-pressures, which could cause distortion of or damage to the membrane filter.

NOTE 14 Membrane filtration of eluents containing remnants of fibrous products can be difficult, as the membrane filter may block.

A.4.2.6.3 Pour plating

With a pour plate-technique, separate aliquots of suspension are mixed with molten agar, at a temperature not exceeding 45°C, which is then allowed to solidify in a plate, e.g. a Petri dish. The pour plate is incubated and the colonies counted.

Pour plating does not separate microorganisms from the eluent. If microbicidal or microbiostatic substances are present, the relevant considerations in A.4.2.6.1 will apply.

A.4.2.6.4 Spread plates

With a spread plate technique, an aliquot of suspension is spread on the surface of a solid medium using a spreader.

The aliquot of suspension which has been spread onto the surface of the medium has to be absorbed so that discrete colonies can develop; the need for absorption determines the volume of the aliquot that can be processed using one plate.

If microbicidal or microbiostatic substances are present, the relevant considerations in A.4.2.6.1 will apply.

A.4.2.6.5 Most Probable Number (MPN) method for serial dilutions

When a sufficient volume of eluent is available, 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. A statistical assessment of the number of dilutions showing growth provides an estimate of the original number of microorganisms. Tables, see for example DeMan[14], have been produced using appropriate statistical assumptions so that the Most Probable Number (MPN) of microorganisms can be directly estimated.

MPN methods are simple to perform, but the range of possible culture conditions which can be applied is limited and the statistical basis for the method makes it more appropriate for general assessment rather than accurate determinations.

If microbicidal or microbiostatic substances are present, the relevant considerations in A.4.2.6.1 will apply.

A.4.2.6.6 Spiral plating

Using an automatic device, an aliquot of a suspension of microorganism is spread on the surface of solid medium. The suspension is spread at a decreasing rate in a spiral track from the center of the culture plate to the periphery.

After suitable incubation, the organism count 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.

If microbicidal or microbiostatic substances are present, the relevant considerations in A.4.2.6.1 will apply.

The spiral plating technique has been shown to give reproducible results which 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 which are well homogenized and free from aggregates of material.

A.4.2.6.7 MPN method for solid items

The MPN method may also be applied to small, discrete items. This depends upon the bioburden per item being sufficiently low and there being such a distribution of that bioburden that a proportion of the items introduced directly into the culture medium does not produce growth on incubation. The results can be assessed as described in A.4.2.6.5. For some products, it may be appropriate to introduce more than one item into each aliquot of growth medium.

If microbicidal or microbiostatic substances are present, the relevant considerations in A.4.2.6.1 will apply.

A.4.2.7 Other methods for detecting microorganisms

Methods other than colony counts may be used for estimating bioburden. These include the measurement of metabolic activity (e.g. impediometry or epifluorescence). Such methods are termed "indirect" because to have meaning relative to the numbers of viable microorganisms as defined previously, they must be calibrated against colony counts. One major limitation of these techniques is the relatively high numbers of microorganisms that need to be suspended in the eluent in a sample. Normally the lower limit of numbers detected exceeds 100 colony-forming units (cfu).

A.4.3 Selection of media and incubation conditions

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

a) No single combination of medium and incubation conditions will enable the growth of all microorganisms. However, some combinations will give more representative results than others.

b) Validation exercises may require the use of a wider range of media and conditions of incubation than those used routinely.

c) Direct plating on selective media may not permit growth of physiologically stressed or damaged microorganisms.

d) The selection of culture conditions should be made with an understanding of the product being tested, likely microbial contamination sources and the nature of the microorganisms likely to be encountered.

Examples of media and incubation conditions are listed in table A.2.

Yeasts and molds may be cultured by reincubation of aerobic bacterial plates of suitable media at the lower temperature cited in table A.2 for an additional three to seven days but this technique requires careful evaluation.

It should be noted that all nonselective anaerobic culture methods may also permit the growth of facultative anaerobic organisms.

A.5 Validation of bioburden techniques

A.5.1 General

The validation of the bioburden estimation methodology ultimately should lead to an insight into the microflora existing on a product. In order to make future bioburden estimations reliable, this part of ISO 11737 requires that all techniques used be validated and the recovery efficiency determined.

A.5.2 Validation of techniques for removal of microorganisms

A.5.2.1 Approaches

There are essentially two approaches available for validation of the efficiency of removal of microorganisms from medical devices. These approaches are

a) repetitive treatment of a sample product;

b) product inoculation with known levels of microorganisms.

The first of these approaches has the advantage of utilizing the naturally occurring microbiological contamination but requires a relatively high initial bioburden. The second approach creates a model system for testing purposes. The use of such a system raises questions as to its applicability to the natural situation. However, this approach can be used for products with low levels of natural contamination.

A.5.2.1.1 Repetitive recovery method

The principle underlying this approach is that the bioburden estimation method should be repeated until there is no significant increase in the accumulated 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.

Table A.2 — Examples of media and incubation conditions

Types of microorganisms	Solid media	Liquid media	Incubation conditions1)
Nonselective aerobic bacteria	(Soybean casein digest agar) Trytone soya agar Nutrient agar Blood agar Glucose tryptone agar	(Soybean casein digest broth) Tryptone soya broth Nutrient broth	30°C to 35°C for 2 days to 5 days
Yeasts and molds	Sabouraud dextrose agar Malt extract agar Rose bengal Chloramphenicol agar (Soybean casein digest agar) Tryptone soya agar	Sabouraud dextrose broth Malt extract broth (Soybean casein digest broth) Tryptone soya broth	20°C to 25°C for 5 days to 7 days
Anaerobic bacteria	Reinforced clostridial agar2) Schaedler agar2) Prereduced blood agar2) Fastidious anaerobe agar2) Wilken-Chalgren agar2)	Robertson's cooked meat broth Fluid thioglycollate broth	30°C to 35°C for 3 days to 5 days

NOTE—This list is not exhaustive.
1) The incubation conditions listed are those which are commonly used for the types of microorganisms listed.
2) Cultured under anaerobic conditions.

A.5.2.1.2 Product inoculation method

An artificial bioburden can be created by inoculating known numbers of selected microorganisms onto product in order to establish recovery efficiencies. The microorganisms may be vegetative cells or spores; the most common approach utilizes aerobic bacterial spores. The use of vegetative microorganisms is difficult in practice because of the loss of viability which frequently occurs on drying.

The microorganisms used for this validation study may be selected to reflect the natural occurring bioburden. The selected microorganisms may include representative:

a) molds;

b) mesophilic vegetative microorganisms (gram positive and/or gram negative);

c) spores of spore-forming gram positive bacteria.

The use of a representative anaerobic spore-former for the validation studies may present major practical difficulties.

Viable counts should be verified at the time of inoculation. After the inoculum is allowed to dry, if appropriate for the particular product, the method selected for removing microorganisms from the particular product is utilized. A ratio of the recovered titre to initial inoculum establishes the recovery efficiency for the particular method and product.

Microbial inoculation has limitations such as encrustation, adhesion of the suspension, clumping and variation in the level of the inoculum, and these limitations should be taken into account when inoculating product.

Inoculation of products made of absorbent materials can be accomplished by immersion into a suspension of chosen microorganisms. This procedure can produce an even distribution of microorganisms on product.

A.5.2.2 Eluent

The eluent should neither promote nor inhibit the growth of microorganisms removed from the product. In order to establish the effect of the eluent, low known numbers of microorganisms should be inoculated onto product and left in the eluent for a time representing a worst case handling time. The bioburden estimation method should then be utilized in order for possible inhibiting or promoting effects to be demonstrated.

A.5.2.3 Physical forces

Physical forces may be used to remove microorganisms from the product (see A.4.2.4). The effects of these forces on the bioburden estimate should be assessed. Known low numbers (approximately 100 cfu) should be exposed to the physical forces to be used. Enumeration of the microorganisms gives a measure of the effects of the physical forces. However, possible effects of the eluent on the survival of microorganisms removed from product should be taken into consideration (see A.5.2.2).

A.5.3 Validation of enumeration methods

A.5.3.1 Validation of enumeration methods should consider

a) the microorganisms involved;

b) the number of contaminating microorganisms expected. This requires considerations such as the need to concentrate or dilute the eluent;

c) the possibility of using metabolic activity to estimate numbers.

A.5.3.2 The validity of the bioburden estimate largely depends upon:

a) the ability of the selected media to support growth (ensuring recovery of the microorganisms comprising the bioburden);

b) the ability of the selected temperature and incubation time to support growth in the selected medium.

A.6 Use of technique

A.6.1 General

When the bioburden estimates are being used to establish the extent of treatment by the sterilization process, it is important that an accurate estimate is obtained. Moreover, in routinely controlling the manufacturing process, it is important to employ a precise method for estimating bioburden to detect changes before a level is reached at which the validity of the sterilization process is affected.

When a general knowledge of the bioburden is required to confirm the adequacy of an established sterilization process, the acquisition of as accurate and precise an estimate as practical is of importance.

The bioburden estimates generated during initial validation provide a baseline for assessing changes; these estimates can be the basis for detecting inadvertent changes or for determining the effect of changes in manufacturing processes or environment. The setting of process control limits and trend analysis can be sensitive methods for detecting changes in bioburden.

A.6.2 Limit setting for process monitoring

The choice of limits for bioburden estimates to be used in process monitoring is based on historical data. The data should be analyzed to see if they fit a recognized mathematical distribution (e.g. normal, Poisson, binomial). Under the guidance of an experienced statistician the data may be transformed (e.g. by taking logarithms, roots or powers) and fitted to a recognized mathematical distribution. If such attempts are successful, confidence limits about an estimate can be calculated. However, an inappropriate transformation to a recognized mathematical distribution may generate a fit which cannot be justified.

In the absence of a fit to a recognized distribution, the easiest and often the most appropriate way of setting limits is to examine the historical data and find the level below which 95% of the counts fall (or 90% or 99%, whichever is chosen). The periodic review of adopted limits, covered in the requirements, is specified in this part of ISO 11737.

A.6.3 Trend analysis for process monitoring

The objective of carrying out a trend analysis is to seek evidence that a process change has occurred, even though the estimates fall within established limits. The analysis is carried out by examining the data for a deviation from the usual random spread of estimates.

The standard principles of statistical process control[20] [for example, the CUSUM[15], [17], [18], [21] (cumulative sum) method of analysis] may be applied.

A feature of a process which may escape detection is a gradual upward drift in counts masked by occasional deviations or known cyclic fluctuations (e.g. seasonal influences). When appropriate, examining rolling averages can be valuable.

Very often, microbiological data run at a satisfactory level over a period of time and then produce a clear "spike," usually of very short duration. The frequency of occurrence of these "spikes" should be monitored to see if the frequency has changed and thereby warrants investigation.

Annex B
(informative)

Guide to methods for validation of microbiological techniques

B.1 Introduction

This annex describes approaches that may be used for the validation of a technique for the estimation of bioburden. Approaches other than those outlined here may be used.

The judgment of suitably trained and qualified personnel needs to be applied in the correct application of these approaches and, in particular, it is necessary to take account of product configuration and situations in which certain contaminants are sought among the bioburden.

B.2 Validation of technique for removal of microorganisms from product

NOTE 15 This annex outlines two approaches to validation of the removal of microorganisms from product which are introduced in A.5.2; B.2.1 describes a repetitive treatment method (see A.5.2.1.1) and B.2.2 describes a method using inoculated product (see A.5.2.1.2).

B.2.1 Validation using repetitive treatment

NOTE 16 This approach uses the bioburden as it occurs naturally on product for the validation process. Sometimes it is referred to as "exhaustive recovery."

B.2.1.1 Before starting the process of validating a technique for removing microorganisms from product, the technique which is to be validated should be defined and documented.

NOTE 17 It is important that, once a validation exercise is started, the technique is not modified. Therefore, in order to define the technique, it may be necessary to undertake preliminary experiments to identify and optimize the technique which will be validated.

B.2.1.2 A number of products, or parts thereof, for which the recovery efficiency is to be determined should be selected. Each product should be individually subjected to the defined technique (see B.2.1.1). This technique is employed to estimate the number of microorganisms on the product.

Having established the estimate on the product, the technique may then be applied again to the same product to establish if further microorganisms are removed. This process of applying the technique to the same product may be repeated for a defined number of repetitions.

NOTE 18 The exact number of repetitions which are applied will depend upon a number of factors, including the nature of the product, the microorganisms which comprise the bioburden and the initial contamination level. Preliminary experiments (see B.2.1.1) may be used to establish the number of repetitions to be applied.

B.2.1.3 In certain products, it may be appropriate to establish if there are viable microorganisms remaining on the product after repetitive treatment. This may be achieved by either:

a) coating the surface of the product with molten recovery medium, allowing the media to solidify and exposing the product to specified culture conditions (see A.4.2.4.9). The colonies formed on incubation are counted, or;

b) immersing the product in liquid recovery medium, exposing to specified culture conditions and examining for growth. If, after immersion in liquid medium and culture, a fraction of the products indicate the presence of viable microorganisms, the results may be utilized for enumeration by the MPN method (see A.4.2.6.7). If, however, all the results show growth, the MPN method cannot be applied and the method of validation should be reconsidered.

B.2.1.4 The number of colonies counted after initial application of the removal technique (see B.2.1.2) is expressed as a fraction of the total number of colonies counted.

NOTE 19 The fraction of the total number of colonies can be calculated for each product and used to establish a recovery efficiency. Subclause B.5.2.1.1 provides a worked example.

B.2.2 Validation using inoculated product

B.2.2.1 Before starting the process of validating a technique for removing microorganisms from product, the technique which is to be validated should be defined and documented.

NOTE 20 It is important that, once a validation exercise is started, the technique is not modified. Therefore, in order to define the technique, it may be necessary to undertake preliminary experiments to identify and optimize the technique which will be validated.

B.2.2.2 A suspension of the microorganisms with which the product is to be inoculated should be prepared and its viable count determined.

NOTE 21 The choice of microorganisms to be used in validating by product inoculation is discussed in A.5.2.1.2. It is important that the microorganisms selected for inoculation are capable of resisting drying and therefore aerobic bacterial spores are commonly used. Spores of *Bacillus subtilis* var. *niger* have been found convenient because of their availability; an aqueous suspension of *B. subtilis* var. *niger* complying with ISO 11138-2 might be suitable.

B.2.2.3 An appropriate dilution of this suspension should be prepared and the viable count of this dilution determined. The inoculum should be of the same order of magnitude as the natural contamination on a product. For items with a low bioburden, a volume of suspension of suitable concentration to deposit 100 viable microorganisms onto the product may be appropriate.

NOTE 22 Preliminary experiments may be necessary to establish the appropriate dilution (see B.2.2.1).

B.2.2.4 A number of sterile products, or parts thereof, for which the recovery efficiency is to be determined should be selected. Each product is inoculated with a volume of the suspension of microorganisms (see B.2.2.3) and, if appropriate for the particular product, allowed to dry under laminar air-flow conditions.

NOTE 23 If the item has been sterilized by ethylene oxide, it should be fully aerated to reduce the influence of any residuals. Any inhibitory effects of substances eluted from the product should be investigated in preliminary experiments (see B.2.2.1 and B.4).

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.

B.2.2.5 The defined technique (see B.2.2.1) is employed to establish the number of inoculated microorganisms which are removed from the product.

B.2.2.6 The number of microorganisms removed is expressed as a fraction of the number inoculated onto the product. This fraction can be calculated for each product (see B.2.2.4) and used to establish a recovery efficiency. Section B.5 provides a worked example.

NOTE 24 The results derived from the validation of bioburden recovery involving direct inoculation should be viewed with caution, as this method may not mimic exactly the true bioburden.

B.3 Evaluation of culture conditions

The culture conditions, i.e. media and incubation conditions, selected for use in bioburden estimations cannot be expected to detect all potential contaminants. In practice, therefore, it is inevitable that the bioburden will be underestimated. Nevertheless, a decision on appropriate culture conditions needs to be made; 7.2 of this part of ISO 11737 requires that the culture conditions are assessed during validation of a technique.

One approach to the assessment of culture conditions consists of rationally selecting the culture conditions based on a knowledge of the manufacturing process, environment and materials and then comparing the microorganisms enumerated under these culture conditions with those detected by alternative combinations

of medium and incubation conditions.

If this approach indicates that a low proportion of the bioburden is being enumerated, the proposed culture conditions should be reconsidered in order to optimize the count obtained.

B.4 Screening for the release of substances adversely affecting bioburden estimates

Screening is aimed at investigating the effects on potentially fragile microorganisms of substances which may be released from the product into a suspending fluid. It is an example of an approach which may be used to assess a technique for compliance with clause 6.2 of this part of ISO 11737; A.4.2.6.1 should also be consulted.

B.4.1 Sterilized products are selected and each should be subjected to the technique for removal of microorganisms to be used routinely. If the removal technique employs an eluent, the procedure in B.4.2 may be followed whereas, if the product is introduced directly into medium, B.4.3 may be more appropriate.

B.4.2 If the removal technique employs an eluent (see A.4.2.5) a defined number of potentially fragile microorganisms is introduced into the eluent. The number of microorganisms used should be approximately 100. Bacteriostasis tests are generally described in pharmacopoeial monographs.

NOTE 25 The pharmacopeial monographs detail which microorganisms may be used, or if an alternative such as *Pseudomonas fluorescens* may be suitable. The resultant suspension is held for a time at least equal to the maximum permitted during bioburden estimations, and the count of viable microorganisms is established.

B.4.3 If the product is to be introduced directly into the recovery medium (for example, as in a MPN estimation; see A.4.2.6.7), the bacteriostasis test described in pharmacopoeial monographs may be used.

In this test, the product is introduced into the medium and incubated for a specified period. A low number of microorganisms (see B.4.2) is then introduced into the medium and incubation continued. After a specified period, the medium is examined for visible growth.

B.4.4 If the number of microorganisms inoculated and the number recovered in B.4.2 differs appreciably, or no growth of the microorganisms is observed in B.4.3, the technique for bioburden estimation should be reconsidered. It may be necessary to introduce a neutralization or filtration stage to remove the inhibitory substance(s) (see A.4.2.6.1).

B.5 Sample calculations of correction factors

B.5.1 Introduction

Two examples are presented in order to illustrate the calculation of a correction factor. The values quoted should not be taken to indicate values which will necessarily be obtained when carrying out validation exercises.

B.5.2 Validation of removal technique

B.5.2.1 Repetitive treatment

B.5.2.1.1 In this example, an idealized set of data for validation by repetitive treatment is shown in table B.1. These data represent five replicates for a medical device.

Table B.1—Colony counts determined from repetitive treatment for replicated of a medical device

Treatment	Replicate count					Mean colony count
	1	2	3	4	5	
1	60	50	70	55	45	56
2	10	12	5	2	3	6.4
3	1	0	2	0	0	0.6
4	0	1	0	0	1	0.4
Agar overlay	10	5	7	4	2	5.6
Total colony count	81	68	84	61	51	69

B.5.2.1.2 From the data in table B.1, the proportions removed can be calculated as follows:

First treatment	60	50	70	55	45
Total	81	68	84	61	51
% Removal	74	74	83	90	88

Average Recovery = 81.8% Range = 74%-90%

NOTE 26 An idealized situation using agar overlay has been included in the calculations. The nature of certain medical devices may preclude the use of agar overlay [see B.2.1.3 a)].

B.5.2.1.3 Using the mean percentage removal, the correction factor for recovery efficiency is:

$$\frac{100}{81.8} = 1.22$$

NOTE 27 In some applications, it may 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.

B.5.2.2 Product inoculation

B.5.2.2.1 For validation, a product inoculation method was selected because preliminary experiments indicated that the bioburden was very low.

B.5.2.2.2 An aqueous suspension of *Bacillus subtilis* var. *niger* was prepared and the viable count of the suspension was determined using optimal culture conditions.

B.5.2.2.3 A dilution of the suspension was prepared such that 0.1 ml aliquots contained 100 spores. A selected portion of the device was inoculated with 0.1 ml of this diluted suspension and allowed to dry under laminar air-flow.

B.5.2.2.4 The inoculated products were subject to the chosen removal technique and the mean number of *B. subtilis* spores removed was 35, with a range from 25 to 40.

B.5.2.2.5 The correction factor for recovery efficiency is therefore:

$$\frac{100}{35} = 2.9$$

B.5.3 Calculation of the bioburden estimate

The bioburden estimate can be established by multiplying the presterilization count by the correction factor obtained in B.2 of this part of ISO 11737.

Annex C
(informative)

Bibliography

[1] ISO/IEC Guide 25:1990, *General requirements for the competence of calibration and testing laboratories.*

[2] ISO 9000-3:1991, *Quality management and quality assurance standards—Part 3: Guidelines for the application of ISO 9001 to the development, supply and maintenance of software.*

[3] ISO 9002:1994, *Quality systems—Model for quality assurance in production, installation and servicing.*

[4] ISO 9004-1:1994, *Quality management and quality system elements—Part 1: Guidelines.*

[5] ISO 11134:1994, *Sterilization of health care products—Requirements for validation and routine control—Industrial moist heat sterilization.*

[6] ISO 11135:1994, Medical devices—Validation and routine control of ethylene oxide sterilization.

[7] ISO 11137:1995, *Sterilization of health care products—Requirements for validation and routine control—Radiation sterilization.*

[8] ISO 11138-2:1994, Sterilization of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilization.

[9] ISO/TR 13409:—[1)] *Sterilization of healthcare products—Substantiation of 25 kGy for radiation sterilization of small or infrequent production batches.*

[10] ISO 13485:—[1)] Quality systems—Medical devices—Particular requirements for the application of ISO 9001.

[11] ISO 13488:—[1)] Quality systems—Medical devices—Particular requirements for the application of ISO 9002.

[12] ASSOCIATION OF BRITISH HEALTH-CARE INDUSTRIES and UK PANEL ON GAMMA & ELECTRON IRRADIATION. *Guidelines of the estimation of microbial contamination; environmental and bioburden monitoring,* 1992.

[13] BONALSKY, J.R. A model system for testing raw materials for microbial content. *Pharm. Technol*., **4** (2), 1980, pp. 49-51.

[14] DEMAN, J.C. MPN tables corrected. *Euro J. Appl. Microbiol.*, **17**, 1983, pp. 301-305.

[15] GOLDSMITH, P.L. and Whitfield, H. Average run length in cumulative chart quality control schemes. *Technometrics*, **3,** 1961, pp. 11-20.

[16] HALLS, N.A., *et al.* The occurrence of atypically high presterilization microbial counts ("spikes") on hypodermic products. *Radiat. Phys. Chem.*, **22** (3-5), 1983, pp.663-666.

[17] JOHNSON, R. A. and BRADSHAW, M. The effect of serial correlation on the performance of CUSUM tests. *Technometrics,* **16**, 1974, pp. 103-112.

[18] LUCAS, J.M. The design and use of V-mask control schemes. *Qual. Technol.*, **8** (1), 1976.

[19] LUNDHOLM, M. Comparison of methods of quantitative determinations of airborne bacteria and evaluation of total viable counts. *Appl. Environ. Microbiol*., July 1982, pp. 179-183.

[20] MAL, O. *SPC and Continuous Improvement,* IFS Publications, 1989.

[21] PAGE, E.S., Cumulative sum charts. *Technometrics,* **3**, 1961, pp. 1-9.

[22] PDA BIOBURDEN RECOVERY VALIDATION TASK FORCE. Technical Report: Bioburden recovery validation. *J. Parenter. Sci. Technol.,* **44** (6), 1990, pp. 324-331.

[23] PULEO, J.R., FAVERO, M.S., and PETERSON, J.J. Use of ultrasonic energy in assessing microbial contamination on surfaces. *Appl. Microbiol*., **15** (6),1967, pp. 1345-1351.

[24] SHIRTZ, J.T. Sterility Testing. *Pharm. Eng.,* November-December, 1987, pp. 35-37.

[25] SOKOLSKI, W.T., and CHIDESTEY, C.G., Improved viable counting method for petroleum-based ointments. *J. Pharm. Sci.*, **53,** 1964, pp. 103-107.

Annotations from 11737-1.pdf

Page 22

Annotation 1; Label: AAMI; Date: 09/29/2000 3:41:41 PM
1) The incubation conditions listed are those which are commonly used for the types of microorganisms listed.

Annotation 2; Label: AAMI; Date: 09/29/2000 3:42:30 PM
2) Cultured under anaerobic conditions.