BRITISH STANDARD

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Sterilization of medical devices —
Estimation of the population of micro-organisms on product

Part 2. Guidance

The European Standard EN 1174-2 : 1996 has the status of a British Standard

ICS 07.100.10; 11.080

Committees responsible for this British Standard

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Association of Contact Lens Manufacturers
Association of the British Pharmaceutical Industry
British Anaesthetic and Respiratory Equipment Manufacturers Association
British Surgical Trades Association
Central Sterilising Club
Department of Health
Hospital Infection Society
Institute of Sterile Services Management
Medical Sterile Products Association
National Physical Laboratory
Panel on Gamma and Electron Irradiation
Parenteral Society
Royal College of Pathologists
Royal Pharmaceutical Society of Great Britain
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National foreword

This Part of BS EN 1174 has been prepared by Technical Committee CH/67, and is the English language version of EN 1174-2 : 1996 *Sterilization of medical devices — Estimation of the population of micro-organisms on product — Part 2: Guidance*, published by the European Committee for Standardization (CEN).

Cross-reference

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EN 1174-1 : 1996	BS EN 1174 *Sterilization of medical devices — Estimation of the population of micro-organisms on product* Part 1 : 1996 *Requirements*

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN title page, pages 2 to 14, an inside back cover and a back cover.

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Sterilization of medical devices — Estimation of the population of micro-organisms on product — Part 2: Guidance

Stérilisation des dispositifs médicaux — Estimation de la population de micro-organismes sur un produit — Partie 2: Lignes directrices

Sterilisation von Medizinprodukten — Schätzung der Population von Mikroorganismen auf einem Produkt — Teil 2: Leitfaden

This European Standard was approved by CEN on 1996-10-19. CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Foreword

This European Standard has been prepared by Technical Committee CEN/TC 204, Sterilization of medical devices, the secretariat of which is held by BSI.

Annex A is informative.

This European Standard has been prepared under a mandate given to CEN/CENELEC by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s).

For relationship with EU Directives(s), see informative Annex ZA, which is an integral part of this standard.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 1997, and conflicting national standards shall be withdrawn at the latest by May 1997.

This standard has been considered by CEN/TC 204 as one of a sequence of European standards concerned with the estimation of the population of micro-organisms (bioburden) on product to be sterilized or after sterilization. EN 1174 has been prepared in three Parts, as follows:

EN 1174 *Sterilization of medical devices — Estimation of the population of micro-organisms on product*

Part 1: *Requirements*

Part 2: *Guidance*

Part 3: *Guide to the methods for validation of microbiological techniques.*

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

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Introduction

Requirements for the estimation of the population of micro-organisms on product (this population is commonly known as the bioburden) during the manufacture of medical devices are specified in EN 1174-1. This Part of EN 1174 contains guidance on the implementation of EN 1174-1. Methods other than those given in the guidance can be used but these alternative methods should be demonstrated as being effective in achieving compliance with the requirements of EN 1174-1.

1 Scope

This Part of this European Standard provides guidance on the implementation of the requirements specified in EN 1174-1. It is aimed at providing a better understanding of EN 1174-1 as well as assisting in implementing its requirements. The guidance given is not intended to be exhaustive, but to highlight important aspects to which attention should be given.

NOTE. This Part of EN 1174-1 is informative and does not contain requirements.

This Part of this European standard is not intended as a checklist for assessing compliance with EN 1174-1.

2 Normative reference

This European Standard incorporates, by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN 1174-1 : 1996 *Sterilization of medical devices — Estimation of the population of micro-organisms on product — Part 1: Requirements*

3 Definitions

For the purposes of this Part of EN 1174, the definitions given in EN 1174-1 : 1996 apply.

4 General

4.1 Operation of the laboratory

In order that the data obtained from performing bioburden[1)] 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 should be formally certified against EN 45001.

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 EN 29004. EN 45001 outlines requirements for a laboratory quality system and particular requirements for quality systems for medical device manufacture are specified in EN 46001 and EN 46002.

4.2 Equipment and materials

4.2.1 *Electronic data processing*

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:

a) the applications software run on the computer system;

b) operations software;

c) data packages in use.

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

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

[1)] See Introduction.

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

1) documentation on development, including the source code, is retained;

2) records of acceptance testing are retained;

3) modifications to programs are documented;

4) 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 either detect or prevent unauthorized program changes.

Software programs which organize, tabulate, 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 will probably be required and these procedures should be documented.

4.2.2 *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.

4.3 Microbiological media

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

The ability of microbiological medium to support growth of micro-organisms 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 micro-organisms. Growth support tests are described in Pharmacopoeial monographs and these monographs detail which micro-organisms may be suitable.

5 Selection of technique

5.1 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 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 EN 1174-1, 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 establish directly the extent of exposure to sterilizing conditions, consideration should be given to including packaging materials within the programme 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 amalgamating products into such groups should be documented and should ensure that the data are representative of all products in the group.

5.2 Elements of bioburden estimation

5.2.1 *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 micro-organisms in the sample. Sampling systems should be consistent to allow comparisons to be made over a period of time.

Generally, micro-organisms 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 **5.2.4.7**, **5.2.4.8** and **5.2.4.9**).

Recovery of micro-organisms 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 whilst in the liquid. Commonly used eluents are discussed in **5.2.5**.

5.2.2 ***Sample selection***

5.2.2.1 In choosing the sample of product for pre-sterilization 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 taken so that the product has undergone all essential stages of production, including possible cleaning and packaging processes. Product selected as indicated in a) above represents the more desirable sample.

Different strategies may be employed in the selection of a sample for bioburden estimation for other purposes, such as validating cleaning or assessing production processes.

5.2.2.2 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 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 important when large products like surgical gowns or external drainage kits are tested.

5.2.2.3 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 have to 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.

5.2.3 ***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 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 (see also **7.4**).

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

5.2.4 ***Treatment***

5.2.4.1 *General*

The degree of adhesion of micro-organisms to surfaces varies with the nature of the surface, the micro-organisms involved and other materials present, e.g. lubricants. The origin of the contamination will also influence the degree of adhesion. To remove micro-organisms, 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 high concentrations may be inhibitory to micro-organisms (see also **5.2.5**).

With certain materials, some micro-organisms may occur as a biofilm. A biofilm is a unique structure in which micro-organisms are encapsulated in a matrix which adheres strongly to surfaces. Micro-organisms in biofilms may exhibit increased resistance to sterilization processes. The formation of biofilms would not be expected commonly in the manufacture of medical devices, although in certain instances, for example with materials of animal origin, biofilms 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 **5.2.4.2** to **5.2.4.10** would be appropriate for liberating micro-organisms 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 treatment (see **6.2.2**).

Any treatment used during bioburden estimation should be reproducible and should avoid conditions that are likely to affect the viability of micro-organisms, 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 micro-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 micro-organisms 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 micro-organisms or changes in the microbial population. The maximum storage time should be specified. Desiccation can be the cause of significant decreases in numbers of micro-organisms and should be considered in the selection of storage conditions and storage times.

5.2.4.2 *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 defined.

This method may yield a suspension having a low concentration of micro-organisms because of the relatively large volume of eluent employed. Techniques such as membrane filtration (see **5.2.6.2**) or pour plating (see **5.2.6.3**) may be required for subsequent enumeration.

5.2.4.3 *Ultrasonication*

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.

This 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, for example implantable pulse generators.

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

5.2.4.4 *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 micro-organisms. 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 micro-organisms.

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

The time and frequency of shaking should be defined.

5.2.4.5 *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 defined. 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 only suitable for small items with regular surfaces.

5.2.4.6 *Flushing*

The eluent is passed through the internal lumen of the product. 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 defined.

5.2.4.7 *Disintegration (blending)*

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. 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 micro-organisms. This method provides a way of dividing an item into sufficiently small parts and the micro-organisms enumerated by an appropriate method.

5.2.4.8 *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 pre-determined sampling surface area. Recovery efficiencies 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 micro-organisms 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 analysed by filtration, plate count or other appropriate means.

Swabs are a useful method of sampling irregularly shaped or relatively inaccessible areas. They are 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 micro-organisms on a sample will be collected by the swab. Some of the micro-organisms 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.

5.2.4.9 *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 the possibility of anaerobic locations are potential disadvantages.

5.2.4.10 *Contact plating*

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

NOTE. 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.

5.2.5 ***Eluents, diluents and transport media***

During bioburden estimation, eluents may be used to remove micro-organisms from the product. Transport media may be used to transfer removed micro-organisms for enumeration and diluents may be employed to obtain suspensions containing micro-organisms 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 micro-organisms does not occur.

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

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

Table 1. Examples of eluents and diluents

Solution	Concentration	Applications
Water	—	Dilution of aqueous samples. Preparation of isotonic solutions of soluble materials prior to counting.
Ringer	1/4 strength	General
Peptone water	0,1 % to 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
Thiosulphate Ringer	1/4 strength	Neutralisation of residual chlorine
Sodium chloride	0,25 % to 0,9 %	General

NOTE. This list is not exhaustive.

A surfactant such as polysorbate 80 may be added to eluents or diluents. A concentration of between 0,01 % and 0,1 % is generally used, depending upon the specific application. The appropriate concentration to be used with any particular treatment should be selected carefully because foaming may occur.

5.2.6 ***Transfer to culture medium***

5.2.6.1 *General*

Treatment will usually produce a suspension of micro-organisms in the eluent which can be examined for the presence of viable micro-organisms by one of the methods described in **5.2.6.2** to **5.2.6.7** below.

Prior to transfer to culture medium, additional treatment may be necessary in order to disrupt aggregates of micro-organisms and thereby reduce variation. In some cases, the method used to remove the micro-organisms 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 chemical inactivation. 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 micro-organism is able to express itself as a visible colony without being affected adversely by its near neighbours.

In addition, the presence of fibres may prevent the formation of discrete colonies and make enumeration difficult.

5.2.6.2 *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 micro-organisms 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 on either an agar surface or an absorbent pad soaked in nutrient medium. Colonies produced on the surface of the membrane filter can be counted and isolated for further identification.

Membrane filtration is particularly useful for suspensions of low concentrations of micro-organisms.

Filtration is useful when the liquid substrate is suspected of containing microbicidal or microbiostatic substances, because the micro-organisms 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 micro-organisms and so it is important that only membrane filters suitable for enumeration of micro-organisms are used. The membrane filter and the eluent should be compatible.

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

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

5.2.6.3 *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 on a plate, e.g. Petri dish. The pour plate is incubated and the colonies counted.

Pour plating does not separate micro-organisms from the eluent. If microbicidal or microbiostatic substances are present the considerations outlined in this regard in **5.2.6.1** will apply.

5.2.6.4 *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 considerations outlined in this regard in **5.2.6.1** will also apply.

5.2.6.5 *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 micro-organisms. Tables, for example DeMan[2)], have been produced using appropriate statistical assumptions so that the Most Probable Number (MPN) of micro-organisms 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 considerations outlined in this regard in **5.2.6.1** will apply.

5.2.6.6 *Spiral plating*

Using an automatic device, an aliquot of a suspension of micro-organism is spread 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.

[2)] DeMan, J.C. (1983) MPN Tables corrected, *European J. Appl. Microbiol*, **17**, 301-305.

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 considerations outlined in this regard in **5.2.6.1** 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 homogenised and free from aggregates of material.

5.2.6.7 *Most Probable Number (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 do not produce growth on incubation. The results can be assessed as described in **5.2.6.5**. 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 considerations outlined in this regard in **5.2.6.1** will apply.

5.2.7 ***Other methods of detecting micro-organisms***

Methods other than colony counts may be used for estimating bioburden, including 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 micro-organisms as defined in **5.2.6**, they have to be calibrated against colony counts. One major limitation of these techniques is the relatively high numbers of micro-organisms that need to be suspended in the eluent in a sample. Normally the lower limit of numbers detected exceeds 100 colony-forming units (cfu).

5.3 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 micro-organisms. 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 micro-organisms.

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 micro-organisms likely to be encountered.

Examples of media and incubation conditions are listed in table 2 (see also **4.3**).

Yeasts and moulds may be cultured by re-incubation of aerobic bacterial plates of suitable media at the lower temperature cited in table 2 for an additional 3 to 7 days but this technique requires careful evaluation.

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

Table 2. Examples of media and incubation conditions[1)]

Types of micro-organism	Solid media	Liquid media	Incubation conditions[2)]
Non-selective aerobic bacteria	Tryptone soya agar (Soybean casein digest agar) Nutrient agar Blood agar base Glucose tryptone agar	Tryptone soya broth (Soybean casein digest broth) Nutrient broth	30 °C to 35 °C for 2 days to 5 days
Yeasts and moulds	Sabouraud dextrose agar Malt extract agar Rose Bengal Chloramphenicol agar Tryptone soya agar (Soybean casein digest agar)	Sabouraud dextrose broth Malt extract broth Tryptone soya broth (Soybean casein digest broth)	20 °C to 25 °C for 5 days to 7 days
Anaerobic bacteria	Reinforced clostridial agar[3)] Schaedler agar[3)] Pre-reduced blood agar[3)] Fastidious anaerobe agar[3)] Wilken-Chalgren agar[3)]	Robertson's cooked meat broth Fluid thioglycollate broth	30 °C to 35 °C for 3 days to 5 days

[1)] This list is not exhaustive.

[2)] The incubation conditions listed indicate conditions which are commonly used for the types of micro-organisms listed.

[3)] Cultured under anaerobic conditions.

6 Validation of bioburden techniques

6.1 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, EN 1174-1 requires that all techniques used are validated and the recovery efficiency determined.

6.2 Validation of technique for removal of micro-organisms

6.2.1 *Approaches*

There are essentially two approaches available for validation of the efficiency of removal of micro-organisms from medical devices. These are:

a) repetitive treatment of a sample product;

b) product inoculation with known levels of micro-organisms.

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 product with low levels of natural contamination.

6.2.2 *Repetitive treatment 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 micro-organisms 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 micro-organisms recovered and the actual number on the product cannot always be demonstrated.

6.2.3 *Product inoculation method*

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

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

a) moulds;

b) mesophilic vegetative micro-organisms (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 product, the method selected for removing micro-organisms 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 micro-organisms. This procedure can produce an even distribution of micro-organisms on product.

6.2.4 *Eluent*

The eluent should neither promote nor inhibit the replication of micro-organisms removed from the product. In order to establish the effect of the eluent, low known numbers of micro-organisms 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.

6.2.5 *Physical forces*

Physical forces may be used to remove micro-organisms from the product (see **5.2.4**). The effects of these forces on the bioburden estimate should be assessed. Known low numbers (approximately 100 colony forming units) should be exposed to the physical forces to be used. Enumeration of the micro-organisms gives a measure of the effects of the physical forces. However, possible effects of the eluent on the survival of micro-organisms removed from product should be taken into consideration (see **6.2.4**).

6.3 Validation of enumeration methods

6.3.1 Validation of enumeration methods should consider the following:

a) the micro-organisms involved;

b) the number of contaminating micro-organisms expected (this requires considerations such as the need to concentrate or dilute the eluent);

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

6.3.2 The validity of the bioburden estimate largely depends upon:

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

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

7 Use of technique

7.1 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.

7.2 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 analysed 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, which is required by EN 1174-1, can be undertaken on this basis.

7.3 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[3], for example the CUSUM[4) 5) 6) 7)] (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 a very short duration. The frequency of occurrence of these 'spikes' should be monitored to see if this frequency has changed and thereby warrants investigation.

7.4 Sampling frequency for process monitoring

During the initial manufacture of a new product or on the introduction of new production processes or equipment, it is common to perform bioburden estimations at a relatively high frequency in order to gain confidence in the control of the process. Once a level of confidence has been attained, the frequency may be reduced so that routine surveillance of the process is achieved. The frequency should be selected in order to detect changes in bioburden over time, for example due to seasonal variations.

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Annex A (informative)
Bibliography

EN 29004	*Quality management and quality system elements*
EN 45001	*General criteria for the operation of testing laboratories*
EN 46001	*Quality systems — Medical devices — Particular requirements for the application of EN 29001*
EN 46002	*Quality systems — Medical devices — Particular requirements for the application of EN 29002*
ISO 9000-3	*Quality management and quality assurance standards — Part 3: Guidelines for the application of ISO 9001 to the development, supply and maintenance of software*

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Annex ZA (informative)

Clauses of this European Standard addressing essential requirements or other provisions of EU Directives

This European standard has been prepared under a mandate given to CEN/CENELEC by the European Commission and the European Free Trade Association and supports essential requirements of EU Directives 90/385/EEC and 93/42/EEC.

WARNING: Other requirements and other EU Directives *may* be applicable to the product(s) falling within the scope of this standard.

The clauses of this standard as detailed in table ZA.1 are likely to support requirements of the Directives 90/385/EEC and 93/42/EEC.

Compliance with the clauses of this standard provides one means of conforming with the specific essential requirements of the Directives concerned and associated EFTA regulations.

Table ZA.1. Correspondence between this European Standard and EU Directives

Clauses/sub-clauses of this European Standard	**Corresponding E Rs of Directive 90/385/EEC**	**Corresponding E Rs of Directive 93/42/EEC**
Clauses 4, 5, 6 and 7	I.1	I.1

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List of references

See national foreword.

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