BRITISH STANDARD

BS EN
1174-3 : 1997

Sterilization of medical devices —

Estimation of the population of micro-organisms on product

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

The European Standard EN 1174-3 : 1996 has the status of a British Standard

ICS 07.100.10; 11.080

Committees responsible for this British Standard

The preparation of this British Standard was entrusted to Technical Committee CH/67, Sterilization of medical devices, upon which the following bodies were represented:

Association of British Health-Care Industries
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
Sterilised Suture Manufacturers Association

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Contents

National foreword

This Part of BS EN 1174 has been prepared by Technical Committee CH/67 and is the English language version of EN 1174-3 : 1996 *Sterilization of medical devices — Estimation of the population of micro-organisms on product — Part 3: Guide to the methods for validation of microbiological techniques*, published by the European Committee for Standardization (CEN).

Cross-reference

Publication referred to	Corresponding British Standard
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 8, an inside back cover and a back cover.

EUROPEAN STANDARD

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Descriptors: Medical equipment, medical devices, sterilization, quality, estimation, contamination, designation, micro-organisms, microbiological analysis, inspection

English version

Sterilization of medical devices — Estimation of the population of micro-organisms on product — Part 3: Guide to the methods for validation of microbiological techniques

Stérilisation des dispositifs médicaux — Estimation de la population de micro-organismes sur un produit — Partie 3: Lignes directrices concernant les méthodes de validation des techniques microbiologiques

Sterilisation von Medizinprodukten — Schätzung der Population von Mikroorganismen auf einem Produkt — Teil 3: Leitfaden zu den Validierungsverfahren für mikrobiologische Methoden

CEN

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

Central Secretariat: rue de Stassart 36, B-1050 Brussels

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.

Annexes A and B are informative.

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

For relationship with EU Directive(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.

Contents

Introduction

This Part of EN 1174 describes approaches that may be used for the validation of a technique for the estimation of bioburden. These general approaches are intended to provide guidance on the implementation of the requirements of EN 1174-1. Approaches other than those outlined here may be used.

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

1 Scope

This Part of EN 1174 gives guidance by describing approaches which may be taken when validating techniques for bioburden estimation.

This guidance is not intended to be exhaustive but is intended to highlight important aspects of methodology to which attention should be given.

This document is informative and does not contain requirements.

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

4 Validation of the technique for removal of micro-organisms from product

NOTE. This document outlines two approaches to validation of the removal of micro-organisms from product which are introduced in **6.2** of EN 1174-2 : 1996. In **4.1** a repetitive treatment method (see **6.2.2** of EN 1174-2 : 1996) is described and in **4.2** a method using inoculated product (see **6.2.3** of EN 1174-2 : 1996) is described.

4.1 Validation using repetitive treatment

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

4.1.1 Before starting the process of validating a technique for removing micro-organisms from product, the technique which is to be validated should be defined and documented.

NOTE. 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.

4.1.2 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 **4.1.1**) to estimate the number of micro-organisms on the product.

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

NOTE. The exact number of repetitions which are applied will depend upon a number of factors including the nature of the product, the micro-organisms which comprise the bioburden and the initial contamination level. Preliminary experiments (see **4.1.1**) may be used to establish the number of repetitions to be applied.

4.1.3 For certain products, to establish if there are viable micro-organisms remaining on the product after repetitive treatment it is recommended to either:

a) coat the surface of the product with molten recovery medium, allowed to solidify and the product exposed to specified culture conditions (see **5.2.4.9** in EN 1174-2 : 1996) before the colonies formed on incubation are counted; or

b) immerse the product in liquid recovery medium, exposed to specified culture conditions and examined for growth.

NOTE. If, after immersion in liquid medium and culture, a fraction of the products indicate the presence of viable micro-organisms, the results may be utilized for enumeration by the Most Probable Number (MPN) method (see **5.2.6.7** in EN 1174-2 : 1996). However, if all the results show growth, the MPN method cannot be applied and the method of validation should be reconsidered.

4.1.4 The number of colonies counted after initial application of the removal technique (see **4.1.2**) is expressed as a fraction of the total number of colonies counted.

NOTE. The fraction of the total number of colonies can be calculated for each product and used to establish a removal efficiency. **A.2.1** to this Part of EN 1174 provides a worked example.

4.2 Validation using inoculated product

4.2.1 Before starting the process of validating a technique for removing micro-organisms from product, the technique which is to be validated should be defined and documented.

NOTE. 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.

4.2.2 A suspension of the micro-organisms with which the product is to be inoculated should be prepared and its viable count determined.

NOTE. The choice of micro-organism to be used when validating by product inoculation is discussed in **6.2.3** of EN 1174-2 : 1996. It is important that the micro-organisms 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 *Bacillus subtilis* var *niger* conforming to prEN 866-2 may be suitable.

4.2.3 An appropriate dilution of this suspension should be prepared and the viable count of this dilution determined.

NOTE. Preliminary experiments may be necessary to establish the appropriate dilution (see **4.2.1**). The viable count of 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 approximately 100 viable micro-organisms on to the product may be appropriate.

4.2.4 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 micro-organisms (see **4.2.3**) and, if appropriate for the particular product, allowed to dry under laminar air flow conditions.

NOTE 1. 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 **4.2.1** and clause **6**).

NOTE 2. 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.

4.2.5 The defined technique (see **4.2.1**) is employed to establish the number of inoculated micro-organisms which are removed from the product.

4.2.6 The number of micro-organisms removed is expressed as a fraction of the number inoculated onto the product.

NOTE 1. This fraction can be calculated for each product (see **4.2.4**) and used to establish a removal efficiency. **A.2.2** to this Part of EN 1174 provides a worked example.

NOTE 2. The results derived from the validation of bioburden recovery method involving direct inoculation should be considered with caution as this method may not mimic exactly the true bioburden.

5 Evaluation of culture conditions

NOTE. 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 should be made on the culture conditions to be employed and **6.2** of EN 1174-1 : 1996 requires that the selected conditions are assessed during validation of a technique. This clause of this Part of EN 1174 describes an approach which may be used to assess if the selection is appropriate.

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

An example of this approach is given in **A.3**.

5.1 Before starting the process of validation of a technique used for validating culture conditions, the conditions to be validated are defined and documented.

NOTE. It is important that once a validation exercise is started, the culture conditions are not modified. Therefore, in order to establish the culture conditions, it may be necessary to undertake preliminary experiments to identify and optimize the conditions to be validated.

5.2 A number of products are selected and each product should be individually subjected to the defined technique (see **5.1**) to remove the bioburden and the culture conditions to be employed routinely are used in estimating the bioburden.

Additionally, a preselected range of additional media and incubation conditions are used to estimate the bioburden.

NOTE. The selection of the additional range of media and incubation conditions is undertaken following consideration of a range of factors such as the manufacturing process used for the product and the micro-organisms which may be expected to be present. The selected range of culture conditions for this evaluation exercise should be documented together with the rationale for those selected. Media and incubation conditions for consideration include those listed in table 2 of EN 1174-2 : 1996.

5.3 Colony counts are performed after defined incubation periods such as 48 h and 5 days. From these counts, a maximum number of recoverable micro-organisms can be determined.

NOTE. The determination should consider the growth of micro-organisms on more than one type of medium. Care should be taken to avoid counting the same micro-organisms on more than one medium.

5.4 The counts of micro-organisms detected using the culture conditions being validated are compared with the maximum number of detectable micro-organisms.

6 Screening for the release of substances adversely affecting bioburden estimates

NOTE. Screening is aimed at investigating the effects on potentially fragile micro-organisms 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 **5.2** of EN 1174-1 : 1996. In addition **5.2.6** of EN 1174-2 : 1996 should be consulted.

6.1 Sterilized products are selected and each should be subjected to the technique for removal of micro-organisms to be used routinely. If the removal technique employs an eluent, the procedure in **6.2** may be followed whereas, if the product is introduced directly into medium, **6.3** may be more appropriate.

6.2 If the removal technique employs an eluent (see **5.2.4** of EN 1174-2 : 1996) a defined number of potentially fragile micro-organisms is introduced into the eluent from **6.1**. The number of micro-organisms used should be approximately 100.

NOTE. The bacteriostasis test described in the European Pharmacopoeia details micro-organisms which may be used or an alternative such as *Pseudomonas fluorescens* may be suitable.

The resultant suspension is held for a time at least equal to the maximum to be permitted during bioburden estimations and the count of viable micro-organisms is established.

6.3 If the product is to be introduced directly into the recovery medium (for example as in an MPN estimation; see **5.2.6.5** of EN 1174-2 : 1996), the bacteriostasis test described in annex V.2.1 of the European Pharmacopoeia may be used.

In this test, the product is introduced into the medium and incubated for a defined period. A low number of micro-organisms (see **6.2** above) is then introduced into the medium and incubation continued. After a defined period, the medium is examined for visible growth.

6.4 If the number of micro-organisms inoculated and the number recovered in **6.2** differs appreciably or if no growth of the micro-organisms is observed in **6.3**, the technique for bioburden estimation should be reconsidered. It may be necessary to introduce a neutralisation or filtration stage to remove the inhibitory substance(s). (See **5.2.6.3** and **5.2.6.5** of EN 1174-2 : 1996).

Annex A (informative)
Worked examples to illustrate the calculation of correction factors

A.1 Introduction

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.

A.2 Validation of removal technique

A.2.1 *Repetitive treatment*

A.2.1.1 In this example, an idealized set of data for validation by repetitive treatment are shown in table A.1. These data represent five replicates for a medical device.

Table A.1 Colony counts determined from repetitive treatment for replicates of a medical device

Treatment	Replicate					Mean colony count
	1	2	3	4	5	
1	60	50	70	55	45	56,0
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[1)]	10	5	7	4	2	5,6
Total colony count	81	68	84	61	51	69,0

[1)] In this idealized situation, an agar overlay has been performed and has been included in the calculation. The nature of certain medical devices may prelude the use of agar overlay (see **5.2.4.9** of EN 1174-2 : 1996).

A.2.1.2 From the data in table A.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 % to 90 %

A.2.1.3 Using the mean percentage removal, the correction factor for removal efficiency would be:

$$\frac{100}{81,8} = 1,22$$

NOTE. 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.

A.2.2 *Product inoculation*

A.2.2.1 For validation, a product inoculation method was selected because preliminary experiments indicated that the bioburden was very low.

A.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.

A.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.

A.2.2.4 The inoculated products were subjected to the chosen removal technique and the mean number of *Bacillus subtilis* spores removed was 35, with a range from 25 to 40.

A.2.2.5 The correction factor for removal efficiency was therefore $\frac{100}{35} = 2,9$.

A.2.3 *Calculation of bioburden estimate*

The bioburden estimate can be established by multiplying the pre-sterilization count by the correction factor in **A.2.1.3** or **A.2.2.5**.

A.3 Recovery conditions

A.3.1 In this example, the use of tryptone soya agar was selected for routine bioburden estimations. Several products were subjected individually to the technique for removal of micro-organisms, the resultant eluents mixed, separated into seven aliquots and each aliquot was filtered through a separate membrane filter. Each filter was placed onto the surface of one of seven preselected recovery media and incubated. After defined time periods, the colonies which had developed on the membrane were counted. The data generated are shown in table A.2.

NOTE. The media and incubations used in an investigation of this type should be selected with care based upon a knowledge of the conditions used in the manufacture of the product and contaminants which may be expected to be present. The examples given in table A.2 are for illustrative purposes only and, in some circumstances, the use of more than one set of culture conditions may be appropriate. Furthermore, this investigation may be repeated on separate batches of a type of medical device to investigate variation between batches.

A.3.2 Each of the three colony types isolated using condition (1) was subcultured using replicate plating onto the culture conditions being validated (condition (7)). This was repeated for conditions (2) to (6) so that all colony types were subcultured onto the culture conditions being validated.

Table A.2 Colony counts determined following incubation under differing preselected culture conditions

Condition number	Media	Incubation conditions	Count per filter	Number of colony types
1	Tryptone soya agar	35 °C to 37 °C, 3 days, aerobic	30	3
2	Sabouraud dextrose agar	20 °C to 25 °C, 5 days, aerobic	2	1
3	Plate count agar	28 °C to 30 °C, 5 days, aerobic	45	4
4	Malt extract agar	20 °C to 25 °C, 5 days, aerobic	15	2
5	Fastidious anaerobe agar	28 °C to 30 °C, 5 days, anaerobic	5	2
6	Yeast extract agar	20 °C to 25 °C, 5 days, aerobic	30	4
7	Tryptone soya agar (proposed routine conditions)	28 °C to 32 °C, 5 days, aerobic	60	5

A.3.3 After incubation one colony type from condition (5) and two from condition (6) did not form a visible colony when using the culture conditions being validated (condition (7)).

A.3.4 Of the five colonies produced in condition (5), three were of the type which did not grow in the conditions being validated. Similarly, there were 10 colonies from condition (6) of the type which did not grow in the conditions being validated. This suggests that the proposed culture conditions are appropriate for routine use.

Annex B (informative)
Bibliography

prEN 866-2 *Biological systems for testing sterilizers — Part 2: Particular systems for use in ethylene oxide sterilizers*

EN 1174-2 : 1996 *Medical devices — Estimation of the population of micro-organisms on product — Part 2: Guidance*

European Pharmacopoeia (Ph.Eur), 2nd Edition, Annex V.2.1, Maisonneuve SA, 57 St Ruffino, France, 1980.

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** and **6**	I.1	I.1

List of references

See national foreword.

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