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Sterilization of health care products — Radiation —

Part 2: Establishing the sterilization dose

Stérilisation des produits de santé — Irradiation —

Partie 2: Établissement de la dose stérilisante

Reference number
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Foreword

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

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 11137-2 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

This first edition, together with ISO 11137-1 and ISO 11137-3, cancels and replaces ISO 11137:1995.

ISO 11137 consists of the following parts, under the general title *Sterilization of health care products — Radiation*:

— *Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices*

— *Part 2: Establishing the sterilization dose*

— *Part 3: Guidance on dosimetric aspects*

This corrected version of ISO 11137-2:2006 incorporates changes in the following subclauses:

4.3.1.3, 5.1.1, 7.1, 7.2.3.2, 7.3.4.2, 7.4, 8.1, 8.2.3.1.1, 8.2.3.3.1, 8.2.6.3, 8.3.3.3.1, 8.3.6.3, 9.2.3.2, 9.2.4, 9.3.4.2, 9.3.5, 9.3.6.2, 9.4.1.2, 9.4.3.2, 9.4.5.2, 9.5.2.2, 9.5.4.2, 9.5.6.2, 10.2.5.2, 10.2.6.1, 10.3.3.2, 10.3.6.4.2, 11.3.

Introduction

This part of ISO 11137 describes methods that may be used to establish the sterilization dose in accordance with one of the two approaches specified in 8.2 of ISO 11137-1:2006. The methods used in these approaches are:

a) dose setting to obtain a product-specific dose;

b) dose substantiation to verify a preselected dose of 25 kGy or 15 kGy.

The basis of the dose setting methods described in this part of ISO 11137 (Methods 1 and 2) owe much to the ideas first propounded by Tallentire (Tallentire, 1973 [17]; Tallentire, Dwyer and Ley, 1971 [18]; Tallentire and Khan, 1978 [19]). Subsequently, standardized protocols were developed (Davis *et al.*, 1981 [8]; Davis, Strawderman and Whitby, 1984 [9]) which formed the basis of the dose setting methods detailed in the AAMI *Recommended Practice for Sterilization by Gamma Radiation* (AAMI 1984, 1991 [4], [6]).

Methods 1 and 2 and the associated sterilization dose audit procedures use data derived from the inactivation of the microbial population in its natural state on product. The methods are based on a probability model for the inactivation of microbial populations. The probability model, as applied to bioburden made up of a mixture of various microbial species, assumes that each such species has its own unique D_{10} value. In the model, the probability that an item will possess a surviving microorganism after exposure to a given dose of radiation is defined in terms of the initial number of microorganisms on the item prior to irradiation and the D_{10} values of the microorganisms. The methods involve performance of tests of sterility on product items that have received doses of radiation lower than the sterilization dose. The outcome of these tests is used to predict the dose needed to achieve a predetermined sterility assurance level, SAL.

Methods 1 and 2 may also be used to substantiate 25 kGy if, on performing a dose setting exercise, the derived sterilization dose for an SAL of 10^{-6} is $\leqslant$ 25 kGy. The basis of the method devised specifically for substantiation of 25 kGy, Method VD_{max}, was put forward by Kowalski and Tallentire (1999) [14]. Subsequent evaluations involving computational techniques demonstrated that the underlying principles were soundly based (Kowalski, Aoshuang and Tallentire, 2000) [13] and field trials confirmed that Method VD_{max} is effective in substantiating 25 kGy for a wide variety of medical devices manufactured and assembled in different ways (Kowalski *et al.*, 2002) [16].

A standardized procedure for the use of VD_{max} for substantiation of a sterilization dose of 25 kGy has been published in the AAMI Technical Information Report *Sterilization of health care products — Radiation sterilization — Substantiation of 25 kGy as a sterilization dose — Method VD_{max}* (AAMI TIR27:2001) [5], a text on which the method described herein is largely based. Method VD_{max} is founded on dose setting Method 1 and, as such, it possesses the high level of conservativeness characteristic of Method 1. In a similar manner to the dose setting methods, it involves performance of tests of sterility on product items that have received a dose of radiation lower than the sterilization dose. The outcomes of these tests are used to substantiate that 25 kGy achieves an SAL of 10^{-6}.

To link the use of VD_{max} for the substantiation of a particular preselected sterilization dose, the numerical value of the latter, expressed in kGy, is included as a superscript to the VD_{max} symbol. Thus, for substantiation of a sterilization dose of 25 kGy the method is designated VD_{max}^{25}.

Method VD_{max}^{15} is based on the same principles as Method VD_{max}^{25} described above. The test procedure is the same as Method VD_{max}^{25}, but VD_{max}^{15} is limited to product with average bioburden $\leqslant$ 1,5. The outcomes of these tests are used to substantiate that 15 kGy achieves a sterility assurance level of 10^{-6}.

This part of ISO 11137 also describes methods that may be used to carry out sterilization dose audits in accordance with ISO 11137-1:2006, Clause 12. Following establishment of the sterilization dose, sterilization dose audits are performed routinely to confirm that the sterilization dose continues to achieve the desired SAL.

Sterilization of health care products — Radiation —

Part 2: Establishing the sterilization dose

1 Scope

This part of ISO 11137 specifies methods of determining the minimum dose needed to achieve a specified requirement for sterility and methods to substantiate the use of 25 kGy or 15 kGy as the sterilization dose to achieve a sterility assurance level, SAL, of 10^{-6}. This part of ISO 11137 also specifies methods of dose auditing in order to demonstrate the continued effectiveness of the sterilization dose.

This part of ISO 11137 defines product families for dose establishment and dose auditing.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 11137-1:2006, *Sterilization of health care products — Radiation — Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices*

ISO 11737-1, *Sterilization of medical devices — Microbiological methods — Part 1: Determination of the population of microorganisms on product*

ISO 11737-2, *Sterilization of medical devices — Microbiological methods — Part 2: Test of sterility performed in the validation of a sterilization process*

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

3 Abbreviations, terms and definitions

For purposes of this document, the terms and definitions given in ISO 11137-1 and the following apply.

3.1 Abbreviations

3.1.1
A
dose to adjust the median ffp dose downwards, to the FFP dose

3.1.2
*CD**
number of positive tests of sterility obtained from tests performed individually on 100 product items irradiated in a Method 2 verification dose experiment

3.1.3
d^*
dose derived from an incremental dose experiment performed on product items drawn from a given production batch

3.1.4
D^*
initial estimate of the dose to provide an SAL of 10^{-2} for the test items

NOTE Generally, it is the median of the 3 d^* values derived for a given product.

3.1.5
D^{**}
final estimate of the dose to provide an SAL of 10^{-2} for the test items, which is used in the calculation of the sterilization dose

3.1.6
DD^*
dose delivered in a Method 2 verification dose experiment

3.1.7
DS
estimate of D_{10} value of microorganisms present on product after exposure to DD^*

3.1.8
D **value**
D_{10} **value**
time or dose required to achieve inactivation of 90 % of a population of the test microorganism under stated conditions

[ISO/TS 11139:2006]

NOTE For the purposes of this document, D_{10} applies to the radiation dose only and not to time.

3.1.9
first fraction positive dose
ffp
lowest dose of an incremental dose series, applied to product items drawn from a given production batch, at which at least one of the associated 20 tests of sterility is negative

3.1.10
First Fraction Positive dose
FFP
dose at which 19 positives out of the 20 tests of sterility are expected to occur, calculated by subtracting A from the median of 3 ffp doses

3.1.11
First No Positive dose
FNP
estimate of the dose to provide an SAL of 10^{-2} for the test items, which is used in the calculation of DS

3.1.12
VD_{max}^{15}
maximal verification dose for a given bioburden, consistent with the attainment of an SAL of 10^{-6} at a specified sterilization dose of 15 kGy

3.1.13
VD_{max}^{25}
maximal verification dose for a given bioburden, consistent with the attainment of an SAL of 10^{-6} at a specified sterilization dose of 25 kGy

3.2 Terms

3.2.1
batch
defined quantity of product, intended or purported to be uniform in character and quality, which has been produced during a defined cycle of manufacture

[ISO/TS 11139:2006]

3.2.2
bioburden
population of viable microorganisms on or in product and/or sterile barrier system

[ISO/TS 11139:2006]

3.2.3
false positive
test result interpreted as growth arising from the product, or portions thereof, tested when either growth resulted from extraneous microbial contamination or turbidity occurred from interaction between the product, or portions thereof, and the test medium

3.2.4
fraction positive
quotient in which the number of positive tests of sterility is given by the numerator and the number of tests performed is given by the denominator

3.2.5
incremental dose
dose within a series of doses applied to a number of product, or portions thereof, and used in a dose setting method to obtain or confirm the sterilization dose

3.2.6
negative test of sterility
test result for which there is no detectable microbial growth from product, or portion thereof, subjected to a test of sterility

3.2.7
packaging system
combination of the sterile barrier system and protective packaging

[ISO/TS 11139:2006]

3.2.8
positive test of sterility
test result for which there is detectable microbial growth from product, or portion thereof, subjected to a test of sterility

3.2.9
sample item portion
SIP
defined portion of a health care product that is tested

3.2.10
sterile barrier system
minimum package that prevents ingress of microorganisms and allows aseptic presentation of product at the point of use

3.2.11
sterility assurance level
SAL
probability of a single viable microorganism occurring on an item after sterilization

[ISO/TS 11139:2006]

NOTE The term sterility assurance level takes a quantitative value, generally 10^{-6} or 10^{-3}. When applying this quantitative value to assurance of sterility, an SAL of 10^{-6} has a lower value but provides a greater assurance of sterility than an SAL of 10^{-3}.

3.2.12
sterilization dose audit
exercise undertaken to confirm the appropriateness of an established sterilization dose

3.2.13
verification dose
dose of radiation predicted to give a predetermined SAL $\geqslant 10^{-2}$ used in establishing the sterilization dose

4 Definition and maintenance of product families for dose setting, dose substantiation and sterilization dose auditing

4.1 General

The establishment of a sterilization dose and the carrying out of sterilization dose audits are activities that are part of process definition (see Clause 8 of ISO 11137-1:2006) and maintaining process effectiveness (see Clause 12 of ISO 11137-1:2006). For these activities, product may be grouped into families; definition of product families is based principally on the number and types of microorganism present on or in product (the bioburden). The type of microorganism is indicative of its resistance to radiation. Variables such as density and product configuration within its packaging system are not considered in the establishment of these product families because they are not factors that influence bioburden.

In using product families in establishing the sterilization dose and for sterilization dose auditing, it is important to be aware of risks such as reduction in the ability to detect an inadvertent change within the manufacturing process that influences the effectiveness of sterilization. Furthermore, the use of a single product to represent the product family might not detect changes that occur in other members of the product family. The risk associated with a reduction in ability to detect changes in other members of the product family should be evaluated and a plan for maintaining product families developed and implemented before proceeding.

NOTE See ISO 14971 for guidance related to risk management.

4.2 Defining product families

4.2.1 The criteria for defining a product family shall be documented. Product shall be assessed against these criteria and the similarities between potential product family members considered. Consideration shall include all product-related variables that affect bioburden, including, but not limited to:

a) nature and sources of raw materials, including the effect, if any, of raw materials that might be sourced from more than one location;

b) components;

c) product design and size;

d) manufacturing process;

e) manufacturing equipment;

f) manufacturing environment;

g) manufacturing location.

The outcome of the assessment and considerations shall be recorded (see 4.1.2 of ISO 11137-1:2006).

4.2.2 Product shall only be included in a product family if it is demonstrated that the product-related variables (see 4.2.1) are similar and under control.

4.2.3 To include product within a product family, it shall be demonstrated that bioburden comprises similar numbers and types of microorganisms.

4.2.4 Inclusion of product from more than one manufacturing location in a product family shall be specifically justified and recorded (see 4.1.2 of ISO 11137-1:2006). Consideration shall be given to the effect on bioburden of:

a) geographic and/or climatic differences between locations;

b) any differences in the control of the manufacturing processes or environment;

c) sources of raw materials and processing adjuvants (e.g. water).

4.3 Designation of product to represent a product family for performance of a verification dose experiment or sterilization dose audit

4.3.1 Product to represent a product family

4.3.1.1 The number and types of microorganism on or in product shall be used as the basis for selecting product to represent a product family.

4.3.1.2 A product family shall be represented by:

a) the master product (see 4.3.2)

or

b) an equivalent product (see 4.3.3)

or

c) a simulated product (see 4.3.4).

4.3.1.3 A formal, documented assessment shall be undertaken to decide which of the three potential representative products in 4.3.1.2 is appropriate. In this assessment, consideration shall be given to the following:

a) numbers of microorganisms comprising the bioburden;

b) types of microorganism comprising the bioburden;

c) the environment in which the microorganisms occur;

d) size of product;

e) number of components;

f) complexity of product;

g) degree of automation during manufacture;

h) manufacturing environment.

4.3.2 Master product

A member of a product family shall only be considered a master product if assessment (see 4.3.1.3) indicates that the member presents a challenge that is greater than that of all other product family members. In some situations, there can be several products within the product family, each of which could be considered as the master product. In such circumstances, any one of these products may be selected as the master product to represent the product family in accordance with 4.3.3.

4.3.3 Equivalent product

A group of product shall only be considered equivalent if assessment (see 4.3.1.3) indicates that group members require the same sterilization dose. Selection of the equivalent product to represent the family shall be either a) at random, or b) according to a planned schedule to include different members of the product family. The manufacturing volume and availability of product should be considered in the selection of the equivalent product to represent the product family.

4.3.4 Simulated product

A simulated product shall only represent a product family if it constitutes an equivalent or greater challenge to the sterilization process than that provided by members of the product family. Simulated product shall be packaged in a manner and with materials used for the actual product.

NOTE A simulated product is not intended for clinical use; it is fabricated solely for the establishment or maintenance of the sterilization dose.

A simulated product may be:

a) one which is similar to the actual product in terms of materials and size, and subjected to similar manufacturing processes; e.g. a piece of the material used for implants which goes through the entire manufacturing process

or

b) a combination of components from product within the product family that would not typically be combined for use; e.g a tubing set containing multiple filters, clamps and stopcocks that are components of other products within the product family.

4.4 Maintaining product families

4.4.1 Periodic review

Review shall be performed at a specified frequency to assure that product families and product used to represent each product family remain valid. Responsibility for reviews of product and/or processes that might affect membership of product families shall be allocated to competent personnel. Such review shall be performed at least annually. The outcome of the review shall be recorded in accordance with 4.1.2 of ISO 11137-1:2006.

4.4.2 Modification to product and/or manufacturing process

Modifications to product, such as raw materials (nature and source), components or product design (including size), and/or modifications to the manufacturing process, such as equipment, environment or location, shall be assessed through a formal, documented change control system. Such modifications can alter the basis on which the product family was defined or the basis on which the selection of product to represent the product family was made. Significant changes can require definition of a new product family or the selection of a different representative product.

4.4.3 Records

Records of product families shall be retained (see 4.1.2 of ISO 11137-1:2006).

4.5 Effect of failure of establishment of sterilization dose or of a sterilization dose audit on a product family

In the event of failure during establishment of the sterilization dose or sterilization dose audit for a product family, all members of that family shall be considered to be affected. Subsequent actions shall apply to all product comprising the product family.

5 Selection and testing of product for establishing and verifying the sterilization dose

5.1 Nature of product

5.1.1 Product for sterilization can consist of:

a) an individual health care product in its packaging system;

b) a set of components presented in a packaging system, which are assembled at the point of use to form the health care product, together with accessories required to use the assembled product;

c) a number of identical health care products in their packaging system;

d) a kit comprising a variety of procedure-related health care products.

Product items for the performance of dose setting and dose substantiation shall be taken in accordance with Table 1.

Table 1 — Nature of product items for establishing and verifying the sterilization dose

Product type	Item for bioburden estimation, verification and/or incremental dose experiment	Rationale
Individual health care product in its packaging system	Individual health care product	Each health care product is used independently in clinical practice.
Set of components in a packaging system	Combination of all components of the product	Components are assembled as a product and used together in clinical practice.
Number of identical health care products in their packaging system	Single health care product taken from the packaging system	Each health care product is used independently in clinical practice; the SAL of an individual health care product within the packaging system meets the selected SAL , although the SAL associated with that packaging system might be higher.
Kit of procedure-related health care products [a]	Each type of health care product comprising the kit	Each health care product is used independently in clinical practice.
NOTE 1 See 5.2 for guidance on the use of SIP for product characterized in 5.1.1 b).		
NOTE 2 See Clause 4 for the use of product families for product characterized in 5.1.1 d).		
[a] In dose setting, the sterilization dose is chosen based on the health care product requiring the highest sterilization dose.		

5.1.2 If the product has a claim of sterility for part of the product, the sterilization dose may be established on the basis of that part only.

EXAMPLE If the product has a label claim of sterility for the fluid path only, the sterilization dose may be established based on bioburden determinations and outcomes of tests of sterility performed on the fluid path.

5.2 Sample item portion (SIP)

5.2.1 For product with an average bioburden equal to or greater than 1,0, whenever practicable, an entire product (SIP equal to 1,0) should be used for testing in accordance with Table 1. When the use of an entire product is not practicable, a selected portion of product (sample item portion) may be substituted. The SIP should be as large a portion of item as practicable in order to manipulate in the laboratory, and should be of a size that can be handled during testing.

5.2.2 For a product with an average bioburden equal to or less than 0,9, an entire product (SIP equal to 1,0) shall be used for testing in accordance with Table 1.

5.2.3 If the bioburden is evenly distributed on and/or in the item, the SIP may be selected from any portion of the item. If the bioburden is not evenly distributed, the SIP shall consist of portions of product selected at random, which proportionally represent each of the materials from which the product is made. If the bioburden distribution is known, the SIP may be selected from the portion of the product that is considered to be the most severe challenge to the sterilization process.

The value of SIP can be calculated on the basis of length, mass, volume or surface area (see Table 2 for examples).

Table 2 — Examples for calculation of SIP

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

5.2.4 The preparation and packaging of a sample item portion shall be carried out under conditions that minimize alterations to bioburden. Environmentally-controlled conditions should be used for preparation of SIPs and, whenever possible, packaging materials should be equivalent to those used for the finished product.

5.2.5 The adequacy of a selected SIP shall be demonstrated. The bioburden of the SIP shall be such that tests of sterility performed individually on 20 non-irradiated SIP items yield a minimum of 17 positive tests of sterility (i.e. 85 % positives). If the criterion is not achieved, a SIP larger than that examined originally and that meets the criterion shall be used. If an entire product is tested (SIP equal to 1,0), the criterion of a minimum of 17 positive tests of sterility observed out of 20 tests of sterility performed does not have to be met.

5.3 Manner of sampling

5.3.1 Product for establishing or auditing the sterilization dose shall be representative of that subjected to routine processing procedures and conditions. Generally, each product item used for a bioburden determination or in the performance of a test of sterility should be taken from a separate packaging system.

5.3.2 The period of time that elapses between the selection of product samples and the determination of bioburden should reflect the time period between completion of the last manufacturing step and sterilization of product. Product items may be selected from product rejected during the manufacturing process provided that they have been subjected to the same processing and conditions as the remainder of production.

5.4 Microbiological testing

5.4.1 Bioburden determinations and tests of sterility shall be conducted in accordance with ISO 11737-1 and ISO 11737-2, respectively.

Soybean Casein Digest Broth, with an incubation temperature of (30 ± 2) °C and an incubation period of 14 days, is generally recommended when a single medium is used for the performance of tests of sterility. If there is reason to suspect that this medium and temperature do not support the growth of microorganisms present, other appropriate media and incubation conditions should be used. See, e.g., Herring et al, 1974 [12]; Favero, 1971 [10]; NHB 5340.1A, 1968 [7].

Whenever practicable, product the should be irradiated in its original form and package system. However, to reduce the possibility of false positives in the test of sterility, an item may be disassembled and repackaged prior to irradiation. Manipulations prior to irradiation are not acceptable if they change the magnitude of the bioburden or its response to radiation (i.e. manipulations that alter the chemical environment in the vicinity of the microorganisms, typically oxygen tension). Materials for repackaging product items for irradiation shall be capable of withstanding the doses delivered and subsequent handling, thereby minimizing the likelihood of contamination.

5.4.2 Bioburden determinations shall be carried out on a product that has undergone the packaging process.

NOTE Generally, it is sufficient to perform a bioburden determination on a product after its removal from its packaging system and to omit the packaging system from the determination.

5.5 Irradiation

5.5.1 Irradiation of a product in establishing and verifying the sterilization dose shall be conducted in an irradiator that has undergone Installation Qualification, Operational Qualification and Performance Qualification, in accordance with ISO 11137-1. For the performance of a verification dose experiment or an incremental dose experiment, sufficient dose mapping shall be carried out to identify the highest and the lowest doses received by product.

5.5.2 Dose measurements and the use of radiation sources shall be in accordance with ISO 11137-1.

NOTE See ISO 11137-3 for guidance on dosimetric aspects of radiation sterilization.

6 Methods of dose establishment

6.1 If a sterilization dose is established in accordance with 8.2.2 a) of ISO 11137-1:2006 (product-specific sterilization dose), it shall be set by one of the following methods:

a) Method 1 for multiple and single batches (see Clause 7),

b) Method 2A (see 8.2),

c) Method 2B (see 8.3)

or

d) a method providing equivalent assurance to that of a), b) or c) above in achieving the specified requirements for sterility.

6.2 If a sterilization dose is established in accordance with 8.2.2 b) of ISO 11137-1:2006, it shall be substantiated by one of the following methods:

a) for product with an average bioburden in the range 0,1 to 1 000 (inclusive)

   1) Method VD_{max}^{25} (see 9.2 or 9.3),

   2) Method 1 (see Clause 7), subject to the derived sterilization dose taking a value $\leqslant$ 25 kGy and achieving an SAL of 10^{-6},

   3) Method 2 (see Clause 8), subject to the derived sterilization dose taking a value $\leqslant$ 25 kGy and achieving an SAL of 10^{-6}

   or

   4) a method providing equivalent assurance to that of 1), 2) or 3) above in achieving maximally an SAL of 10^{-6} (see 3.2.11, NOTE);

b) for product with an average bioburden in the range 0,1 to 1,5 (inclusive) by

   1) Method VD_{max}^{15} (see 9.4 or 9.5),

   2) Method 1 (see Clause 7), subject to the derived sterilization dose taking a value $\leqslant$ 15 kGy and achieving an SAL of 10^{-6},

   3) Method 2 (see Clause 8), subject to the derived sterilization dose taking a value $\leqslant$ 15 kGy and achieving an SAL of 10^{-6}

   or

   4) a method providing equivalent assurance to that of 1), 2) or 3) above in achieving maximally an SAL of 10^{-6} (see 3.2.11, NOTE);

c) for product with an average bioburden $<$ 0,1 by

   1) Method VD_{max}^{25} (see 9.2 or 9.3),

   2) Method VD_{max}^{15} (see 9.4 or 9.5),

   3) Method 2 (see Clause 8), subject to the derived sterilization dose taking a value $\leqslant$ 15 kGy achieving an SAL of 10^{-6}

   or

   4) a method providing equivalent assurance to that of 1), 2) or 3) above in achieving maximally an SAL of 10^{-6} (see 3.2.11, NOTE).

7 Method 1: dose setting using bioburden information

7.1 Rationale

This method of establishing a sterilization dose depends upon experimental verification that the radiation resistance of the bioburden is less than or equal to the resistance of a microbial population having the standard distribution of resistances (SDR).

A rationalized choice has been made for the SDR. The SDR specifies resistances of microorganisms in terms of D_{10} values and the probability of occurrence of values in the total population (see Table 3). Using computational methods, the individual doses required to achieve values of SAL of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} for increasing levels of average bioburden having the SDR, have been calculated. The calculated values of dose for given average bioburdens are tabulated in Tables 5 and 6.

Table 3 — Standard distribution of resistances (SDR) used in Method 1
(Whitby and Gelda, 1979 [20])

D_{10} (kGy)	1,0	1,5	2,0	2,5	2,8	3,1	3,4	3,7	4,0	4,2
Probability (%)	65,487	22,493	6,302	3,179	1,213	0,786	0,350	0,111	0,072	0,007

In practice, determination is made of the average bioburden. The dose that gives an SAL of 10^{-2} at this average bioburden is read from Table 5 or Table 6. This dose is designated the verification dose and it represents the dose that will reduce a microbial population having the SDR to a level that gives an SAL of 10^{-2}. One hundred product items are then exposed to the selected verification dose and each item is individually subjected to a test of sterility. If there are not more than two positive tests out of the 100 tests, Table 5 or Table 6 is again entered at the average bioburden to provide the sterilization dose for any desired SAL.

The rationale for allowing two positives is based upon the assumption that the probabilities of occurrence of numbers of positives around an average of one positive are distributed according to the Poisson distribution. With this distribution, there is a probability of 92 % that zero, one or two positives will occur. See Table 4.

Table 4 — Expected probability of positives from 100 tests at 10^{-2} SAL

Number positives	0	1	2	3	4	5	6	7	8
Probability (%)	36,6	37,0	18,5	6,1	1,5	0,3	0,05	0,006	0,000 7

NOTE Table B1 of ISO 11137:1995, giving verification and sterilization doses for Method 1, was compiled using regularly increasing doses to give corresponding increasing average bioburden values. The dose increment was 0,1 kGy and the average bioburden values increased in a non-regular fashion and included both whole and fractional numbers (i.e. 104; 112,6; 121,9; 131,9; etc.). In order to improve the table, making it easier to use and interpret, the average bioburden values in Table 5 of this part of ISO 11137 are expressed as regularly-increasing whole numbers. The incremental increases in the bioburden values are chosen to yield increases in the verification dose of around 0,1 kGy, the verification doses being rounded to one place of decimals. Regular increases in average bioburden values have been similarly included in Table 6.

7.2 Procedure for Method 1 for product with an average bioburden ⩾ 1,0 for multiple production batches

7.2.1 General

In applying Method 1, the six stages below shall be followed.

NOTE For a worked example, see 11.1.

7.2.2 Stage 1: Select SAL and obtain samples of product

7.2.2.1 Record the SAL for the intended use of the product.

7.2.2.2 Select at least 10 product items from each of three independent production batches, in accordance with 5.1, 5.2 and 5.3.

7.2.3 Stage 2: Determine average bioburden

7.2.3.1 Decide if a correction factor is to be applied in the determination of bioburden.

NOTE The method for determination of bioburden described in ISO 11737-1 applies a correction factor, derived from the validation of the bioburden technique, to a viable count. The performance of dose establishment using Method 1 can use this viable count without the application of the correction factor. When a correction factor is not used, the bioburden may be underestimated. Failure to apply the bioburden correction factor could increase the risk of failure of the verification dose experiment.

7.2.3.2 Determine the bioburden of each of the selected product items and calculate:

a) the average bioburden per item for each of the three batches (batch average);

b) the average bioburden per item for all selected product items (overall average bioburden).

NOTE Bioburden is generally determined on individual product items, but when the bioburden is low (e.g. < 10), it is permissible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIP; SIPs should not be pooled, rather a larger SIP should be chosen.

7.2.3.3 Compare the three batch averages with the overall average bioburden and determine whether any one of the batch averages is two or more times greater than the overall average bioburden.

7.2.4 Stage 3: Obtain verification dose

Obtain the dose for an SAL of 10^{-2} from Table 5 using one of the following,

a) the highest batch average bioburden, if one or more batch averages is $\geqslant 2 \times$ (overall average bioburden)

or

b) the overall average bioburden, if each of the batch averages is $< 2 \times$ (overall average bioburden).

Designate this dose as the verification dose.

Use the SIP average bioburden to determine the verification dose if SIPs are to be used in the performance of the tests of sterility.

Use the closest tabulated average bioburden greater than the calculated average bioburden if the average bioburden is not given in Table 5.

7.2.5 Stage 4: Perform verification dose experiment

7.2.5.1 Select 100 product items from a single batch of product. The 100 product items for the performance of Stage 4 may be selected from one of the batches for which a bioburden determination was carried out in Stage 2, or from a fourth batch manufactured under conditions that are representative of normal production. The ability of the product to support microbial growth should be taken into account in selecting the batch to be used.

7.2.5.2 Irradiate the product items at the verification dose. Determine the dose. If the highest dose to the product items exceeds the verification dose by more than 10 %, and the sterilization dose is to be established using Method 1, the verification dose experiment shall be repeated. If the arithmetic mean of the highest and lowest doses delivered to product items is less than 90 % of the verification dose, the verification dose experiment may be repeated. If this mean dose is less than 90 % of the verification dose and, on performance of the tests of sterility, acceptable results are observed (see 7.2.6.1), the verification experiment need not be repeated.

7.2.5.3 Subject each irradiated product item individually to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility.

7.2.6 Stage 5: Interpretation of results

7.2.6.1 Accept verification if there are no more than two positive tests of sterility from the 100 tests carried out.

7.2.6.2 Do not accept verification if there are more than two positive tests of sterility.

If this outcome can be ascribed to incorrect performance in the determination of bioburden, non-application of the correction factor in the determination of the bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, the verification dose experiment may be repeated following implementation of corrective action.

If this outcome cannot be ascribed to a cause addressed by corrective action, this method of dose setting is not valid and an alternative method for establishing a sterilization dose shall be used (see Clause 6).

7.2.7 Stage 6: Establish sterilization dose

7.2.7.1 If the entire product is used and verification is accepted, obtain the sterilization dose for the product from Table 5 using the closest tabulated average bioburden that is greater than or equal to the calculated average bioburden and reading the dose necessary to achieve the desired SAL.

7.2.7.2 If a SIP of less than 1,0 is used and verification is accepted, calculate the average bioburden for the entire product by dividing the SIP average bioburden by the SIP value. Obtain the sterilization dose for the product from Table 5 using the closest tabulated average bioburden that is greater than or equal to the calculated average bioburden for the entire product and reading the dose necessary to achieve the desired SAL.

Table 5 — Radiation dose (kGy) required to achieve a given SAL for an average bioburden ⩾ 1,0 having the standard distribution of resistances

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
1,0	3,0	5,2	8,0	11,0	14,2
1,5	3,3	5,7	8,5	11,5	14,8
2,0	3,6	6,0	8,8	11,9	15,2
2,5	3,8	6,3	9,1	12,2	15,6
3,0	4,0	6,5	9,4	12,5	15,8
3,5	4,1	6,7	9,6	12,7	16,1
4,0	4,3	6,8	9,7	12,9	16,2
4,5	4,4	7,0	9,9	13,1	16,4
5,0	4,5	7,1	10,0	13,2	16,6
5,5	4,6	7,2	10,2	13,4	16,7
6,0	4,7	7,3	10,3	13,5	16,9
6,5	4,8	7,4	10,4	13,6	17,0
7,0	4,8	7,5	10,5	13,7	17,1
7,5	4,9	7,6	10,6	13,8	17,2
8,0	5,0	7,7	10,7	13,9	17,3
8,5	5,1	7,8	10,8	14,0	17,4
9,0	5,1	7,8	10,8	14,1	17,5

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
9,5	5,2	7,9	10,9	14,1	17,6
10	5,2	8,0	11,0	14,2	17,6
11	5,3	8,1	11,1	14,3	17,8
12	5,4	8,2	11,2	14,5	17,9
13	5,5	8,3	11,3	14,6	18,0
14	5,6	8,4	11,4	14,7	18,1
15	5,7	8,5	11,5	14,8	18,2
16	5,8	8,5	11,6	14,9	18,3
17	5,8	8,6	11,7	15,0	18,4
18	5,9	8,7	11,8	15,1	18,5
19	5,9	8,8	11,9	15,1	18,6
20	6,0	8,8	11,9	15,2	18,7
22	6,1	9,0	12,1	15,4	18,8
24	6,2	9,1	12,2	15,5	19,0
26	6,3	9,2	12,3	15,6	19,1
28	6,4	9,3	12,4	15,7	19,2
30	6,5	9,4	12,5	15,8	19,3

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
32	6,6	9,4	12,6	15,9	19,4
34	6,6	9,5	12,7	16,0	19,5
36	6,7	9,6	12,8	16,1	19,6
38	6,8	9,7	12,8	16,2	19,7
40	6,8	9,7	12,9	16,2	19,8
42	6,9	9,8	13,0	16,3	19,8
44	6,9	9,9	13,0	16,4	19,9
46	7,0	9,9	13,1	16,5	20,0
48	7,0	10,0	13,2	16,5	20,0
50	7,1	10,0	13,2	16,6	20,1
55	7,2	10,2	13,4	16,7	20,3
60	7,3	10,3	13,5	16,9	20,4
65	7,4	10,4	13,6	17,0	20,5
70	7,5	10,5	13,7	17,1	20,6
75	7,6	10,6	13,8	17,2	20,7
80	7,7	10,7	13,9	17,3	20,8
85	7,7	10,8	14,0	17,4	20,9
90	7,8	10,8	14,1	17,5	21,0
95	7,9	10,9	14,1	17,5	21,1
100	8,0	11,0	14,2	17,6	21,2
110	8,1	11,1	14,3	17,8	21,3
120	8,2	11,2	14,5	17,9	21,5
130	8,3	11,3	14,6	18,0	21,6
140	8,4	11,4	14,7	18,1	21,7
150	8,5	11,5	14,8	18,2	21,8
160	8,5	11,6	14,9	18,3	21,9
170	8,6	11,7	15,0	18,4	22,0
180	8,7	11,8	15,1	18,5	22,1
190	8,8	11,9	15,1	18,6	22,2
200	8,8	11,9	15,2	18,7	22,3
220	9,0	12,1	15,4	18,8	22,4
240	9,1	12,2	15,5	19,0	22,6
260	9,2	12,3	15,6	19,1	22,7
280	9,3	12,4	15,7	19,2	22,8
300	9,4	12,5	15,8	19,3	22,9
325	9,5	12,6	15,9	19,4	23,1
350	9,6	12,7	16,0	19,5	23,2

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
375	9,7	12,8	16,2	19,7	23,3
400	9,7	12,9	16,2	19,8	23,4
425	9,8	13,0	16,3	19,8	23,5
450	9,9	13,1	16,4	19,9	23,6
475	10,0	13,1	16,5	20,0	23,7
500	10,0	13,2	16,6	20,1	23,7
525	10,1	13,3	16,7	20,2	23,8
550	10,2	13,4	16,7	20,3	23,9
575	10,2	13,4	16,8	20,3	24,0
600	10,3	13,5	16,9	20,4	24,0
650	10,4	13,6	17,0	20,5	24,2
700	10,5	13,7	17,1	20,6	24,3
750	10,6	13,8	17,2	20,7	24,4
800	10,7	13,9	17,3	20,8	24,5
850	10,8	14,0	17,4	20,9	24,6
900	10,8	14,1	17,5	21,0	24,7
950	10,9	14,1	17,5	21,1	24,8
1 000	11,0	14,2	17,6	21,2	24,9
1 050	11,0	14,3	17,7	21,3	24,9
1 100	11,1	14,4	17,8	21,3	25,0
1 150	11,2	14,4	17,8	21,4	25,1
1 200	11,2	14,5	17,9	21,5	25,2
1 250	11,3	14,5	18,0	21,5	25,2
1 300	11,3	14,6	18,0	21,6	25,3
1 350	11,4	14,6	18,1	21,7	25,3
1 400	11,4	14,7	18,1	21,7	25,4
1 450	11,5	14,8	18,2	21,8	25,5
1 500	11,5	14,8	18,2	21,8	25,5
1 550	11,6	14,9	18,3	21,9	25,6
1 600	11,6	14,9	18,3	21,9	25,6
1 650	11,7	14,9	18,4	22,0	25,7
1 700	11,7	15,0	18,4	22,0	25,7
1 750	11,7	15,0	18,5	22,1	25,8
1 800	11,8	15,1	18,5	22,1	25,8
1 850	11,8	15,1	18,6	22,2	25,9
1 900	11,9	15,1	18,6	22,2	25,9
1 950	11,9	15,2	18,6	22,2	25,9

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
2 000	11,9	15,2	18,7	22,3	26,0
2 100	12,0	15,3	18,8	22,4	26,1
2 200	12,1	15,4	18,8	22,4	26,1
2 300	12,1	15,4	18,9	22,5	26,2
2 400	12,2	15,5	19,0	22,6	26,3
2 500	12,2	15,6	19,0	22,6	26,4
2 600	12,3	15,6	19,1	22,7	26,4
2 700	12,3	15,7	19,1	22,8	26,5
2 800	12,4	15,7	19,2	22,8	26,5
2 900	12,4	15,8	19,3	22,9	26,6
3 000	12,5	15,8	19,3	22,9	26,6
3 200	12,6	15,9	19,4	23,0	26,8
3 400	12,7	16,0	19,5	23,1	26,9
3 600	12,8	16,1	19,6	23,2	26,9
3 800	12,8	16,2	19,7	23,3	27,0
4 000	12,9	16,3	19,8	23,4	27,1
4 200	13,0	16,3	19,8	23,5	27,2
4 400	13,0	16,4	19,9	23,5	27,3
4 600	13,1	16,5	20,0	23,6	27,3
4 800	13,2	16,5	20,0	23,7	27,4
5 000	13,2	16,6	20,1	23,7	27,5
5 300	13,3	16,7	20,2	23,8	27,6
5 600	13,4	16,8	20,3	23,9	27,7
5 900	13,5	16,8	20,4	24,0	27,8
6 200	13,5	16,9	20,4	24,1	27,8
6 500	13,6	17,0	20,5	24,2	27,9
6 800	13,7	17,0	20,6	24,2	28,0
7 100	13,7	17,1	20,7	24,3	28,1
7 400	13,8	17,2	20,7	24,4	28,1
7 700	13,8	17,2	20,8	24,4	28,2
8 000	13,9	17,3	20,8	24,5	28,3
8 500	14,0	17,4	20,9	24,6	28,4
9 000	14,1	17,5	21,0	24,7	28,5
9 500	14,1	17,6	21,1	24,8	28,5
10 000	14,2	17,6	21,2	24,9	28,6
10 500	14,3	17,7	21,3	24,9	28,7
11 000	14,4	17,8	21,3	25,0	28,8

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
11 500	14,4	17,8	21,4	25,1	28,9
12 000	14,5	17,9	21,5	25,2	28,9
13 000	14,6	18,0	21,6	25,3	29,1
14 000	14,7	18,1	21,7	25,4	29,2
15 000	14,8	18,2	21,8	25,5	29,3
16 000	14,9	18,3	21,9	25,6	29,4
17 000	15,0	18,4	22,0	25,7	29,5
18 000	15,1	18,5	22,1	25,8	29,6
19 000	15,1	18,6	22,2	25,9	29,7
20 000	15,2	18,7	22,3	26,0	29,8
21 000	15,3	18,8	22,4	26,1	29,9
22 000	15,4	18,8	22,4	26,1	29,9
23 000	15,4	18,9	22,5	26,2	30,0
24 000	15,5	19,0	22,6	26,3	30,1
25 000	15,6	19,0	22,6	26,4	30,1
26 000	15,6	19,1	22,7	26,4	30,2
27 000	15,7	19,1	22,8	26,5	30,3
28 000	15,7	19,2	22,8	26,5	30,3
29 000	15,8	19,3	22,9	26,6	30,4
30 000	15,8	19,3	22,9	26,6	30,4
32 000	15,9	19,4	23,0	26,8	30,6
34 000	16,0	19,5	23,1	26,9	30,7
36 000	16,1	19,6	23,2	26,9	30,8
38 000	16,2	19,7	23,3	27,0	30,8
40 000	16,3	19,8	23,4	27,1	30,9
42 000	16,3	19,8	23,5	27,2	31,0
44 000	16,4	19,9	23,5	27,3	31,1
46 000	16,5	20,0	23,6	27,3	31,2
48 000	16,5	20,0	23,7	27,4	31,2
50 000	16,6	20,1	23,7	27,5	31,3
54 000	16,7	20,2	23,9	27,6	31,4
58 000	16,8	20,3	24,0	27,7	31,5
62 000	16,9	20,4	24,1	27,8	31,7
66 000	17,0	20,5	24,2	27,9	31,8
70 000	17,1	20,6	24,3	28,0	31,9
75 000	17,2	20,7	24,4	28,2	32,0
80 000	17,3	20,8	24,5	28,3	32,1

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
85 000	17,4	20,9	24,6	28,4	32,2
90 000	17,5	21,0	24,7	28,5	32,3
95 000	17,6	21,1	24,8	28,5	32,4
100 000	17,6	21,2	24,9	28,6	32,5
110 000	17,8	21,3	25,0	28,8	32,6
120 000	17,9	21,5	25,2	28,9	32,8
130 000	18,0	21,6	25,3	29,1	32,9
140 000	18,1	21,7	25,4	29,2	33,0
150 000	18,2	21,8	25,5	29,3	33,1
160 000	18,3	21,9	25,6	29,4	33,3
170 000	18,4	22,0	25,7	29,5	33,4
180 000	18,5	22,1	25,8	29,6	33,4
190 000	18,6	22,2	25,9	29,7	33,5
200 000	18,7	22,3	26,0	29,8	33,6
220 000	18,8	22,4	26,1	29,9	33,8
240 000	19,0	22,6	26,3	30,1	33,9
260 000	19,1	22,7	26,4	30,2	34,1
280 000	19,2	22,8	26,5	30,3	34,2
300 000	19,3	22,9	26,6	30,4	34,3
320 000	19,4	23,0	26,8	30,6	34,4
340 000	19,5	23,1	26,9	30,7	34,5

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
380 000	19,7	23,3	27,0	30,8	34,7
400 000	19,8	23,4	27,1	30,9	34,8
420 000	19,8	23,5	27,2	31,0	34,9
440 000	19,9	23,5	27,3	31,1	35,0
460 000	20,0	23,6	27,3	31,2	35,0
480 000	20,0	23,7	27,4	31,2	35,1
500 000	20,1	23,7	27,5	31,3	35,2
540 000	20,2	23,9	27,6	31,4	35,3
580 000	20,3	24,0	27,7	31,5	35,4
620 000	20,4	24,1	27,8	31,7	35,5
660 000	20,5	24,2	27,9	31,8	35,6
700 000	20,6	24,3	28,0	31,9	35,7
750 000	20,7	24,4	28,2	32,0	35,9
800 000	20,8	24,5	28,3	32,1	36,0
850 000	20,9	24,6	28,4	32,2	36,1
900 000	21,0	24,7	28,5	32,3	36,2
950 000	21,1	24,8	28,5	32,4	36,3
1 000 000	21,2	24,9	28,6	32,5	36,3

NOTE 1 The presence of high bioburden levels in this table is not intended to imply that such levels are the norm.

NOTE 2 Tabulated values are used in Stages 3, 4 and 6 of Method 1 dose setting.

7.3 Procedure for Method 1 for product with an average bioburden ⩾ 1,0 for a single production batch

7.3.1 Rationale

This method is an adaptation of Method 1 and is intended to be used for the establishment of a sterilization dose for a single production batch only. It is the method for establishing a sterilization dose depending upon experimental verification that the radiation resistance of the bioburden is less than or equal to the resistance of a microbial population having an SDR.

7.3.2 General

In applying this adaptation of Method 1, the six stages below shall be followed.

NOTE For a worked example, see 11.1.

7.3.3 Stage 1: Select SAL and obtain samples of product

7.3.3.1 Record the SAL for the intended use of the product.

7.3.3.2 Select at least 10 product items from the single batch in accordance with 5.1, 5.2 and 5.3.

7.3.4 Stage 2: Determine average bioburden

7.3.4.1 Decide if a correction factor is to be applied in the determination of bioburden.

NOTE The method for determination of bioburden described in ISO 11737-1 applies a correction factor, derived from the validation of the bioburden technique, to a viable count. The performance of dose establishment using Method 1 can use this viable count without the application of the correction factor. When a correction factor is not used the bioburden may be underestimated. Failure to apply the bioburden correction factor could increase the risk of failure of the verification dose experiment.

7.3.4.2 Determine the bioburden of each of the selected product items and calculate the average bioburden per item for all selected product items (overall average bioburden).

NOTE Bioburden is generally determined on individual product items, but when the bioburden is low (e.g. < 10), it is permissible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIP; SIPs should not be pooled, rather a larger SIP should be chosen.

7.3.5 Stage 3: Obtain verification dose

Obtain the dose for an SAL of 10^{-2} from Table 5 using the average bioburden. Designate this dose as the verification dose.

Use the SIP average bioburden to determine the verification dose, if SIPs are to be used in the performance of the tests of sterility.

Use the closest tabulated average bioburden greater than the calculated average bioburden, if the average bioburden is not given in Table 5.

7.3.6 Stage 4: Perform verification dose experiment

7.3.6.1 Select 100 product items from the single batch of product.

7.3.6.2 Irradiate the product items at the verification dose. Determine the dose. If the highest dose to the product items exceeds the verification dose by more than 10 %, and the sterilization dose is to be established using Method 1, the verification dose experiment shall be repeated. If the arithmetic mean of the highest and lowest doses delivered to product items is less than 90 % of the verification dose, the verification dose experiment may be repeated. If this mean dose is less than 90 % of the verification dose and, on performance of the tests of sterility, acceptable results are observed (see 7.3.7.1), the verification experiment need not be repeated.

7.3.6.3 Subject each irradiated product item individually to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility.

7.3.7 Stage 5: Interpretation of results

7.3.7.1 Accept verification if there are no more than two positive tests of sterility from the 100 tests carried out.

7.3.7.2 Do not accept verification if there are more than two positive tests of sterility.

If this outcome can be ascribed to incorrect performance in the determination of bioburden, non-application of the correction factor in the determination of the bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, the verification dose experiment may be repeated following implementation of corrective action.

If this outcome cannot be ascribed to a cause addressed by corrective action, this method of dose setting is not valid and an alternative method for establishing a sterilization dose shall be used (see Clause 6).

7.3.8 Stage 6: Establish sterilization dose

7.3.8.1 If the entire product is used and verification is accepted, obtain the sterilization dose for the product from Table 5 using the closest tabulated average bioburden that is greater than or equal to the calculated average bioburden and reading the dose necessary to achieve the desired SAL.

7.3.8.2 If a SIP of less than 1,0 is used and verification is accepted, calculate the average bioburden for the entire product by dividing the SIP average bioburden by the SIP value. Obtain the sterilization dose for the product from Table 5 using the closest tabulated average bioburden that is greater than or equal to the calculated average bioburden for the entire product and reading the dose necessary to achieve the desired SAL.

7.4 Procedure for Method 1 for product with an average bioburden in the range 0,1 to 0,9 for multiple or single production batches

For a product with an average bioburden within the range 0,1 to 0,9 inclusive, the procedure for dose establishment using Method 1 given above for multiple (see 7.2) or single (see 7.3) batches shall be followed, except:

a) an entire product shall be used for testing in accordance with Table 1;

b) a correction factor shall be used in the determination of bioburden;

c) Table 6 shall be entered in order to obtain the dose providing an SAL of 10^{-2} (the verification dose) and the sterilization dose for the selected SAL.

NOTE 1 For a worked example, see 11.1.

NOTE 2 Tabulated values are used in Stages 3, 4 and 6 of Method 1 dose setting.

Table 6 — Radiation dose (kGy) required to achieve a given SAL for an average bioburden in the range 0,1 to 0,9 having the standard distribution of resistances

Average bioburden	Sterility assurance level SAL					Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
0,10	1,3	3,0	5,2	8,0	11,0	0,45	2,3	4,4	7,0	9,9	13,1
0,15	1,5	3,3	5,7	8,5	11,5	0,50	2,4	4,5	7,1	10,0	13,2
0,20	1,7	3,6	6,0	8,8	11,9	0,60	2,5	4,7	7,3	10,3	13,5
0,25	1,9	3,8	6,3	9,1	12,2	0,70	2,7	4,8	7,5	10,5	13,7
0,30	2,0	4,0	6,5	9,4	12,5	0,80	2,8	5,0	7,7	10,7	13,9
0,35	2,1	4,1	6,7	9,6	12,7	0,90	2,9	5,1	7,8	10,8	14,1
0,40	2,2	4,3	6,8	9,7	12,9						
NOTE For an average bioburden with the range > 0,9 and < 1,0, enter Table 5 at an average bioburden of 1,0.											

8 Method 2: Dose setting using fraction positive information from incremental dosing to determine an extrapolation factor

8.1 Rationale

With Method 2, information is obtained about the resistance to radiation of microorganisms , as they occur on the product. The method uses the results of tests of sterility conducted on product items that have been exposed to a series of incremental doses to estimate the dose at which one in 100 product items is expected to be non-sterile (that is, an SAL of 10^{-2}). The microorganisms surviving exposure to such a dose should have a more homogeneous D_{10} value than the initial bioburden. From the incremental dose experiment, an estimate is made of this D_{10} value, and it is used for extrapolation to SAL values below 10^{-2} in order to determine the sterilization dose.

The validity of the calculated sterilization dose generally depends upon the validity of the extrapolation beyond the dose expected to achieve an SAL of 10^{-2}. In extensive tests of the experimental protocol using computer simulation of inactivation of microorganisms on the product, the validity of this extrapolation has been confirmed for populations having distributions of resistance that were established experimentally. An elaboration of the rationale outlined above, together with results from the computer simulation, is contained in Davis, Strawderman and Whitby, 1984 [9].

The following text describes two procedures designated Method 2A and Method 2B. Method 2A is the method that has been generally applied, whereas Method 2B has been developed for products with a consistent and very low bioburden. Conditions that must be met in order to use Method 2B are specified in 8.3.1.1.

Bioburden determination is not used in establishing the sterilization dose using Method 2. However, bioburden determination is required as part of the routine monitoring of product (see 7.3 and 12.1 of ISO 11137-1:2006).

Calculations for A, DS and the sterilization dose are not the same for Methods 2A and 2B; therefore, close attention is necessary to ensure the use of the appropriate formulae.

Dose calculations should be made with data that are reported to one place of decimals. The sterilization dose may be rounded (using standard rounding procedures) to one place of decimals.

NOTE 1 In the following procedures and examples, notation is lower case when it refers to results derived from product taken from a single batch. Notation is upper case when it refers to results derived from product taken from all three batches.

NOTE 2 Method 2B requires that the entire product (SIP = 1,0) be used, whereas for Method 2A, either the entire product or a portion of product (SIP < 1,0) may be used.

8.2 Procedure for Method 2A

8.2.1 General

In applying Method 2A, the five stages below shall be followed.

NOTE For worked examples, see 11.2.2 and 11.2.3.

8.2.2 Stage 1: Select SAL and obtain samples of product

8.2.2.1 Record the SAL for the intended use of the product.

8.2.2.2 Select at least 280 product items from each of three independent production batches in accordance with 5.1, 5.2 and 5.3. Additional product may be needed to validate the adequacy of an SIP < 1. See 5.2.5.

8.2.3 Stage 2: Perform incremental dose experiment

8.2.3.1 General

8.2.3.1.1 For each of 3 production batches, irradiate 20 product items at each of a series of at least 9 doses, starting at 2 kGy and increasing in nominal increments of 2 kGy. Determine each incremental dose. The highest dose at each nominal incremental dose is used subsequently in the identification of first fraction positive dose (ffp) and d^*. This dose may vary from the nominal incremental dose by ± 1,0 kGy or ± 10 %, whichever is greater. If the arithmetic mean of the highest and lowest doses at a given incremental dose is less than the value taken at the lower limit, the irradiation of 20 further product items at this particular incremental dose may be carried out.

8.2.3.1.2 Subject the irradiated product items, individually, to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility.

8.2.3.1.3 Obtain the following from the results of this experiment:

a) A and First Fraction Positive dose (FFP) (see 8.2.3.2);

b) D* (see 8.2.3.3);

c) CD* batch (see 8.2.3.4).

8.2.3.2 A and FFP

8.2.3.2.1 For each of three production batches, determine the lowest dose from the incremental dose series at which at least one of the 20 tests of sterility is negative. Designate this dose as ffp for the particular batch and find the median ffp of the three. If 2 or 3 batches exhibit the same ffp, choose the dose for the batch showing the higher or highest number of positives as the median ffp.

8.2.3.2.2 Obtain the value of A from Table 7 using the number of positive tests of sterility at the median ffp.

Table 7 — Values of A for different numbers of positive tests of sterility at median ffp (Method 2A)

Number of positive tests of sterility at median ffp	A (kGy)	Number of positive tests of sterility at median ffp	A (kGy)
19	0,00	9	0,79
18	0,13	8	0,87
17	0,22	7	0,95
16	0,31	6	1,05
15	0,38	5	1,15
14	0,45	4	1,28
13	0,52	3	1,43
12	0,58	2	1,65
11	0,65	1	2,00
10	0,72	0	2,00

NOTE See Equation (1) for formula for calculating A.

$$A = (2\,\text{kGy})\frac{\left\{\log_{10}(\log_e 20) - \log_{10}\left[\log_e(20/n)\right]\right\}}{\left\{\log_{10}(\log_e 20) - \log_{10}\left[\log_e(20/19)\right]\right\}} \quad (1)$$

where n is the number of tests of sterility that are negative. (See Davis *et al.*, 1981 [8].)

8.2.3.2.3 Calculate FFP from Equation (2).

$$\text{FFP} = \text{median ffp} - A \quad (2)$$

8.2.3.3 D*

8.2.3.3.1 For each of the three production batches, determine d* by either

a) finding the lower of two consecutive doses at which all tests of sterility are negative, followed by no more than one further positive test in any of the remaining tests in the incremental dose series

or

b) finding the dose at which one positive in 20 tests of sterility occurs, immediately preceded by one, and only one, incremental dose at which all tests are negative and followed by incremental doses at which all tests are negative.

8.2.3.3.2 If the criteria in 8.2.3.3.1 a) or b) are not met with each of the three production batches, the incremental dose experiment is invalid. In this circumstance, performance of the incremental dose experiment may be repeated after investigation of the methodology of the experiment and implementation of corrective action(s).

8.2.3.3.3 Designate D^* as follows,

a) if the highest batch d^* exceeds the median batch d^* by < 5 kGy, the median batch d^* becomes D^*

or

b) if the highest batch d^* exceeds the median batch d^* by $\geqslant 5$ kGy, the highest batch d^* becomes D^*.

8.2.3.4 CD^* batch

Determine the batch for which $d^* = D^*$ and designate this as CD^* batch. If more than one batch has a d^* equal to D^*, one of these batches may be designated at random as CD^* batch. Retained product items from a CD^* batch are used in Stage 3 of Method 2A. Storage conditions of the retained product from the three batches should be such that microbial growth is prevented. Where this is not practicable, a fourth batch may be taken as the CD^* batch.

8.2.4 Stage 3: Perform verification dose experiment

8.2.4.1 Irradiate 100 product items from CD^* batch at a dose of D^*. Determine the dose and designate the highest dose delivered to the product items as DD^*. DD^* may vary from D^* by + 1,0 kGy or + 10 %, whichever is greater. If the arithmetic mean of the highest and lowest doses delivered to product items is less than 90 % of D^*, the irradiation may be repeated with a further 100 product items taken from the CD^* batch. If this mean dose is less than 90 % of D^* and, on performance of the test of sterility, acceptable results are observed (see 8.2.5), the verification dose experiment need not be repeated.

8.2.4.2 Subject the irradiated product items individually to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility. Designate this value as CD^*.

8.2.5 Stage 4: Consideration of results

Obtain the First No Positive dose (FNP) from the results of this experiment as follows:

a) if $CD^* \leqslant 2$, FNP = DD^*;

b) if $2 < CD^* < 10$, FNP = DD^* + 2,0 kGy,

c) if $9 < CD^* < 16$, FNP = DD^* + 4,0 kGy

or

d) if $CD^* > 15$, the cause should be determined, corrective action implemented and D^* redetermined.

8.2.6 Stage 5: Establish sterilization dose

8.2.6.1 Determine DS from FFP and FNP using Equation (3) or Equation (4) depending on the difference between FNP and FFP.

When (FNP – FFP) $<$ 10 kGy use

$$DS = 2 + 0{,}2\ (\text{FNP} - \text{FFP}) \qquad (3)$$

NOTE In using Equation (3), if (FNP – FFP) is < 0, set (FNP – FFP) = 0.

When (FNP – FFP) $\geqslant$ to 10 kGy use

$$DS = 0{,}4\ (\mathrm{FNP} - \mathrm{FFP}) \tag{4}$$

8.2.6.2 Establish D^{**} using Equation (5).

$$D^{**} = DD^{*} + \left[\log\left(CD^{*}\right)\right]\left(DS\right) \tag{5}$$

NOTE If $CD^{*} = 0$, set [log (CD^{*})] = 0.

8.2.6.3 Calculate the sterilization dose, using Equation (6).

$$\text{sterilization dose} = D^{**} + \left[-\log\left(\mathrm{SAL}\right) - \log\left(\mathrm{SIP}\right) - 2\right]\left(DS\right) \tag{6}$$

where:

D^{**} is the final estimate of the dose that will provide a 10^{-2} SAL;

SAL is the preselected sterility assurance level;

SIP is the portion of product (sample item portion) used for determining D^{**} and DS;

DS is an estimate of the dose required to inactivate 90 % of the microorganisms surviving DD^{*}.

Dose calculations should be made with data that are reported to one place of decimals. The sterilization dose may be rounded (using standard rounding procedures) to one place of decimals.

NOTE The term log (SIP) in Equation (6) provides the appropriate factor for correction for a portion of the product being used for dose setting.

8.3 Procedure for Method 2B

8.3.1 General

8.3.1.1 In applying Method 2B, the following three requirements shall be satisfied:

a) the entire product is utilized (SIP = 1,0);

b) after irradiation at any of the incremental doses, the number of positive tests of sterility observed does not exceed 14;

c) FNP does not exceed 5,5 kGy.

8.3.1.2 In applying Method 2B, the five stages below shall be followed.

NOTE For worked examples, see 11.2.4.

8.3.2 Stage 1: Select SAL and obtain samples of product

8.3.2.1 Record the SAL for the intended use of the product.

8.3.2.2 Select at least 260 product items from each of three independent production batches in accordance with 5.1, 5.2 and 5.3.

8.3.3 Stage 2: Perform incremental dose experiment

8.3.3.1 General

8.3.3.1.1 For each of 3 production batches, irradiate 20 product items from each of a series of at least 8 doses, starting at 1 kGy and increasing in nominal increments of 1 kGy. Determine each incremental dose. The highest dose at each nominal incremental dose is used subsequently in the identification of the ffp and d^*. This dose may vary from the nominal incremental dose by ± 0,5 kGy or ± 10 %, whichever is greater. If the arithmetic mean of the highest and lowest doses at a given incremental dose is less than the value taken by the lower limit, the irradiation of 20 further product items at this particular incremental dose may be carried out.

8.3.3.1.2 Subject the irradiated product items individually to a test of sterility in accordance with ISO 11737 2 (see 5.4.1) and record the number of positive tests of sterility.

8.3.3.1.3 Obtain the following from the results of this experiment:

a) A and FFP (see 8.3.3.2);

b) D^* (see 8.3.3.3);

c) CD^* batch (see 8.3.3.4).

8.3.3.2 A and FFP

8.3.3.2.1 For each of three production batches, determine the lowest dose from the incremental dose series at which at least one of the 20 tests of sterility is negative. Designate this dose as ffp for the particular batch and find the median ffp of the three. If 2 or 3 batches exhibit the same ffp, choose the dose for the batch showing the higher or highest number of positives as the median ffp.

8.3.3.2.2 Obtain the value of A from Table 8 using the number of positive tests of sterility at the median ffp.

Table 8 — Values of A for different numbers of positive tests of sterility at median ffp (Method 2B)

Number of positive tests of sterility at median ffp	A (kGy)	Number of positive tests of sterility at median ffp	A (kGy)
14	0,22	6	0,52
13	0,26	5	0,58
12	0,29	4	0,64
11	0,32	3	0,72
10	0,36	2	0,82
9	0,40	1	1,00
8	0,44	0	1,00
7	0,48		

NOTE See Equation (7) for a formula for calculating A.

$$A = (1\,\text{kGy})\frac{\left\{\log_{10}\left(\log_e 20\right) - \log_{10}\left[\log_e\left(20/n\right)\right]\right\}}{\left\{\log_{10}\left(\log_e 20\right) - \log_{10}\left[\log_e\left(20/19\right)\right]\right\}} \qquad (7)$$

where n is the number of tests of sterility that are negative. (See Davis *et al.*, 1981 [8].)

8.3.3.2.3 Calculate FFP from Equation (2), see 8.2.3.2.3.

8.3.3.3 D^*

8.3.3.3.1 For each of the three production batches, determine d^* by either

a) finding the lower of two consecutive doses at which all tests of sterility are negative, followed by no more than one further positive test in any of the remaining tests in the incremental dose series

or

b) finding the dose at which one positive in 20 tests of sterility occurs, immediately preceded by one, and only one, incremental dose at which all tests are negative and followed by incremental doses at which all tests are negative.

8.3.3.3.2 If the criteria in 8.3.3.3.1 a) or b) are not met with each of the three production batches, the incremental dose experiment is invalid. In this circumstance, performance of the incremental dose experiment may be repeated after investigation of the methodology of the experiment and implementation of corrective action(s).

8.3.3.3.3 Designate D^* as follows:

a) if the highest batch d^* exceeds the median batch d^* by < 5 kGy, the median batch d^* becomes D^*

or

b) if the highest batch d^* exceeds the median batch d^* by $\geqslant 5$ kGy, the highest batch d^* becomes D^*.

8.3.3.4 CD^* **batch**

Determine the batch for which $d^* = D^*$ and designate this as CD^* batch. If more than one batch has a d^* equal to D^*, one of these batches may be designated at random as CD^* batch. Retained product items from CD^* batch are used in Stage 3 of Method 2B. Storage conditions of the retained product from the three batches should be such that microbial growth is prevented. Where this is not practicable, a fourth batch may be taken as the CD^* batch.

8.3.4 Stage 3: Perform verification dose experiment

8.3.4.1 Irradiate 100 product items from the CD^* batch at a dose of D^*. Determine the dose and designate the highest dose delivered to the product items as DD^*. DD^* may vary from D^* by + 1,0 kGy or + 10 %, whichever is greater. If the arithmetic mean of the highest and lowest doses delivered to product items is less than 90 % of D^*, the irradiation may be repeated with a further 100 product items taken from the CD^* batch. If this mean dose is less than 90 % of D^* and, on the performance of the test of sterility, acceptable results are observed (see 8.3.5), the verification dose experiment need not be repeated.

8.3.4.2 Subject the irradiated product items individually to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility. Designate this value as CD^*.

8.3.5 Stage 4: Consideration of results

Obtain FNP from the results of this experiment as follows:

a) if $CD^* \leqslant 2$, FNP = DD^*,

b) if $2 < CD^* < 10$, FNP = DD^* + 2,0 kGy,

c) if $9 < CD^* < 16$, FNP = DD^* + 4,0 kGy

or

d) if $CD^* > 15$, the cause should be determined, corrective action implemented and D^* redetermined.

8.3.6 Stage 5: Establish sterilization dose

8.3.6.1 Determine DS from FFP and FNP using Equation (8) depending on the difference between FNP and FFP.

$$DS = 1{,}6 + 0{,}2\,(\mathrm{FNP} - \mathrm{FFP}) \tag{8}$$

NOTE In using Equation (8), if (FNP – FFP) < 0, set (FNP – FFP) = 0.

8.3.6.2 Establish D^{**} using Equation (5) – see 8.2.6.2.

NOTE If $CD^{*} = 0$, set [log (CD^{*})] = 0.

8.3.6.3 Calculate the sterilization dose using Equation (9).

$$\text{sterilization dose} = D^{**} + \left[-\log(\mathrm{SAL}) - 2\right](DS) \tag{9}$$

where:

D^{**} is the final estimate of the dose that will provide an SAL value of 10^{-2};

SAL is the preselected sterility assurance level;

DS is an estimate of the dose required to inactivate 90 % of the microorganisms surviving DD^{*}.

9 Method VD_{max} — Substantiation of 25 kGy or 15 kGy as the sterilization dose

9.1 Rationale

Operationally, this method of substantiation for a selected sterilization dose is similar to dose setting Method 1 (see Clause 7); it also requires a determination of bioburden and the performance of a verification dose experiment.

In carrying out substantiation, the method verifies that bioburden present on product prior to sterilization is less resistant to radiation than a microbial population of maximal resistance consistent with the attainment of an SAL of 10^{-6} at the selected sterilization dose; verification is conducted at an SAL of 10^{-1} with 10 product items irradiated in the performance of the verification dose experiment. The dose corresponding to this SAL (maximal verification dose, VD_{max}) is characteristic of both the bioburden level and the associated maximal resistance. In establishing the maximal resistance for a particular bioburden level, due account has been taken of the various resistance components of the SDR (see Table 3), the latter being the basis of Method 1. Components of the SDR of high resistance that have significant effect on the attainment of an SAL of 10^{-6} have been used to define the maximal resistances on which this substantiation method is based. In this way, the level of conservativeness of the SDR, and thus of Method 1, is preserved. See Kowalski and Tallentire, 1999 [14]; Kowalski, Aoshuang and Tallentire, 2000 [13]; and Kowalski and Tallentire, 2003 [15].

In practice, a determination is made of the average bioburden. The VD_{max} dose corresponding to this average is read from a table; it is the dose at which the verification dose experiment is carried out. Ten product items, or portions thereof, are exposed to the VD_{max} dose and each item is subjected individually to a test of sterility. If there is no more than one positive test of sterility in the 10 tests, the pre-selected sterilization dose is substantiated.

The VD_{max} methods given in this part of ISO 11137 are for selected sterilization doses of 25 kGy and 15 kGy. The method for 25 kGy is applicable to product having an average bioburden less than or equal to 1 000 (see 9.2 or 9.3 and Table 9), whereas that for 15 kGy applies only to product with bioburden $\leqslant$ 1,5 (see 9.4 or 9.5 and Table 10). The inclusion of Method VD_{max} for 15 kGy provides an alternative to Method 1 for dose establishment for product of low average bioburden. To distinguish the two applications of Method VD_{max} and their associated sets of values of verification dose, a superscript of “25” or “15” has been added to the term VD_{max} where appropriate, viz. VD_{max}^{25} and VD_{max}^{15}.

NOTE Inspection of the values of VD_{max}^{25} for the various levels of average bioburden given in Table 9 reveals a change in the relationship between the bioburden level and the value of VD_{max}. With increasing bioburden up to a level of 80, values progressively increase, as might be expected. However, at a bioburden of 80, VD_{max}^{25} takes a maximum, and for higher bioburden levels, the corresponding VD_{max} values decline. A similar increase, followed by a decrease, is seen with VD_{max}^{15} values (see Table 10). This behaviour is not the result of an error in either the tables or the calculation of the VD_{max} values. It is an inevitable outcome of building into Method VD_{max} the same degree of conservativeness as that in Method 1 (see Kowalski and Tallentire, 2003 [15]).

9.2 Procedure for Method VD_{max}^{25} for multiple production batches

9.2.1 General

9.2.1.1 This method shall only be used if the average bioburden of product is $\leqslant$ 1 000.

9.2.1.2 In applying VD_{max}^{25} for product with an average bioburden $\leqslant$ 0,9, the entire product item shall be used in accordance with Table 9, whereas for product with an average bioburden $>$ 0,9, a SIP may be used.

9.2.1.3 In applying Method VD_{max}^{25}, the five stages below shall be followed.

NOTE For worked examples, see 11.3.

9.2.2 Stage 1: Obtain samples of product

Select at least 10 product items from each of three independent production batches in accordance with 5.1, 5.2 and 5.3.

9.2.3 Stage 2: Determine average bioburden

9.2.3.1 Apply the correction factor (see ISO 11737-1) in the determination of bioburden.

9.2.3.2 Determine the bioburden of each of the selected product items and calculate:

a) the average bioburden per item for each of the three batches (batch average);

b) the average bioburden per item for the selected product items (overall average bioburden).

NOTE Bioburden is generally determined on individual product items, but when the bioburden is low (e.g. $<$ 10), it is permissible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIP; SIPs should not be pooled, rather a larger SIP should be chosen.

9.2.3.3 Compare the three batch averages to the overall average bioburden and determine whether any one of the batch averages is two or more times greater than the overall average bioburden.

9.2.4 Stage 3: Obtain VD_{max}^{25}

Obtain VD_{max}^{25} from Table 9 using one of the following:

a) the highest batch average, if one or more batch averages is $\geqslant 2 \times$ (overall average bioburden)

or

b) the overall average bioburden, if each of the batch averages is $< 2 \times$ (overall average bioburden).

For an SIP = 1,0, if the average bioburden is not given in Table 9, use the closest tabulated average bioburden greater than the calculated average bioburden.

For an SIP $<$ 1,0, calculate the average bioburden for the entire product item (SIP = 1,0) by dividing the SIP average bioburden by the SIP decimal value. If the calculated average bioburden is not given in Table 9, use the closest tabulated average bioburden greater than the calculated average bioburden to locate the value of SIP = 1,0 VD_{max}^{25} and the corresponding SIP dose reduction factor.

NOTE Use of a SIP $<$ 1,0 is not permitted for product with an average bioburden $\leqslant$ 0,9 (see 9.2.1.2).

Table 9 — Values of VD_{max}^{25} and SIP dose reduction factors for levels of average bioburden ⩽ 1 000 CFU

Average bioburden	SIP = 1,0 VD_{max}^{25} (kGy)	SIP dose reduction factor (kGy)
⩽ 0,1	0,0	n/a [a]
0,15	0,9	n/a [a]
0,20	1,4	n/a [a]
0,25	1,8	n/a [a]
0,30	2,2	n/a [a]
0,35	2,5	n/a [a]
0,40	2,7	n/a [a]
0,45	2,9	n/a [a]
0,50	3,1	n/a [a]
0,60	3,4	n/a [a]
0,70	3,6	n/a [a]
0,80	3,8	n/a [a]
0,90	4,0	n/a [a]
1,0	4,2	4,17
1,5	4,8	4,05
2,0	5,2	3,97
2,5	5,5	3,91
3,0	5,7	3,86
3,5	5,9	3,82
4,0	6,1	3,79
4,5	6,2	3,76
5,0	6,3	3,73
5,5	6,5	3,71
6,0	6,6	3,69
6,5	6,7	3,67
7,0	6,7	3,65
7,5	6,8	3,64
8,0	6,9	3,62
8,5	7,0	3,61
9,0	7,0	3,59
9,5	7,1	3,58
10	7,1	3,57
11	7,2	3,55
12	7,3	3,53
13	7,4	3,51
14	7,5	3,50
15	7,6	3,48
16	7,6	3,47
17	7,7	3,46
18	7,8	3,45
19	7,8	3,43
20	7,9	3,42
22	8,0	3,40
24	8,1	3,39
26	8,1	3,37
28	8,2	3,36
30	8,3	3,34
35	8,4	3,31
40	8,6	3,29
45	8,7	3,27
50	8,8	3,25
55	8,9	3,23
60	8,9	3,21
65	9,0	3,20
70	9,1	3,19
75	9,1	3,17
80	9,2	3,15
85	9,1	3,11
90	9,1	3,08
95	9,1	3,05
100	9,0	3,01
110	9,0	2,96
120	9,0	2,91
130	8,9	2,86
140	8,9	2,83
150	8,9	2,79
160	8,8	2,76
170	8,8	2,72
180	8,8	2,69
190	8,7	2,67
200	8,7	2,64
220	8,7	2,60
240	8,6	2,56
260	8,6	2,52
280	8,6	2,49
300	8,6	2,46
325	8,5	2,43
350	8,5	2,40
375	8,5	2,37
400	8,4	2,34
425	8,4	2,32
450	8,4	2,30
475	8,4	2,28
500	8,4	2,26
525	8,3	2,24
550	8,3	2,22
575	8,3	2,21
600	8,3	2,19
650	8,3	2,16
700	8,2	2,14
750	8,2	2,12
800	8,2	2,09
850	8,2	2,07
900	8,1	2,05
950	8,1	2,04
1 000	8,1	2,02

NOTE If VD_{max}^{25} = 0,0 kGy, product items are not irradiated.

[a] Not applicable; in the range of average bioburden ⩽ 0,9, the entire product (SIP = 1,0) is used and hence the SIP Dose Reduction Factor is not given.

Use Equation (10) to calculate the SIP VD_{max}^{25} (see Kowalski and Tallentire 2003 [15]).

$$\text{SIP } VD_{max}^{25} = (\text{SIP} = 1{,}0\ VD_{max}^{25}) + (\text{SIP dose reduction factor} \times \log \text{SIP}) \quad (10)$$

9.2.5 Stage 4: Perform verification dose experiment

9.2.5.1 Select 10 product items from a single batch of product. The 10 product items for the performance of Stage 4 may be selected from one of the batches for which a bioburden determination was carried out in Stage 2, or from a fourth batch manufactured under conditions that are representative of normal production. The ability of the product to support microbial growth should be taken into account in selecting the batch to be used.

9.2.5.2 Irradiate 10 product items at VD_{max}^{25} obtained from Table 9 or derived using Equation (10), whichever is appropriate. Determine the dose. The highest dose to the product items may not exceed VD_{max}^{25} by more than 10 %. If the arithmetic mean of the highest and lowest doses delivered to product items is < 90 % of VD_{max}^{25}, the verification dose experiment may be repeated. If this mean dose is < 90 % of VD_{max}^{25} and, on performance of the test of sterility, acceptable results are observed (see 9.2.6), the verification experiment need not be repeated.

NOTE If $VD_{max}^{25} = 0{,}0$ kGy, product items are not irradiated.

9.2.5.3 Subject the product items (see 9.2.5.2) individually to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility.

9.2.6 Stage 5: Interpretation of results

9.2.6.1 Accept verification if there is no more than one positive test of sterility from the 10 tests carried out and thereby substantiate 25 kGy as the sterilization dose.

9.2.6.2 Perform a confirmatory verification dose experiment (see 9.2.7) if there are two positive tests of sterility in the 10 tests carried out.

9.2.6.3 Do not accept verification if there are more than two positive tests of sterility.

If this outcome can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, the verification dose experiment may be repeated following implementation of corrective action.

If this outcome cannot be ascribed to a cause addressed by corrective action, this method of dose substantiation is not valid and an alternative method for substantiation of 25 kGy as the sterilization dose shall be used (see Clause 6).

9.2.7 Confirmatory verification dose experiment

9.2.7.1 General

If a confirmatory verification dose experiment is to be carried out (see 9.2.6.2), the three stages below (9.2.7.2, 9.2.7.3 and 9.2.7.4) shall be followed.

9.2.7.2 Stage 1: Obtain samples of product

Select at least 10 product items from a single batch of product. The 10 product items for the performance of confirmatory verification dose experiment may be selected from one of the batches on which a bioburden determination was carried out in Stage 2 (see 9.2.3), from a fourth batch used in Stage 4 (see 9.2.5) or from a batch manufactured under conditions that are representative of normal production. The ability of the product to support microbial growth should be taken into account in selecting the batch to be used.

9.2.7.3 Stage 2: Perform confirmatory verification dose experiment

9.2.7.3.1 Irradiate 10 product items at VD_{max}^{25} as determined in 9.2.4. Determine the dose. If the highest dose to the product items exceeds VD_{max}^{25} by more than 10 %, the verification dose experiment shall be repeated. If the arithmetic mean of the highest and lowest doses delivered to product items is < 90 % of VD_{max}^{25}, the confirmatory verification dose experiment may be repeated. If this mean dose is < 90 % of VD_{max}^{25} and, on performance of the tests of sterility, acceptable results are observed (see 9.2.7.4), the verification experiment need not be repeated.

9.2.7.3.2 Subject each irradiated product item individually to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility.

9.2.7.4 Stage 3: Interpretation of results

9.2.7.4.1 Accept verification and thereby substantiate 25 kGy as the sterilization dose if there are no positive tests of sterility from the 10 tests carried out, giving a total of 2 positive tests of sterility obtained from the original verification and confirmatory verification dose experiments.

9.2.7.4.2 Do not accept verification if there are any positive tests of sterility.

If this outcome can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, the confirmatory verification dose experiment may be repeated following implementation of corrective action.

If this outcome cannot be ascribed to a cause addressed by corrective action, this method of substantiation of 25 kGy as the sterilization dose is not valid and an alternative method for substantiation of 25 kGy as the sterilization dose shall be used (see Clause 6).

9.3 Procedure for Method VD_{max}^{25} for a single production batch

9.3.1 Rationale

This method is an adaptation of Method VD_{max}^{25} and is intended to be used only for the substantiation of 25 kGy as the sterilization dose for a single production batch.

9.3.2 General

9.3.2.1 This method shall only be used if average bioburden of product is $\leqslant 1\,000$.

9.3.2.2 In applying VD_{max}^{25} for product with an average bioburden $\leqslant 0{,}9$, the entire product item shall be used in accordance with Table 9, whereas for product with an average bioburden $> 0{,}9$, a SIP may be used.

9.3.2.3 In applying this adaptation of Method VD_{max}^{25}, the five stages below shall be followed.

9.3.3 Stage 1: Obtain samples of product

Select at least 10 product items from the single batch in accordance with 5.1, 5.2 and 5.3.

9.3.4 Stage 2: Determine average bioburden

9.3.4.1 Apply a correction factor (see ISO 11737-1) in the determination of bioburden.

9.3.4.2 Determine the bioburden of each of the selected product items and calculate the average bioburden.

NOTE Bioburden is generally determined on individual product items, but when the bioburden is low (e.g. < 10), it is permissible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIP; SIPs should not be pooled, rather a larger SIP should be chosen.

9.3.5 Stage 3: Obtain VD_{max}^{25}

Obtain VD_{max}^{25} from Table 9.

a) For an SIP = 1,0, if the average bioburden is not given in Table 9, use the closest tabulated average bioburden greater than the calculated average bioburden.

b) For an SIP < 1,0, calculate the average bioburden for the entire product item (SIP = 1,0) by dividing the SIP average bioburden by the SIP decimal value. If the calculated average bioburden is not given in Table 9, use the closest tabulated average bioburden greater than the calculated average bioburden to locate the value of SIP = 1,0 VD_{max}^{25} and the corresponding SIP dose reduction factor.

NOTE Use of an SIP < 1,0 is not permitted for product with an average bioburden ⩽ 0,9 (see 9.3.2.2).

Use Equation (10) to calculate the SIP VD_{max}^{25} (see 9.2.4).

9.3.6 Stage 4: Perform verification dose experiment

9.3.6.1 Select 10 product items from the single batch of product.

9.3.6.2 Irradiate the 10 product items, or portions thereof if appropriate, at VD_{max} obtained from Table 9 or derived using Equation (10), whichever is appropriate. Determine the dose. If the highest dose to the product items exceeds the verification dose by more than 10 %, and the sterilization dose is to be substantiated using VD_{max}^{25}, the verification dose experiment shall be repeated. If the arithmetic mean of the highest and lowest doses delivered to product items is < 90 % of the VD_{max}^{25}, the verification dose experiment may be repeated. If this mean dose is < 90 % of VD_{max}^{25} and, on performance of the test of sterility, acceptable results are observed (see 9.3.7.1), the verification experiment need not be repeated.

NOTE If VD_{max}^{25} = 0,0 kGy, product items are not irradiated.

9.3.6.3 Subject each irradiated product item (see 9.3.6.2) individually to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility.

9.3.7 Stage 5: Interpretation of results

9.3.7.1 Accept verification and thereby substantiate 25 kGy as the sterilization dose if there is no more than one positive test of sterility from the 10 tests carried out.

9.3.7.2 Perform a confirmatory verification dose experiment (see 9.2.7) if there are two positive tests of sterility in the 10 tests carried out.

9.3.7.3 Do not accept verification if there are more than two positive tests of sterility.

If this outcome can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, the verification dose experiment may be repeated following implementation of corrective action.

If this outcome cannot be ascribed to a cause addressed by corrective action, this method of substantiation of 25 kGy as the sterilization dose is not valid and an alternative method for substantiation of 25 kGy as the sterilization dose shall be used (see Clause 6).

9.4 Procedure for Method VD_{max}^{15} for multiple production batches

9.4.1 General

9.4.1.1 This method shall only be used if average bioburden of product is ⩽ 1,5.

9.4.1.2 In applying Method VD_{max}^{15}, an entire product item (SIP = 1,0) shall be used in accordance with Table 1.

9.4.1.3 In applying Method VD_{max}^{15}, the five stages below shall be followed.

NOTE For worked examples, see 11.3.

9.4.2 Stage 1: Obtain samples of product

Select at least 10 product items from each of three independent production batches in accordance with 5.1, 5.2 and 5.3.

9.4.3 Stage 2: Determine average bioburden

9.4.3.1 Apply a correction factor (see ISO 11737-1) in the determination of bioburden.

9.4.3.2 Determine the bioburden of each of the selected product items and calculate:

a) the average bioburden per item for each of the three batches (batch average);

b) the average bioburden per item for the selected product items (overall average bioburden).

NOTE Bioburden is generally determined on individual product items, but when the bioburden is low (e.g. < 10), it is permissible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIP; SIPs should not be pooled, rather a larger SIP should be chosen.

9.4.3.3 Compare the three batch averages to the overall average bioburden and determine whether any one of the batch averages is two or more times greater than the overall average bioburden.

9.4.4 Stage 3: Obtain VD_{max}^{15}

Obtain VD_{max}^{15} from Table 10 using one of the following:

a) the highest batch average, if one or more batch averages $\geqslant 2 \times$ (overall average bioburden)

or

b) the overall average bioburden, if each of the batch averages is $< 2 \times$ (overall average bioburden).

If the average bioburden is not given in Table 10, use the closest tabulated average bioburden greater than the calculated average bioburden.

Table 10 — Values of VD_{max}^{15} for levels of average bioburden $\leqslant$ 1,5

Average bioburden	SIP = 1,0 VD_{max}^{15} (kGy)	Average bioburden	SIP = 1,0 VD_{max}^{15} (kGy)
$\leqslant$ 0,1	0,0	0,50	1,8
0,15	0,5	0,60	2,0
0,20	0,9	0,70	2,2
0,25	1,1	0,80	2,3
0,30	1,3	0,90	2,2
0,35	1,5	1,0	2,1
0,40	1,6	1,5	1,7
0,45	1,7		

NOTE If VD_{max}^{15} = 0,0 kGy, product items are not irradiated.

9.4.5 Stage 4: Perform verification dose experiment

9.4.5.1 Select 10 product items from a single batch of product. The 10 product items for the performance of Stage 4 may be selected from one of the batches for which a bioburden determination was carried out in Stage 2, or from a fourth batch manufactured under conditions which are representative of normal production. The ability of the product to support microbial growth should be taken into account in selecting the batch to be used.

9.4.5.2 Irradiate 10 product items at VD_{max}^{15} obtained from Table 10. Determine the dose. If the highest dose to the product items exceeds VD_{max}^{15} by more than + 0,1 kGy or + 10 %, whichever is greater, and the sterilization dose is to be substantiated using VD_{max}^{15}, the verification dose experiment shall be repeated. If the arithmetic mean of the highest and lowest doses delivered to product items is less than 90 % of VD_{max}^{15}, the verification dose experiment may be repeated. If this mean dose is less than 90 % of VD_{max}^{15} and, on performance of the test of sterility, acceptable results are observed (see 9.4.6), the verification experiment need not be repeated.

NOTE If VD_{max}^{15} = 0,0 kGy, product items are not irradiated.

9.4.5.3 Subject each irradiated product item (see 9.4.5.2) individually to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility.

9.4.6 Stage 5: Interpretation of results

9.4.6.1 Accept verification and thereby substantiate 15 kGy as the sterilization dose if there is no more than one positive test of sterility from the 10 tests carried out.

9.4.6.2 Perform a confirmatory verification dose experiment (see 9.4.7) if there are two positive tests of sterility in the 10 tests carried out.

9.4.6.3 Do not accept verification if there are more than two positive tests of sterility.

If this outcome can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, the verification dose experiment may be repeated following implementation of corrective action.

If this outcome cannot be ascribed to a cause addressed by corrective action, this method of substantiation of 15 kGy as the sterilization dose is not valid and an alternative method for substantiation of 15 kGy as the sterilization dose shall be used (see Clause 6).

If any of these circumstances apply, then the verification dose experiment may be repeated.

9.4.7 Confirmatory verification dose experiment

9.4.7.1 General

If a confirmatory verification dose experiment is to be carried out (see 9.4.6.2), the three stages below (9.4.7.2, 9.4.7.3 and 9.4.7.4) shall be followed.

9.4.7.2 Stage 1: Obtain samples of product

Select at least 10 product items from a single batch of product. The 10 product items for the performance of confirmatory verification dose experiment may be selected from one of the batches on which a bioburden determination was carried out in Stage 2 (see 9.4.3), from a fourth batch used in Stage 4 (see 9.4.5) or from a batch manufactured under conditions which are representative of normal production. The ability of the product to support microbial growth should be taken into account in selecting the batch to be used.

9.4.7.3 Stage 2: Perform confirmatory verification dose experiment

9.4.7.3.1 Irradiate the 10 product items at VD_{max}^{15} as determined in 9.4.4. Determine the dose. If the highest dose to the product items exceeds the verification dose by more than 10 %, and the sterilization dose is to be established using VD_{max}^{15}, the verification dose experiment shall be repeated. If the arithmetic mean of the highest and lowest doses delivered to product items is < 90 % of VD_{max}^{15}, the confirmatory verification dose experiment may be repeated. If this mean dose is < 90 % of VD_{max}^{15} and, on performance of the tests of sterility, acceptable results are observed (see 9.4.7.4), the verification experiment need not be repeated.

9.4.7.3.2 Subject each irradiated product item individually to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility.

9.4.7.4 Stage 3: Interpretation of results

9.4.7.4.1 Accept verification and thereby substantiate 15 kGy as the sterilization dose if there are no positive tests of sterility from the 10 tests carried out, giving a total of 2 positive tests of sterility obtained from the original verification and confirmatory verification dose experiments.

9.4.7.4.2 Do not accept verification if there are more than two positive tests of sterility.

If this outcome can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, the confirmatory verification dose experiment may be repeated following implementation of corrective action.

If this outcome cannot be ascribed to a cause addressed by corrective action, this method of substantiation of 15 kGy as the sterilization dose is not valid and an alternative method for substantiation of 15 kGy as the sterilization dose shall be used (see Clause 6).

9.5 Procedure for Method VD_{max}^{15} for a single production batch

9.5.1 Rationale

This method is an adaptation of Method VD_{max}^{15} and is intended to be used only for the substantiation of 15 kGy as the sterilization dose for a single production batch.

9.5.2 General

9.5.2.1 This method shall only be used if average bioburden of product is ⩽ 1,5.

9.5.2.2 In applying Method VD_{max}^{15}, an entire product (SIP = 1,0) shall be used in accordance with Table 1.

9.5.2.3 In applying this adaptation of Method VD_{max}^{15}, the five stages below (9.5.3 to 9.5.7) shall be followed.

9.5.3 Stage 1: Obtain samples of product

Select at least 10 product items from the single batch in accordance with 5.1, 5.2 and 5.3.

9.5.4 Stage 2: Determine average bioburden

9.5.4.1 Apply the correction factor (see ISO 11737-1) in the determination of bioburden.

9.5.4.2 Determine the bioburden of each of the selected product items and calculate the average bioburden.

NOTE Bioburden is generally determined on individual product items, but when the bioburden is low (e.g. < 10), it is permissible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIP; SIPs should not be pooled, rather a larger SIP should be chosen.

9.5.5 Stage 3: Obtain VD_{max}^{15}

Obtain VD_{max}^{15} from Table 10. If the average bioburden is not given in Table 10, use the closest tabulated average bioburden greater than the calculated average bioburden.

9.5.6 Stage 4: Perform verification dose experiment

9.5.6.1 Select 10 product items from the single batch of product.

9.5.6.2 Irradiate the product items at VD_{max}^{15} obtained from Table 10. Determine the dose. If the highest dose to the product items exceeds the verification dose by more than ± 0,1 kGy or ± 10 %, whichever is greater, and the sterilization dose is to be substantiated using VD_{max}^{15}, the verification dose experiment shall be repeated. If the arithmetic mean of the highest and lowest doses delivered to product items is ≤ 90 % of VD_{max}^{15}, the verification dose experiment may be repeated.

NOTE If VD_{max}^{15} = 0,0 kGy, product items are not irradiated.

9.5.6.3 Subject each irradiated product item (see 9.5.6.2) individually to a test of sterility in accordance with ISO 11737-2 (see 5.4.1) and record the number of positive tests of sterility.

9.5.7 Stage 5: Interpretation of results

9.5.7.1 Accept verification if there is no more than one positive test of sterility from the 10 tests carried out and thereby substantiate 15 kGy as the sterilization dose.

9.5.7.2 Perform a confirmatory verification dose experiment (see 9.4.7) if there are two positive tests of sterility in the 10 tests carried out.

9.5.7.3 Do not accept verification if there are more than two positive tests of sterility.

If this outcome can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, the verification dose experiment may be repeated following implementation of corrective action.

If this outcome cannot be ascribed to a cause addressed by corrective action, this method of substantiation of 15 kGy as the sterilization dose is not valid and an alternative method for substantiation of 15 kGy as the sterilization dose shall be used (see Clause 6).

If any of these circumstances apply, then the verification dose experiment may be repeated.

10 Auditing sterilization dose

10.1 Purpose and frequency

Once the sterilization dose has been established, periodic audits shall be carried out to confirm the continued appropriateness of the sterilization dose. The frequency at which audits are performed shall be in accordance with 12.1 of ISO 11137-1:2006. Dose audits are not required during periods in which product is not produced. A review of environmental and manufacturing controls, together with determinations of bioburden should be conducted in conjunction with sterilization dose audits. If the review indicates lack of control, appropriate action should be taken.

10.2 Procedure for auditing a sterilization dose established using Method 1 or Method 2

10.2.1 General

10.2.1.1 For the performance of a sterilization dose audit for a sterilization dose established using Method 1 or Method 2, use a SIP equivalent to that used in establishing the original sterilization dose.

10.2.1.2 In applying a sterilization dose audit, the four stages below (10.2.2 to 10.2.5) shall be followed.

NOTE For worked examples, see 11.4 and 11.5.

10.2.2 Stage 1: Obtain samples of product

Select at least 110 product items from a single batch of product in accordance with 5.1, 5.2 and 5.3.

10.2.3 Stage 2: Determine average bioburden

Determine the bioburden of each of at least 10 product items and calculate the average bioburden. If a correction factor (see ISO 11737-1) was used in establishing the original sterilization dose, use the same correction factor in the sterilization dose audit.

NOTE 1 Bioburden is generally determined on individual product items but when the bioburden is low (e.g. < 10), it is possible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIP; SIPs should not be pooled, rather a larger SIP should be chosen.

NOTE 2 Bioburden data are not intended to be used in obtaining the verification dose for the sterilization dose audit. These data are used for process monitoring and control (e.g. trend analysis, investigation of sterilization dose audit failures or reduction in sterilization dose audit frequency).

10.2.4 Stage 3: Perform verification dose experiment

10.2.4.1 Irradiate 100 product items at the verification dose or D^{**} found in the original or any subsequent dose setting exercise, as appropriate. Determine the dose. If the highest dose to the product items exceeds the verification dose or D^{**} by more than 10 %, the verification dose experiment shall be repeated. If the arithmetic mean of the highest and lowest doses delivered to product items is < 90 % of the verification dose or D^{**}, the sterilization dose audit may be repeated. If this mean dose is < 90 % of the verification dose and, on performance of the test of sterility, acceptable results are observed (see 10.2.5), the sterilization dose audit need not be repeated.

10.2.4.2 Subject each irradiated product item (see 10.2.4.1) individually to a test of sterility using the media and incubation conditions used in the original dose setting experiment and record the number of positive tests of sterility.

10.2.5 Stage 4: Interpretation of results

10.2.5.1 If there are no more than two positive tests of sterility from the 100 tests carried out, accept verification.

10.2.5.2 If three or four positive tests of sterility are obtained in the 100 tests, and the outcome cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, augment the sterilization dose immediately (see 10.2.6). Repeat the sterilization dose audit using a further 100 product items and the same verification dose or D^{**} as that used in the original sterilization dose audit. Interpret the results of the repeat sterilization dose audit in accordance with 10.2.5.5.

10.2.5.3 If 5 to 15 positive tests of sterility are obtained in the 100 tests, the sterilization dose is inadequate; the sterilization dose shall be augmented immediately (see 10.2.6).

If the occurrence of 5 or more positive tests of sterility can be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, implement corrective action and repeat the sterilization dose audit. Interpret the results in accordance with 10.2.5.5.

If the occurrence of 5 or more positive tests of sterility can be ascribed to a specific bioburden-related cause, implement corrective action and repeat the sterilization dose audit. Interpret the results in accordance with 10.2.5.5.

If the occurrence of 5 or more positive tests of sterility cannot be ascribed to any one or more of the above causes, verification is not accepted; the previously established sterilization dose is not valid. Re-establish the sterilization dose using another method (see Clause 6) and augment the sterilization dose until re-establishment of the sterilization dose is completed.

10.2.5.4 If more than 15 positive tests of sterility are obtained, the sterilization dose shall not be augmented. If this outcome cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, sterilization at the previously established sterilization dose shall be discontinued and sterilization shall not resume until the sterilization dose is re-established using another method (see Clause 6).

10.2.5.5 Interpret results of the repeat sterilization dose audit, performed in accordance with 10.2.5.2 or 10.2.5.3, as follows:

a) if no more than two positive tests of sterility are obtained in the 100 tests, and a review of environmental and manufacturing controls and bioburden determination indicates no values outside established specifications, use of the original sterilization dose may be resumed;

b) if three or four positive tests of sterility are obtained in the 100 tests, re-establish the sterilization dose immediately and continue to use the augmented sterilization dose until re-establishment of the sterilization dose is completed;

c) if 5 to 15 positive tests of sterility are obtained in the 100 tests, re-establish the sterilization dose using another method (see Clause 6) and continue to use the augmented sterilization dose until re-establishment of the sterilization does is completed;

d) if more than 15 positive tests of sterility are obtained in the 100 tests, the sterilization dose shall not be augmented; sterilization at the previously established sterilization dose shall be discontinued and sterilization shall not resume until the sterilization dose is re-established using another method (see Clause 6).

10.2.6 Augmentation of a sterilization dose established using Method 1, Method 2A or Method 2B

10.2.6.1 General

The method for augmentation of the sterilization dose, established using Method 1, Method 2A or Method 2B, is based on a method propounded by Herring, 1999 [11]. It uses the information from the failed sterilization dose audit and the principles underlying Method 2, together with a conservative estimate of the resistance of the most radiation-resistant component of the microbial population of the product.

10.2.6.2 Stage 1: Analyse data from the failed sterilization dose audit

a) Identify the highest dose measured in performing the sterilization dose audit. Designate this value the “maximum audit dose”.

b) Record the number of positive tests of sterility found in the sterilization dose audit (see 10.2.5.2 and 10.2.5.3). Designate this value the “number of audit positives”.

10.2.6.3 Stage 2: Determine extrapolation factor

a) Determine the value of E using Equation (11) or Equation (12), depending on the number of audit positives.

If the number of audit positives is 3 to 9 inclusive, use Equation (11).

E = "maximum audit dose" + 2 kGy (11)

If the number of audit positives is 10 to 15 inclusive, use Equation (12).

E = "maximum audit dose" + 4 kGy (12)

b) Calculate the extrapolation factor using Equation (13) or Equation (14), depending on the value of $(E - 1)$.

If $(E - 1)$ is $\leqslant 9$, use Equation (13).

extrapolation factor = 2 + 0,2$(E - 1)$ (13)

If $(E - 1)$ is > 9 and $\leqslant 15$, use Equation (14).

extrapolation factor = 0,4$(E - 1)$ (14)

If the calculation using Equation (13) or Equation (14) gives a value greater than 4,2 kGy, set the extrapolation factor = 4,2 kGy.

10.2.6.4 Stage 3: Calculate adjusted dose (the dose to achieve an SAL value of 10^{-2})

Calculate the adjusted dose using Equation (15).

adjusted dose = "maximum audit dose" + [log ("number of audit positives")] (extrapolation factor) (15)

10.2.6.5 Stage 4: Calculate augmented sterilization dose

For Method 1 and Method 2A, calculate the augmented sterilization dose using Equation (16).

augmented sterilization dose = adjusted dose + [–log (SAL) – log (SIP) – 2] (extrapolation factor) (16)

For Method 2B, calculate the augmented sterilization dose using Equation (17).

augmented sterilization dose = adjusted dose + [–log (SAL) – 2] (extrapolation factor) (17)

10.3 Procedure for auditing a sterilization dose substantiated using VD_{max}

10.3.1 General

10.3.1.1 For the performance of a sterilization dose audit for a sterilization dose established using Method VD_{max}, use a SIP equivalent to that used in substantiating the original sterilization dose.

10.3.1.2 In applying the sterilization dose audit, the four stages below (10.3.2 to10.3.5) shall be followed.

NOTE For worked examples, see 11.6.

10.3.2 Stage 1: Obtain samples of product

Select at least 20 product items from a single batch of product in accordance with 5.1, 5.2 and 5.3.

10.3.3 Stage 2: Determine average bioburden

10.3.3.1 Apply the same correction factor (see ISO 11737-1) in the determination of bioburden as that used in substantiating the original sterilization dose.

10.3.3.2 Determine the bioburden of each of at least 10 product items and calculate the average bioburden.

NOTE 1 Bioburden is generally determined on individual product items, but when the bioburden is low (e.g. < 10), it is permissible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIP; SIPs should not be pooled, rather a larger SIP should be chosen.

NOTE 2 Bioburden data are not intended to be used in obtaining the verification dose for the sterilization dose audit. These data are used for process monitoring and control (e.g. trend analysis, investigation of sterilization dose audit failures or reduction in sterilization dose audit frequency).

10.3.4 Stage 3: Perform verification dose experiment

10.3.4.1 Irradiate 10 product items, or portions thereof, if appropriate, used in the original substantiation exercise, at VD_{max}^{25} or VD_{max}^{15}, whichever is applicable. Determine the dose. The highest dose to the product items may not exceed VD_{max} by more than + 0,1 kGy or + 10 %, whichever is greater. If the arithmetic mean of the highest and lowest doses delivered to product items is < 90 % of VD_{max}, the verification dose experiment may be repeated with a further 10 product items. If this mean dose is < 90 % of VD_{max} and, on performance of the tests of sterility, acceptable results are observed (see 10.3.5), the verification dose experiment need not be repeated. If the highest dose exceeds VD_{max} by more than 10 %, the verification dose experiment may be repeated following corrective action.

10.3.4.2 Subject each of the irradiated product items, or portions thereof, individually to a test of sterility using the media and incubation conditions used in the original dose substantiation exercise. Record the number of positive tests of sterility.

10.3.5 Stage 4: Interpretation of results

10.3.5.1 Accept the sterilization dose audit if no more than one positive test of sterility is obtained in the 10 tests carried out.

10.3.5.2 Perform a confirmatory sterilization dose audit (see 10.3.6) if there are two positive tests of sterility in the 10 tests carried out.

10.3.5.3 If three or more positive tests of sterility are obtained in the 10 tests carried out, and this outcome cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, the sterilization dose is inadequate.

a) If three to six positive tests of sterility are obtained in the 10 tests carried out, and this outcome cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, augment the dose immediately(see 10.3.7). Sterilization at the previously established sterilization dose shall be discontinued, and the sterilization dose shall be augmented until the sterilization dose is re-established using another method (see Clause 6).

b) If seven or more positive tests of sterility are obtained in the 10 tests carried out, and this outcome cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, discontinue sterilization at the previously established dose. The sterilization dose shall not be augmented, and sterilization shall not resume until the sterilization dose is re-established using another method (see Clause 6).

If the occurrence of three or more positive tests of sterility can be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, implement corrective action and repeat the sterilization dose audit. Interpret the results in accordance with 10.3.5.

When the cause of the failure can be attributed to a change in the manufacturing process, environment or components, it might be possible to determine the time frame in which the change occurred and therefore

identify the affected product batches. Any effect on the SAL should be assessed for the batches already released and a decision made on the risk associated with their continued use. The assessment of the effect on the SAL may not occur until the sterilization dose is re-established.

10.3.6 Confirmatory sterilization dose audit

10.3.6.1 General

10.3.6.1.1 For the performance of a sterilization dose audit for a sterilization dose established using Method VD_{max}, use a SIP equivalent to that used in substantiating the original sterilization dose.

10.3.6.1.2 In applying the confirmatory sterilization dose audit, the three stages below shall be followed.

10.3.6.2 Stage 1: Obtain samples of product

Select at least 10 product items from a single batch of product in accordance with 5.1, 5.2 and 5.3. The 10 product items for the performance of confirmatory sterilization dose audit may be selected from either the batch used in 10.3.2 for the verification dose experiment carried out in the original sterilization dose audit (see 10.3.2) or a second batch manufactured under conditions that are representative of normal production. The ability of the product to support microbial growth should be taken into account in selecting the batch.

10.3.6.3 Stage 2: Perform confirmatory verification dose experiment

10.3.6.3.1 Irradiate 10 product items at VD_{max}^{25} or VD_{max}^{15} obtained originally, whichever is applicable (see 9.2 and 9.3 or 9.4 and 9.5, respectively). Determine the dose. The highest dose to the product items may not exceed VD_{max} by more than + 0,1 kGy or + 10 %, whichever is greater. If the arithmetic mean of the highest and lowest doses delivered to product items is < 90 % of VD_{max}, the confirmatory sterilization dose audit may be repeated. If this mean dose is < 90 % of VD_{max} and, on performance of the tests of sterility, acceptable results are observed (see 10.3.6.4), the verification experiment need not be repeated. If the highest dose exceeds the verification dose by more than 10 %, the verification dose experiment may be repeated following corrective action.

10.3.6.3.2 Subject each irradiated product item individually to a test of sterility using the media and incubation conditions used in the original dose substantiation exercise and record the number of positive tests of sterility.

10.3.6.4 Stage 3: Interpretation of results

10.3.6.4.1 Accept verification and thereby substantiate the sterilization dose if there are no positive tests of sterility from the 10 tests carried out, giving a total of 2 positive tests of sterility obtained from the verification and confirmatory verification dose experiments.

10.3.6.4.2 If one or more positive tests of sterility are obtained in the 10 tests carried out in the confirmatory verification dose experiment, and this outcome cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, the sterilization dose is inadequate.

a) If one to four positive tests of sterility are obtained in the 10 tests carried out in the confirmatory verification dose experiment, and this outcome cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, augment the sterilization dose immediately (see 10.3.7). Sterilization at the previously established sterilization dose shall be discontinued, and the sterilization dose shall be augmented until the sterilization dose is re-established using another method (see Clause 6).

b) If five or more positive tests of sterility are obtained in the 10 tests carried out in the confirmatory verification dose experiment, and this outcome cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, discontinue sterilization at the previously established sterilization dose. The sterilization dose shall not be augmented, and sterilization shall not resume until the sterilization dose is re-established using another method (see Clause 6).

If the occurrence of one or more positive tests of sterility can be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose, implement corrective action and repeat the sterilization dose audit. Interpret the results in accordance with 10.3.5.

When the cause of the failure can be attributed to a change in the manufacturing process, environment or components, it might be possible to determine the time frame in which the change occurred and therefore identify the affected product batches. Any effect on the SAL should be assessed for the batches already released and a decision made on the risk associated with their continued use. The assessment of the effect on the SAL may not occur until the sterilization dose is re-established.

10.3.7 Augmentation of a sterilization dose substantiated using Method VD_{max}^{25} or VD_{max}^{15}

10.3.7.1 VD_{max}^{25}

10.3.7.1.1 From Table 11, obtain the dose augmentation value corresponding to the average bioburden as determined according to 10.3.3. If the average bioburden is not given in Table 11, use the closest tabulated average bioburden greater than the calculated average bioburden to obtain the dose augmentation value. Use this latter value in Equation (18) to calculate the augmented 25 kGy sterilization dose.

augmented sterilization dose (kGy) = 25 kGy + dose augmentation value (18)

Table 11 — Method VD_{max}^{25} augmentation values for average bioburden ⩽ 1 000

Average bioburden	Dose augmentation value (kGy)	Average bioburden	Dose augmentation value (kGy)	Average bioburden	Dose augmentation value (kGy)	Average bioburden	Dose augmentation value (kGy)
⩽ 0,1	5,0	6,5	3,7	40	3,3	240	3,3
0,15	4,8	7,0	3,7	45	3,3	260	3,3
0,20	4,7	7,5	3,6	50	3,2	280	3,3
0,25	4,6	8,0	3,6	55	3,2	300	3,3
0,30	4,6	8,5	3,6	60	3,2	325	3,3
0,35	4,5	9,0	3,6	65	3,2	350	3,3
0,40	4,5	9,5	3,6	70	3,2	375	3,3
0,45	4,4	10	3,6	75	3,2	400	3,3
0,50	4,4	11	3,6	80	3,2	425	3,3
0,60	4,3	12	3,5	85	3,2	450	3,3
0,70	4,3	13	3,5	90	3,2	475	3,3
0,80	4,2	14	3,5	95	3,2	500	3,3
0,90	4,2	15	3,5	100	3,2	525	3,3
1,0	4,2	16	3,5	110	3,2	550	3,3
1,5	4,0	17	3,5	120	3,2	575	3,3
2,0	4,0	18	3,4	130	3,2	600	3,3
2,5	3,9	19	3,4	140	3,2	650	3,4
3,0	3,9	20	3,4	150	3,2	700	3,4
3,5	3,8	22	3,4	160	3,2	750	3,4
4,0	3,8	24	3,4	170	3,2	800	3,4
4,5	3,8	26	3,4	180	3,2	850	3,4
5,0	3,7	28	3,4	190	3,3	900	3,4
5,5	3,7	30	3,3	200	3,3	950	3,4
6,0	3,7	35	3,3	220	3,3	1 000	3,4

10.3.7.1.2 Often the cause of the dose audit failure cannot be identified. In these circumstances, it is not possible to assess the extent to which the SAL of previously sterilized batches has been affected. Augmentation of the dose should begin with the next batch to be sterilized and no action taken with already released batches.

10.3.7.2 VD_{max}^{15}

From Table 12, obtain the dose augmentation value corresponding to the average bioburden calculated in 10.3.3. If the average bioburden is not given in Table 12, use the closest tabulated average bioburden greater than the calculated average bioburden. Use this latter value in Equation (19) to calculate the augmented sterilization dose.

augmented sterilization dose = 15 kGy + dose augmentation value (19)

Table 12 — Method VD_{max}^{15} augmentation values for average bioburden $\leqslant$ 1,5

Average bioburden	Dose augmentation value (kGy)	Average bioburden	Dose augmentation value (kGy)	Average bioburden	Dose augmentation value (kGy)	Average bioburden	Dose augmentation value (kGy)
$\leqslant$ 0,1	3,0	0,30	2,7	0,50	2,6	0,90	2,6
0,15	2,9	0,35	2,7	0,60	2,6	1,0	2,6
0,20	2,8	0,40	2,7	0,70	2,6	1,5	2,7
0,25	2,8	0,45	2,7	0,80	2,6		

11 Worked examples

11.1 Worked examples for Method 1

Three worked examples are given for Method 1. The first is for a product that could be tested for verification using the entire product item (SIP = 1,0), and with an end use requiring an SAL of 10^{-3} (Table 13). The second is for a product with an end use requiring an SAL of 10^{-6} that was too large to be tested easily, so a portion of the product (SIP < 1,0) was used (Table 14). The third is for a product that could be tested for verification using the entire product item (SIP = 1,0), with an end use requiring an SAL of 10^{-6}, and with a bioburden of < 1,0 (Table 15).

Table 13 — Determination of sterilization dose (Method 1, SIP = 1,0)

Term	Value	Comment
Stage 1		
SAL	10^{-3}	For the example, the product end use required an SAL of 10^{-3}.
SIP	1,0	The entire product was chosen for bioburden determination and the verification dose experiment.
Stage 2		
Overall average bioburden	382	Batch average bioburdens of 360, 402 and 384 were observed from the three batches tested, for an overall average bioburden of 382. None of the individual batch averages was twice the overall average of 382, therefore 382 was used to determine the verification dose.
Stage 3		
Verification dose	9,7 kGy	As an average bioburden of 382 is not listed in Table 5, the next larger tabulated bioburden of 400 is used to obtain the verification dose.
Stage 4		
Verification dose experiment	10,4 kGy	The highest dose to the product items was within the specified dose range (i.e. $\leqslant$ 10,7 kGy).
Stage 5		
Interpretation of results	1 positive	The verification dose was within the specified dose range (i.e. $<$ 10,7 kGy) and the test-of-sterility results were acceptable (i.e. $\leqslant$ 2 positives); therefore, verification is accepted.
Stage 6		
Sterilization dose for an SAL of 10^{-3}	12,9 kGy	The 10^{-3} sterilization dose for an average product bioburden of 382 is 12,9 kGy, from Table 5 [a].

[a] As a calculated average bioburden of 382 is not listed in the table, the next larger tabulated bioburden of 400 is used.

Table 14 — Determination of sterilization dose (Method 1, SIP < 1,0)

Term	Value	Comment
Stage 1		
SAL	10^{-6}	For the example, the product end use required an SAL of 10^{-6}.
SIP	0,05	As the product was too large to be subjected to a test of sterility, a 1/20 portion was selected for dose setting.
Stage 2		
Overall SIP average bioburden	59	The bioburden results from the SIPs for the three individual batches gave average results of 50, 62 and 65, giving an overall SIP average bioburden of 59. Counts $\geqslant$ 2 cfu per SIP on 85 % of the product items were obtained, demonstrating the adequacy of the SIP. None of the individual batch average SIP bioburdens was twice the overall SIP average bioburden, and therefore 59 was used to select the verification dose.
Stage 3		
Verification dose	7,3 kGy	As an average bioburden of 59 is not listed in the Table 5, the next larger tabulated bioburden of 60 is used to obtain the verification dose.
Stage 4		
Verification dose experiment	7,7 kGy	The highest dose to the product items was within the specified dose range (i.e. $\leqslant$ 8,0 kGy).
Stage 5		
Interpretation of results	2 positives	The verification dose was within the specified dose range (i.e. $<$ 8,0 kGy) and the test-of-sterility results were acceptable (i.e. $\leqslant$ 2 positives); therefore, verification is accepted.
Stage 6		
Average bioburden for entire product	1 180	The average bioburden for the entire product was calculated as 59/0,05 = 1 180.
Sterilization dose for an SAL of 10^{-6}	25,2 kGy	The 10^{-6} sterilization dose for an entire product average bioburden of 1 180 is 25,2 kGy, from Table 5 [a].

[a] As a calculated average bioburden of 1 180 is not listed in the table, the next larger tabulated bioburden of 1 200 is used.

Table 15 — Determination of sterilization dose (Method 1, SIP = 1,0, bioburden < 1,0)

Term	Value	Comment
Stage 1		
SAL	10^{-6}	For the example, the product end use required an SAL of 10^{-6}.
SIP	1,0	For bioburden values < 1,0, it is required to use the entire product item for bioburden determination and the verification dose experiment.
Stage 2		
Overall average bioburden	0,63	The bioburden results from the three individual batches gave average results of 0,6, 0,6, and 0,7, giving an overall average bioburden of 0,63. None of the individual batch average bioburdens was twice the overall average bioburden, and therefore 0,63 was used to select the verification dose.
Stage 3		
Verification dose	2,7 kGy	The average bioburden of 0,63 is not listed in Table 6; the next larger tabulated value of 0,70 is used to obtain the verification dose.
Stage 4		
Verification dose experiment	2,6 kGy	The highest dose to the product items was within the specified dose range (i.e. ⩽ 3,0 kGy).
Stage 5		
Interpretation of results	2 positives	The verification dose was within the specified dose range (i.e. < 3 kGy) and the test-of-sterility results were acceptable (i.e. ⩽ 2 positives); therefore, verification is accepted.
Stage 6		
Sterilization dose for an SAL of 10^{-6}	13,7 kGy	The 10^{-6} sterilization dose for an average product bioburden of 0,63 is 13,7 kGy, from Table 6 [a].

[a] As a calculated average bioburden of 0,63 is not listed in the table, the next larger tabulated average bioburden of 0,70 is used.

11.2 Worked examples for Method 2

11.2.1 General

Two worked examples are given for Method 2A, one for a product that could be tested using the entire product unit (SIP = 1,0), given in Tables 16, 17, 18, 19 and 20, and a second for a product that had to be tested using a portion of product (SIP < 1,0), given in Tables 21, 22, 23, 24 and 25. One worked example is given for Method 2B, which has as one of its requirements that the whole product be used, given in Tables 26, 27, 28, 29 and 30.

In the following examples, notation is lower case when it refers to results derived from product taken from a single batch and upper case when it refers to results derived from product taken from all three batches.

11.2.2 Worked example for Method 2A (SIP = 1,0)

11.2.2.1 Stage 1: Select SAL and obtain samples of product

11.2.2.1.1 The product end use required an SAL of 10^{-6}. The entire product was used for dose setting (SIP = 1,0), and 280 product items were chosen at random from each of three batches.

11.2.2.1.2 The allocation of product for the incremental dose experiment is shown in Table 16.

Table 16 — Number of product items for irradiation at various incremental doses

Batch No.	Nominal incremental dose (kGy)									Product held for Stage 3 experiment	Total product required
	2	4	6	8	10	12	14	16	18		
1	20	20	20	20	20	20	20	20	20	100	280
2	20	20	20	20	20	20	20	20	20	100	280
3	20	20	20	20	20	20	20	20	20	100	280

11.2.2.2 Stage 2: Perform incremental dose experiment

Table 17 provides an example of data from an incremental dose series, and Table 18 shows values derived from such a series.

Table 17 — Typical data derived from incremental dose experiment (number of positive tests of sterility from 20 tests performed on individual product items)

Batch No.		Nominal dose (kGy)								
		2	4	6	8	10	12	14	16	18
1	Delivered dose (kGy)	2,2	5,0	5,3	9,0	9,2	11,6	15,0	16,2	19,3
	Number of positives	20	5	2	0	0	0	0	0	0
2	Delivered dose (kGy)	2,6	3,2	6,6	8,0	9,7	13,0	13,8	15,8	17,9
	Number of positives	11	7	0	0	1	0	0	0	0
3	Delivered dose (kGy)	2,3	4,2	5,9	7,5	10,7	11,4	13,7	17,5	17,1
	Number of positives	18	7	2	2	0	0	0	0	0
NOTE	Doses were delivered independently and are within ± 1,0 kGy or ± 10 % of the nominal doses, whichever is greater.									

Table 18 — Stage 2 calculations

Term	Value	Comment
Batch 1 ffp Batch 2 ffp Batch 3 ffp	5,0 kGy 2,6 kGy 2,3 kGy	A batch ffp is the first incremental dose where at least one of the 20 tests of sterility is negative.
A	0,65 kGy	Find the number of positive tests of sterility at the median ffp and use Table 7 to determine A. For the example, the number of positive tests of sterility at median ffp (2,6 kGy) is 11, so A is 0,65 kGy.
FFP	1,95 kGy	FFP is the median of the three batch ffps minus A. For the example, FFP = 2,6 kGy – 0,65 kGy = 1,95 kGy.
Batch 1 d* Batch 2 d* Batch 3 d*	9,0 kGy 6,6 kGy 10,7 kGy	d* for a batch is the dose of a) or b), where a) is the lower dose of two consecutive incremental doses at which no positive tests of sterility occur, followed by no more than one further positive test of sterility; b) is the first incremental dose at which 1 positive test of sterility occurs, immediately preceded by one, and only one, dose at which no positive tests of sterility occur, and followed by all negative tests of sterility.
D*	9,0 kGy	D* is the median of the three batch d*s, except when any batch has a d* which exceeds the median d* by 5,0 kGy or more. If the exception is observed, D* is taken to be the maximum of the batch d*s.
CD* batch	Batch 1	The CD* batch is the batch which has d* equal to D*. If more than one d* is equal to D*, choose one at random as the CD* batch.

11.2.2.3 Stage 3: Perform verification dose experiment

Table 19 shows values derived from the Stage 3 experiment.

Table 19 — Stage 3 calculations

Term	Value	Comment
D*	9,0 kGy	From Stage 2 experiment.
DD*	8,0 kGy	DD* is the dose delivered in the Stage 3 experiment. DD* is acceptable if it is less than + 1,0 kGy or + 10 % of D*, whichever is greater.
CD*	2	CD* is the number of positive tests of sterility observed in the Stage 3 experiment.
FNP	8,0 kGy	If CD* is 2 positive tests of sterility or less, FNP is equal to DD*. If 2 < CD* < 10 positive tests of sterility, FNP = DD* + 2,0 kGy. If 9 < CD* < 16 positive tests of sterility, FNP = DD* + 4,0 kGy. If CD* is > 15 positive tests of sterility, D* should be redetermined.

11.2.2.4 Stages 4 and 5: Consideration of results and establishing the sterilization dose

Table 20 shows the calculations used to establish the sterilization dose.

Table 20 — Stage 4 calculations to establish sterilization dose

Term	Value	Comment
CD^*	2	From Stage 3 experiment.
DD^*	8,0 kGy	From Stage 3 experiment.
FNP	8,0 kGy	From Stage 3 experiment.
FFP	1,95 kGy	From Stage 2 experiment.
FNP – FFP	6,05 kGy	For the example: FNP – FFP = 8,0 kGy – 1,95 kGy = 6,05 kGy NOTE If (FNP – FFP) is < 0, set (FNP – FFP) = 0.
DS	3,21 kGy	When (FNP – FFP) is < 10, DS = 2 + 0,2(FNP – FFP) [Equation (3)] When (FNP – FFP) is $\geqslant 10$, DS = 0,4(FNP – FFP) [Equation (4)] For the example: DS = 2 kGy + 0,2 (6,05 kGy) = 3,21 kGy
D^{**}	9,0 kGy	$D^{**} = DD^* + [\log(CD^*)](DS)$ [Equation (5)] NOTE If $CD^* = 0$, set [log (CD^*)] = 0. For the example: D^{**} = 8,0 kGy + [log(2)] × (3,21 kGy) = 8,0 kGy + (0,3010)(3,21 kGy) = 8,97 kGy = 9,0 kGy
SAL	10^{-6}	From Stage 1 decision.
SIP	1,0	From Stage 1 decision.
Sterilization dose for an SAL of 10^{-6}	21,8 kGy	Sterilization dose = D^{**} + [– log(SAL) – log(SIP) –2](DS) [Equation (6)] For the example: Sterilization dose = 9,0 kGy + (6 – 0 – 2) × (3,21 kGy) = 9,0 kGy + (4) × (3,21 kGy) = 21,8 kGy

11.2.3 Worked example for Method 2A (SIP < 1,0)

11.2.3.1 Stage 1: Select SAL and obtain samples of product

11.2.3.1.1 The product end use required an SAL of 10^{-3}. The product was too large for testing in dose setting, so a portion of the product (SIP $< 1,0$) was used, and 300 product items were chosen at random from each of three batches.

11.2.3.1.2 The allocation of product for the incremental dose experiment is shown in Table 21.

Table 21 — Number of product items for irradiation at various incremental doses

Batch No.	Nominal incremental dose (kGy)										Product held for Stage 3 experiment	Total product required
	0	2	4	6	8	10	12	14	16	18		
1	20	20	20	20	20	20	20	20	20	20	100	300
2	20	20	20	20	20	20	20	20	20	20	100	300
3	20	20	20	20	20	20	20	20	20	20	100	300

11.2.3.2 Stage 2: Perform incremental dose experiment

Table 22 provides an example of data from an incremental dose series; Table 23 shows values derived from such a series.

Table 22 — Typical data derived from incremental dose experiment (number of positive tests of sterility from 20 tests performed on individual SIPs)

Batch No.		Nominal dose (kGy)									
		0	2	4	6	8	10	12	14	16	18
1	Delivered dose (kGy)	0,0	1,8	3,7	6,3	7,8	10,9	12,8	14,2	15,2	18,0
	Number of positives	20	17	1	0	0	0	0	0	0	0
2	Delivered dose (kGy)	0,0	1,5	3,9	5,7	8,5	9,9	11,3	14,5	17,3	18,4
	Number of positives	20	20	3	0	0	0	0	0	0	0
3	Delivered dose (kGy)	0,0	2,5	3,5	6,1	7,3	10,2	12,4	12,7	14,8	17,7
	Number of positives	20	9	0	1	0	0	0	0	0	0
NOTE 1	Doses were delivered independently and are within ± 1,0 kGy or ± 10 % of the nominal doses, whichever is greater.										
NOTE 2	When non-irradiated SIPs were subjected individually to a test of sterility, at least 17 positives were observed for each batch.										