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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

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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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 second edition cancels and replaces the first edition (ISO 11137-2:2006), which has been technically revised.

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*

Introduction

This part of ISO 11137 describes methods that can 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:

— dose setting to obtain a product-specific dose;

— 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[19][20][21]. Subsequently, standardized protocols were developed[10][11], which formed the basis of the dose setting methods detailed in the AAMI Recommended Practice for Sterilization by Gamma Radiation[6][8].

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 can 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 less than or equal to 25 kGy. The basis of the method devised specifically for substantiation of 25 kGy, Method VD_{max}, was put forward by Kowalski and Tallentire[16]. Subsequent evaluations involving computational techniques demonstrated that the underlying principles were soundly based[15] 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[18].

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}[7], 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 kilograys, is included as a superscript to the VD_{max} symbol. Thus, for substantiation of a sterilization dose of 25 kGy, the method is designated Method VD_{max}^{25}.

Method VD_{max}^{15} is based on the same principles as Method VD_{max}^{25}. The test procedure is similar to that of Method VD_{max}^{25}, but Method VD_{max}^{15} is limited to product with an average bioburden less than or equal to 1,5. The outcomes of the associated tests of sterility are used to substantiate that 15 kGy achieve a sterility assurance level of 10^{-6}.

This part of ISO 11137 also describes methods that can 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 for 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 sterilization dose audit used to demonstrate the continued effectiveness of the sterilization dose.

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

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 a population of microorganisms on products*

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

3 Terms, definitions and abbreviated terms

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

3.1 Terms and definitions

3.1.1
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, definition 2.1]

3.1.2
bioburden
population of viable microorganisms on or in product and/or sterile barrier system

[ISO/TS 11139:2006, definition 2.2]

3.1.3
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

3.1.4
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

3.1.5
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

3.1.6
negative test of sterility
test result for which there is no detectable microbial growth from product, or portions thereof, subjected to a test of sterility

3.1.7
packaging system
combination of the sterile barrier system and protective packaging

[ISO/TS 11139:2006, definition 2.28]

3.1.8
positive test of sterility
test result for which there is detectable microbial growth from product, or portions thereof, subjected to a test of sterility

3.1.9
sample item portion
SIP
defined portion of a health care product that is tested

3.1.10
standard distribution of resistances
SDR
reference set of resistances of microorganisms and corresponding probabilities of occurrence

3.1.11
sterile barrier system
minimum package that prevents ingress of microorganisms and allows aseptic presentation of product at the point of use

3.1.12
sterility assurance level
SAL
probability of a single viable microorganism occurring on an item after sterilization

NOTE The term SAL 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}.

[ISO/TS 11139:2006, definition 2.46]

3.1.13
sterilization dose audit
exercise undertaken to confirm the appropriateness of an established sterilization dose

3.1.14
test of sterility
technical operation performed as part of development, validation, or requalification to determine the presence or absence of viable microorganisms on product or portions thereof

[ISO/TS 11139:2006, definition 2.54]

3.1.15
verification dose
dose of radiation predicted to give a predetermined SAL greater than or equal to 10^{-2} used in establishing the sterilization dose

3.2 Abbreviated terms

3.2.1
A
dose to adjust the median ffp dose downwards to the FFP dose

3.2.2
CD^*
number of positive tests of sterility obtained from tests performed individually on 100 product items irradiated in a Method 2 verification dose experiment

3.2.3
d^*
dose derived from an incremental dose experiment performed on product items drawn from a given production batch

3.2.4
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 three d^* values derived for a given product.

3.2.5
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

3.2.6
DD^*
highest dose delivered in a Method 2 verification dose experiment

3.2.7
DS
estimate of the D_{10} value of microorganisms present on product after exposure to DD^*

3.2.8
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, definition 2.11]

NOTE For the purposes of this part of ISO 11137, D_{10} applies to the radiation dose only and not to time.

3.2.9
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

3.2.10
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 three ffp doses

3.2.11
First No Positive dose
FNP
estimate of the dose to provide an SAL of 10^{-2} for the test items, that is used in the calculation of DS

3.2.12
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

3.2.13
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

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 numbers and types of microorganisms 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 for establishing the sterilization dose and for carrying out sterilization dose audits, 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 processes;

e) manufacturing equipment;

f) manufacturing environment;

g) manufacturing location.

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

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 ISO 11137-1:2006, 4.1.2). 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 microorganisms 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) number of microorganisms comprising the bioburden;

b) types of microorganisms comprising the bioburden;

c) 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 family, either a) at random, or b) according to a documented procedure to include the different products that could be considered as master products.

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 documented procedure 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 might 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 that 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 ensure 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 a review shall be performed at least annually. The outcome of the review shall be recorded in accordance with ISO 11137-1:2006, 4.1.2.

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 ISO 11137-1:2006, 4.1.2).

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 performance of the 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 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 sterilization dose establishment shall be taken in accordance with Table 1.

Table 1 — Nature of product items for establishing 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 overall SAL associated with the 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 establishment, 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 can 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 can 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 greater than or equal to 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 (SIP) may be substituted. The SIP should be as large a portion of the item as practicable and should be of a size that can be handled during testing.

5.2.2 For a product with an average bioburden less than or equal to 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 either a) portions of product selected at random that proportionally represent each of the materials from which the product is made, or b) 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 an SIP

Basis for SIP	Product
Length	Tubing (consistent diameter) Rolls of bandage
Mass	Powders Gowns
Volume	Liquids
Surface area	Surgical drapes Tubing (variable diameter)

5.2.4 The preparation and packaging of an SIP shall be carried out under conditions that minimize alterations to bioburden. Environmentally controlled conditions should be used for the 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 either at least 17 of the 20 non-irradiated SIPs yield positive tests of sterility, or a bioburden of 1 or more is found on at least 85 % of 20 or more SIPs. If neither of these criteria is met, an SIP that is different from that examined originally and that meets one of the above criteria shall be used. If an entire product is tested (SIP equal to 1,0), the criteria specified above do not apply.

5.2.6 The same portion of product item (SIP) should be used in the performance of tests of sterility when carrying out the verification dose experiment as that used in the determination of bioburden when obtaining the verification dose.

NOTE If a different portion of product item (SIP) is used in the performance of tests of sterility from that used in the determination of bioburden, caution should be exercised when obtaining the verification and sterilization doses.

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. 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 taking of product from production and the performance of the verification dose experiment 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 References [9], [12] and [14]).

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). Whenever practicable, for the performance of a verification dose experiment, product should be irradiated in its original form and in its packaging system. However, to reduce the possibility of false positives in carrying out tests of sterility, product may be disassembled and repackaged prior to irradiation. Materials for repackaging product 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 product in establishing 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.

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

5.5.3 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 delivered to product.

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) Methods 2A and 2B (see Clause 8), or

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

6.2 If a sterilization dose of 25 kGy is established in accordance with 8.2.2 b) of ISO 11137-1:2006 (dose substantiation), it shall be substantiated by one of the following methods:

a) Method VD_{max}^{25} (see 9.2 and 9.3), for product with an average bioburden less than or equal to 1 000,

b) Method 1 (see Clause 7), subject to the derived sterilization dose taking a value less than or equal to 25 kGy and achieving maximally an SAL of 10^{-6},

c) Method 2 (see Clause 8), subject to the derived sterilization dose taking a value less than or equal to 25 kGy and achieving an SAL of 10^{-6},

d) a method providing equivalent assurance to that of a), b) or c) above in achieving maximally an SAL of 10^{-6}.

6.3 If a sterilization dose of 15 kGy is established in accordance with 8.2.2 b) of ISO 11137-1:2006 (dose substantiation), it shall be substantiated by one of the following methods:

a) Method VD_{max}^{15} (see 9.4 and 9.5), for product with an average bioburden less than or equal to 1,5,

b) Method 1 (see Clause 7), subject to the derived sterilization dose taking a value less than or equal to 15 kGy and achieving maximally an SAL of 10^{-6},

c) Method 2 (see Clause 8), subject to the derived sterilization dose taking a value less than or equal to 15 kGy and achieving an SAL of 10^{-6}, or

d) a method providing equivalent assurance to that of a), b) or c) above in achieving maximally an SAL of 10^{-6}.

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 dose values for given average bioburdens are tabulated in Tables 5 and 6.

NOTE Table B.1 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 decimal place. Regular increases in average bioburden values have been similarly included in Table 6.

Table 3 — Standard distribution of resistances (SDR) used in Method 1 (see Reference [10])

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, the average bioburden is determined. 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 as 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, the average bioburden in Table 5 or Table 6 is again used 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 92 % probability that zero, one or two positives will occur (see Table 4).

Table 4 — Probabilities of occurrence of numbers of positives around an average of one, distributed according to the Poisson distribution

Number of 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

7.2 Procedure for Method 1 for product with an average bioburden greater than or equal to 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 10 product items from each of three independent production batches, in accordance with 5.1, 5.2 and 5.3.

NOTE Additional product might be needed to validate the adequacy of an SIP of less than one (see 5.2.5).

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 ISO 11737-1 utilizes a correction factor derived from the validation of the bioburden technique to compensate for incomplete removal of microorganisms from product. The performance of dose establishment using Method 1 may use a bioburden determination without the application of the correction factor. When a correction factor is not used, the bioburden might 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 of product items (batch average bioburden);

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

NOTE 1 Bioburden is generally determined on individual product items unless the bioburden is low (e.g. less than 10), in which case it is permissible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIPs, which should not be pooled; instead a larger SIP should be chosen (see 5.2.5).

NOTE 2 When no colonies are observed in the determination of bioburden, this is sometimes expressed as being below the limit of detection. Use of the limit of detection as a bioburden value in calculating average bioburden could lead to an overestimation. Overestimation could affect the validity of the verification dose experiment.

7.2.3.3 Compare the three batch average bioburdens with the overall average bioburden and determine whether any one of the batch average bioburdens 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 as the average bioburden:

a) if a batch average bioburden is two or more times greater than the overall average bioburden, use the highest batch average bioburden, or

b) if each of the batch average bioburdens is less than two times the overall average bioburden, use the overall average bioburden.

If the average bioburden is not given in Table 5, use the closest tabulated value greater than the average bioburden.

Designate this dose as the verification dose.

If SIPs are to be used in the performance of tests of sterility, use the highest SIP batch average bioburden or the overall SIP average bioburden, as appropriate, to obtain the verification dose.

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 on which a bioburden determination was carried out in Stage 2, or from a fourth batch manufactured under conditions that are representative of normal production (see 5.3).

7.2.5.2 Irradiate these product items at the verification dose.

The highest dose to product items shall not exceed the verification dose by more than 10 %.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of the verification dose.

Determine the dose delivered (see 5.5).

If the highest dose to product items exceeds the verification dose by more than 10 %, the verification dose experiment shall be repeated.

If the arithmetic mean of the highest and lowest doses 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 tests of sterility, acceptable results are observed (see 7.2.6.1), the verification dose experiment need not be repeated.

7.2.5.3 Subject each irradiated product item individually to a test of sterility (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 If no more than two positive tests of sterility are obtained from the 100 tests carried out, accept verification.

7.2.6.2 If three or more positive tests of sterility are obtained, do not accept verification.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, or a specific bioburden-related cause, implement corrective action and repeat the verification dose experiment using a further 100 product items and the same verification dose as that used in the verification dose experiment that was not accepted. Interpret the results of the repeat verification dose experiment in accordance with 7.2.6.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, or a specific bioburden-related cause, 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 by entering the table at the tabulated value equal to the average bioburden used in Stage 3. Alternatively, if the average bioburden is not given in Table 5, enter the table at the closest tabulated value greater than the average bioburden and read the dose necessary to achieve the desired SAL.

7.2.7.2 If an SIP of less than 1,0 is used and verification is accepted, calculate the average bioburden for the entire product by dividing the highest SIP batch average bioburden or the SIP overall average bioburden by the SIP value, as appropriate. Obtain the sterilization dose for the product from Table 5 by entering the table at the tabulated value equal to the average bioburden for the entire product. Alternatively, if the average bioburden is not given in Table 5, enter the table at the closest tabulated value greater than the average bioburden for the entire product and read the dose necessary to achieve the desired SAL.

Table 5 — Radiation dose (kGy) required to achieve a given SAL for an average bioburden greater than or equal to 1,0, which has the standard distribution of resistances (SDR)

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
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

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
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
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

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
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
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

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
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
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

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-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
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

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
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
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

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
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
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

Average bioburden	Sterility assurance level SAL				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
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 greater than or equal to 1,0 for a single production batch

7.3.1 Rationale

This method is an adaptation of Method 1 for multiple production batches given in 7.2. It is intended to be used for the establishment of a sterilization dose for a single production batch only. The method 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 SDR.

7.3.2 General

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

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

NOTE Additional product might be needed to validate the adequacy of an SIP of less than one (see 5.2.5).

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 performance of dose establishment using Method 1 may use a bioburden determination without the application of the correction factor. When a correction factor is not used, the bioburden might 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.

NOTE 1 Bioburden is generally determined on individual product items unless the bioburden is low (e.g. less than 10), in which case it is permissible to pool the 10 product items for the determination of average bioburden. This guidance does not apply to SIPs, which should not be pooled; instead a larger SIP should be chosen (see 5.2.5).

NOTE 2 When no colonies are observed in the determination of bioburden, this is sometimes expressed as being below the limit of detection. Use of the limit of detection as a bioburden value in calculating average bioburden could lead to an overestimation. Overestimation could affect the validity of the verification dose experiment.

7.3.5 Stage 3: Obtain verification dose

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

If the average bioburden is not given in Table 5, use the closest tabulated value greater than the average bioburden.

Designate this dose as the verification dose.

If SIPs are to be used in the performance of the tests of sterility, use the SIP average bioburden to obtain the verification dose.

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 these product items at the verification dose.

The highest dose to product items shall not exceed the verification dose by more than 10 %.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of the verification dose.

Determine the dose delivered (see 5.5).

If the highest dose to product items exceeds the verification dose by more than 10 %, the verification dose experiment shall be repeated.

If the arithmetic mean of the highest and lowest doses 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 tests of sterility, acceptable results are observed (see 7.3.7.1), the verification dose experiment need not be repeated.

7.3.6.3 Subject each irradiated product item individually to a test of sterility (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 If no more than two positive tests of sterility are obtained from the 100 tests carried out, accept verification.

7.3.7.2 If three or more positive tests of sterility are obtained, do not accept verification.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, or a specific bioburden-related cause, implement corrective action and repeat the verification dose experiment. Use a further 100 product items and the same verification dose as that used in the verification dose experiment that was not accepted. Interpret the results of the repeat verification dose experiment in accordance with 7.3.7.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of the verification dose, or a specific bioburden-related cause, this method of dose setting is not valid. 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 by entering the table at the tabulated value equal to the average bioburden used in Stage 3. Alternatively, if the average bioburden is not given in Table 5, enter the table at the closest tabulated value greater than the average bioburden and read the dose necessary to achieve the desired SAL.

7.3.8.2 If an 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 by entering the table at the tabulated value equal to the average bioburden for the entire product. Alternatively, if this average bioburden is not given in Table 5, enter the table at the closest tabulated value greater than the average bioburden for the entire product and read 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 Values derived from Table 6 are used in Stages 3, 4 and 6 of Method 1 dose setting.

NOTE 2 For a worked example, see 11.1.

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

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 within 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 regarding the resistance to radiation of microorganisms as they occur on 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 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 Reference [11].

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

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

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 ISO 11137-1:2006, 7.3 and 12.1).

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 decimal place. The sterilization dose may be rounded (using standard rounding procedures) to one decimal place.

NOTE 2 In the following procedures and 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.

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 280 product items from each of three independent production batches, in accordance with 5.1, 5.2 and 5.3.

NOTE Additional product might be needed to validate the adequacy of an SIP less than one (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 three production batches, irradiate 20 product items at each of a series of at least nine doses, starting at 2 kGy and increasing in nominal increments of 2 kGy.

For a given incremental dose, the highest dose to product items shall not exceed the nominal incremental dose by more than 10 % or 1,0 kGy, whichever is greater.

For a given incremental dose, the arithmetic mean of the highest and lowest doses to product items shall not be less than 90 % of the nominal incremental dose or the nominal incremental dose minus 1,0 kGy, whichever is the lesser.

Determine the dose delivered at each of the incremental doses (see 5.5).

If, for a given incremental dose, the highest dose to product items exceeds the nominal incremental dose by more than 10 % or 1,0 kGy, whichever is greater, irradiation of 20 further product items at the particular incremental dose shall be carried out.

If, for a given incremental dose, the arithmetic mean of the highest and lowest doses to product items is less than 90 % of the nominal incremental dose or the nominal incremental dose minus 1,0 kGy, whichever is the lesser, irradiation of 20 further product items at the particular incremental dose shall be carried out.

8.2.3.1.2 Subject each irradiated product item individually to a test of sterility (see 5.4.1) and record the number of positive tests of sterility found at each incremental dose.

8.2.3.1.3 Obtain the following from the results of this experiment:

a) A and 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 two or three 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		

NOTE The values of A were calculated using Equation (1).

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

where n is the number of tests of sterility that are negative (see Reference [10]).

8.2.3.2.3 Calculate FFP using 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.

8.2.3.3.3 Designate D^* as follows:

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

b) if the highest batch d^* exceeds the median batch d^* by greater than or equal to 5 kGy, the highest batch d^* becomes D^*.

8.2.3.4 CD^* batch

Determine the batch for which d^* equals 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 the CD^* batch are used in Stage 3 of Method 2A. Storage conditions of the retained product from the three batches should take into account the ability of product to support microbial growth. If necessary, a fourth batch shall be taken as the CD^* batch.

8.2.4 Stage 3: Perform verification dose experiment

8.2.4.1 Irradiate 100 product items from the CD^* batch at a dose of D^*.

The highest dose to product items should not exceed D^* by more than 10 % or 1,0 kGy, whichever is greater.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of D^* or less than D^* minus 1,0 kGy, whichever dose is the lesser.

Determine the dose delivered (see 5.5). Designate the highest dose delivered as DD^*.

NOTE Actions in regard to the upper and lower dose limits are dependent on the value taken by CD^* (see 8.2.4.2).

8.2.4.2 Subject each irradiated product item individually to a test of sterility (see 5.4.1) and record the number of positive tests of sterility. Designate this value as CD^*.

NOTE 1 CD^* is used to determine FNP (see 8.2.5) and DS (see 8.2.6).

If CD^* is equal to zero and DD^* exceeds D^* by more than 10 % or 1,0 kGy, whichever is greater, the verification dose experiment shall be repeated.

If CD^* is 1 to 15 inclusive and DD^* exceeds D^* by more than 10 % or 1,0 kGy, whichever is greater, the verification dose experiment need not be repeated.

NOTE 2 A repeat verification dose experiment may, however, be carried out to obtain a value of DD^* lower than that found originally which, in turn, would give low FNP and DS values.

NOTE 3 CD^* values of 1 to 15 inclusive, together with DD^*, provide an estimate of the dose that achieves a 10^{-2} SAL. An acceptance of DD^* that exceeds D^* by more than 10 % or 1,0 kGy, whichever is greater, is permitted as the resulting values of FNP and DS will give conservative values of D^{**} and the sterilization dose.

If CD^* is greater than 15 and the arithmetic mean of DD^*, and the lowest dose to product items is less than 90 % of D^* or less than D^* minus 1,0 kGy, whichever dose is the lesser, the verification dose experiment may be repeated. If this mean is not less than 90 % of D^* or not less than D^* minus 1,0 kGy, whichever dose is the lesser, the cause for the occurrence of more than 15 positive tests of sterility should be investigated, corrective action implemented and D^* redetermined.

8.2.5 Stage 4: Interpretation of results

Obtain FNP from the results of this experiment as follows:

a) if CD^* is less than or equal to 2, let FNP equal DD^*;

b) if CD^* is more than 2 and less than 10, let FNP equal DD^* + 2,0 kGy;

c) if CD^* is more than 9 and less than 16, let FNP equal DD^* + 4,0 kGy; or

d) if CD^* is greater than 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) is less than 10 kGy, use

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

NOTE In using Equation (3), if (FNP – FFP) is less than zero, set (FNP – FFP) to zero.

When (FNP – FFP) is greater than or equal to 10 kGy, use

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

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^{*} equals zero, set [log (CD^{*})] to zero.

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

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

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 decimal place. The sterilization dose may be rounded (using standard rounding procedures) to one decimal place.

NOTE The term log (SIP) in Equation (6) provides the appropriate correction factor 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 equal to 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 a worked example, 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 260 product items from each of three independent production batches, in accordance with 5.1 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 the three production batches, irradiate 20 product items at each of a series of at least eight doses, starting at 1 kGy and increasing in nominal increments of 1 kGy.

For an incremental dose of 1 kGy, the highest dose to product items shall not exceed 1,2 kGy and, for other incremental doses, this dose shall not exceed the nominal incremental dose by more than 10 % or 0,5 kGy, whichever is greater.

For an incremental dose of 1 kGy, the arithmetic mean of the highest and lowest doses to product items shall not be less than 0,8 kGy and, for other incremental doses, the mean dose shall not be less than 90 % of the nominal incremental dose or the nominal incremental dose minus 0,5 kGy, whichever is the lesser.

Determine the dose delivered at each of the incremental doses (see 5.5).

If, for an incremental dose of 1 kGy, the highest dose to product items exceeds 1,2 kGy and, for other incremental doses, this dose exceeds the nominal incremental dose by more than 10 % or 0,5 kGy, whichever is greater, irradiation of 20 further product items at the particular incremental dose shall be carried out.

If, for an incremental dose of 1 kGy, the arithmetic mean of the highest and lowest doses to product items is less than 0,8 kGy and, for other incremental doses, this mean dose is less than 90 % of the nominal incremental dose or the nominal incremental dose minus 0,5 kGy, whichever is the lesser, irradiation of 20 further product items at the particular incremental dose shall be carried out.

8.3.3.1.2 Subject each irradiated product item individually to a test of sterility (see 5.4.1) and record the number of positive tests of sterility found at each incremental dose.

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 two or three 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		
7	0,48		

NOTE The values of A were calculated using Equation (7).

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

where n is the number of tests of sterility that are negative (see Reference [10]).

8.3.3.2.3 Calculate FFP using 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.

8.3.3.3.3 Designate D^* as follows:

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

b) if the highest batch d^* exceeds the median batch d^* by greater than or equal to 5 kGy, the highest batch d^* becomes D^*.

8.3.3.4 CD^* **batch**

Determine the batch for which d^* equals 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 the CD^* batch are used in Stage 3 of Method 2B. Storage conditions of the retained product from the three batches should take into account the ability of product to support microbial growth. If necessary, a fourth batch shall 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*.

The highest dose to product items should not exceed D* by more than 10 % or 1,0 kGy, whichever is greater.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of D* or less than D* minus 1,0 kGy, whichever dose is the lesser.

Determine the dose delivered (see 5.5). Designate the highest dose delivered as DD*.

NOTE Actions in regard to the upper and lower dose limits are dependent on the value taken by CD* (see 8.3.4.2).

8.3.4.2 Subject each irradiated product item individually to a test of sterility (see 5.4.1) and record the number of positive tests of sterility. Designate this value as CD*.

NOTE 1 CD* is used to determine FNP (see 8.3.5) and DS (see 8.3.6).

If CD* is equal to zero and DD* exceeds D* by more than 10 % or 1,0 kGy, whichever is greater, the verification dose experiment shall be repeated.

If CD* is 1 to 15 inclusive and DD* exceeds D* by more than 10 % or 1,0 kGy, whichever is greater, the verification dose experiment need not be repeated.

NOTE 2 A repeat verification dose experiment may, however, be carried out to obtain a value of DD* lower than that found originally which, in turn, would give low FNP and DS values.

NOTE 3 CD* values of 1 to 15 inclusive, together with DD*, provide an estimate of the dose that achieves a 10^{-2} SAL. An acceptance of DD* that exceeds D* by more than 10 % or 1,0 kGy, whichever is greater, is permitted as the resulting values of FNP and DS will give conservative values of D** and the sterilization dose.

If CD* is greater than 1 and the arithmetic mean of DD*, and the lowest dose to product items is less than 90 % of D*, or less than D* minus 1,0 kGy, whichever dose is the lesser, the verification dose experiment may be repeated. If this mean is not less than 90 % of D* or not less than D* minus 1,0 kGy, whichever dose is the lesser, the cause for the occurrence of more than 15 positive tests of sterility should be investigated, corrective action implemented and D* re-determined.

8.3.5 Stage 4: Interpretation of results

Obtain FNP from the results of this experiment as follows:

a) if CD* is less than or equal to 2, let FNP equal DD*;

b) if CD* is more than 2 and less than 10, let FNP equal DD* + 2,0 kGy;

c) if CD* is more than 9 and less than 16, let FNP equal DD* + 4,0 kGy; or

d) if CD* is greater than 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).

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

NOTE In using Equation (8), if (FNP – FFP) is less than zero, set (FNP – FFP) to zero.

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

8.3.6.3 Calculate the sterilization dose using Equation (9).

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

where:

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

SAL is the preselected sterility assurance level;

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 decimal place. The sterilization dose may be rounded (using standard rounding procedures) to one decimal place.

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 by 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 sterilization dose, 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 References [15], [16] and [17]).

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 preselected 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 a bioburden less than or equal to 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, e.g. 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 neither the result of an error in the tables nor 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 Reference [17]).

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 batch average bioburden of product is less than or equal to 1 000.

NOTE All three batch average bioburden values (see 9.2.3.2) shall be less than or equal to 1 000.

9.2.1.2 In applying Method VD_{max}^{25} for product with an average bioburden less than or equal to 0,9, the entire product item shall be used in accordance with Table 1, whereas for product with an average bioburden greater than 0,9, an SIP may be used (see 5.2.5).

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

NOTE For a worked example, see 11.3.

9.2.2 Stage 1: Obtain samples of product

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

NOTE Additional product might be needed to validate the adequacy of an SIP of less than one (see 5.2.5).

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 of product items (batch average bioburden);

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

NOTE 1 Bioburden is generally determined on individual product items, unless the bioburden is low (e.g. less than 10), in which case it is permissible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIPs, which should not be pooled; instead, a larger SIP should be chosen (see 5.2.5).

NOTE 2 When no colonies are observed in the determination of bioburden, this is sometimes expressed as being below the limit of detection. Use of the limit of detection as a bioburden value in calculating average bioburden could lead to an overestimation. Overestimation could affect the validity of the verification dose experiment.

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

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

Obtain the value of SIP equal to 1,0 VD_{max}^{25} from Table 9 using one of the following as the average bioburden:

a) if a batch average bioburden is two or more times greater than the overall average bioburden, use the highest batch average bioburden, or

b) if each of the batch average bioburdens is less than two times the overall average bioburden, use the overall average bioburden.

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

For an SIP of less than 1,0, calculate the average bioburden for the entire product item (SIP equal to 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 value greater than the average bioburden to locate the value of the SIP equal to 1,0 VD_{max}^{25} and the corresponding SIP dose reduction factor.

NOTE Use of an SIP of less than 1,0 is not permitted for product with an average bioburden less than or equal to 0,9 (see 9.2.1.2).

Use Equation (10) to calculate the SIP VD_{max}^{25} (see Reference [17]).

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

Table 9 — Values of VD_{max}^{25} and SIP dose reduction factors for levels of average bioburden less than or equal to 1 000 CFU

Average bioburden	SIP equal to 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 SIP equal to 1,0 VD_{max}^{25} is equal to 0,0 kGy, product items are not irradiated.

[a] Not applicable; in the range of average bioburden less than or equal to 0,9, the entire product (SIP equal to 1,0) is used and hence the SIP dose reduction factor is not given.

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 on which a bioburden determination was carried out in Stage 2, or from a fourth batch manufactured under conditions that are representative of normal production (see 5.3).

9.2.5.2 Irradiate these product items at VD_{max}^{25} obtained from Table 9 or derived using Equation (10), whichever is appropriate.

The highest dose to product items shall not exceed VD_{max}^{25} by more than 10 %.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of VD_{max}^{25}.

Determine the dose delivered (see 5.5).

If the highest dose to 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 to product items is less than 90 % of VD_{max}^{25}, the verification dose experiment may be repeated. If this mean dose is less than 90 % of VD_{max}^{25} and, on performance of tests of sterility, acceptable results are observed (see 9.2.6.1), the verification dose experiment need not be repeated.

9.2.5.3 Subject each irradiated product item individually to a test of sterility (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 If no more than one positive test of sterility is obtained from the 10 tests carried out, accept verification and thereby substantiate 25 kGy as the sterilization dose.

9.2.6.2 If two positive tests of sterility are obtained, perform a confirmatory verification dose experiment (see 9.2.7).

9.2.6.3 If three or more positive tests of sterility are obtained, do not accept verification as the sterilization dose might be inadequate.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{25}, or a specific bioburden-related cause, implement corrective action and repeat the verification dose experiment using a further 10 product items and the same VD_{max}^{25} as that used in the verification dose experiment that was not accepted. Interpret the results of the repeat verification dose experiment in accordance with 9.2.6.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{25}, or a specific bioburden-related cause, the selected sterilization dose of 25 kGy is not substantiated and an alternative method for establishing a 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 shall be followed.

9.2.7.2 Stage 1: Obtain samples of product

Select 10 product items from a single batch of product. The 10 product items for the performance of the 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 the fourth batch used in Stage 4 (see 9.2.5) or from a batch manufactured under conditions that are representative of normal production (see 5.3).

9.2.7.3 Stage 2: Perform confirmatory verification dose experiment

9.2.7.3.1 Irradiate these product items at VD_{max}^{25} as determined in 9.2.4.

The highest dose to product items shall not exceed VD_{max}^{25} by more than 10 %.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of VD_{max}^{25}.

Determine the dose delivered (see 5.5).

If the highest dose to product items exceeds VD_{max}^{25} by more than 10 %, the confirmatory verification dose experiment shall be repeated.

If the arithmetic mean of the highest and lowest doses to product items is less than 90 % of VD_{max}^{25}, the confirmatory verification dose experiment may be repeated. If this mean dose is less than 90 % of VD_{max}^{25} and, on performance of tests of sterility, acceptable results are observed (see 9.2.7.4.1), the confirmatory verification dose experiment need not be repeated.

9.2.7.3.2 Subject each irradiated product item individually to a test of sterility (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 If there are no positive tests of sterility from the 10 tests carried out, giving a total of two positive tests of sterility obtained from the original and confirmatory verification dose experiments performed in carrying out substantiation of 25 kGy, accept confirmatory verification and thereby substantiate 25 kGy as the sterilization dose.

9.2.7.4.2 If any positive tests of sterility are obtained, do not accept confirmatory verification as the sterilization dose might be inadequate.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{25}, or a specific bioburden-related cause, implement corrective action and repeat the confirmatory verification dose experiment using a further 10 product items and the same VD_{max}^{25} as that used originally. Interpret the results of the repeat confirmatory verification dose experiment in accordance with 9.2.7.4.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{25}, or a specific bioburden-related cause, the selected sterilization dose of 25 kGy is not substantiated and an alternative method for establishing a 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 less than or equal to 1 000.

9.3.2.2 In applying Method VD_{max}^{25} for product with an average bioburden less than or equal to 0,9, the entire product item shall be used in accordance with Table 1, whereas for product with an average bioburden greater than 0,9, an SIP may be used (see 5.2.5).

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

NOTE Additional product might be needed to validate the adequacy of an SIP less than one (see 5.2.5).

9.3.4 Stage 2: Determine average bioburden

9.3.4.1 Apply the 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 1 Bioburden is generally determined on individual product items unless the bioburden is low (e.g. less than 10), in which case it is permissible to pool the 10 product items for the determination of average bioburden. This guidance does not apply to SIPs, which should not be pooled; instead, a larger SIP should be chosen (see 5.2.5).

NOTE 2 When no colonies are observed in the determination of bioburden, this is sometimes expressed as being below the limit of detection. Use of the limit of detection as a bioburden value in calculating average bioburden could lead to an overestimation. Overestimation could affect the validity of the verification dose experiment.

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

Obtain the value of SIP equal to 1,0 VD_{max}^{25} from Table 9 using the average bioburden.

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

For an SIP less than 1,0, calculate the average bioburden for the entire product item (SIP equal to 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 value greater than the average bioburden to locate the value of SIP equal to 1,0 VD_{max}^{25} and the corresponding SIP dose reduction factor.

NOTE Use of an SIP of less than 1,0 is not permitted for product with an average bioburden less than or equal to 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 these product items at VD_{max}^{25} obtained from Table 9 or derived using Equation (10), whichever is appropriate.

The highest dose to product items shall not exceed VD_{max}^{25} by more than 10 %.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of VD_{max}^{25}.

Determine the dose delivered (see 5.5).

If the highest dose to 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 to product items is less than 90 % of VD_{max}^{25}, the verification dose experiment may be repeated. If this mean dose is less than 90 % of VD_{max}^{25} and, on performance of tests of sterility, acceptable results are observed (see 9.3.7.1), the verification dose experiment need not be repeated.

9.3.6.3 Subject each irradiated product item individually to a test of sterility (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 If no more than one positive test of sterility is obtained from the 10 tests carried out, accept verification and thereby substantiate 25 kGy as the sterilization dose.

9.3.7.2 If two positive tests of sterility are obtained, perform a confirmatory verification dose experiment (see 9.3.8).

9.3.7.3 If three or more positive tests of sterility are obtained, do not accept verification as the sterilization dose might be inadequate.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{25}, or a specific bioburden-related cause, implement corrective action and repeat the verification dose experiment using a further 10 product items and the same VD_{max}^{25} as that used in the verification dose experiment that was not accepted. Interpret the results of the repeat verification dose experiment in accordance with 9.3.7.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{25}, or a specific bioburden-related cause, the selected sterilization dose of 25 kGy is not substantiated and an alternative method for establishing a sterilization dose shall be used (see Clause 6).

9.3.8 Confirmatory verification dose experiment

9.3.8.1 General

If a confirmatory verification dose experiment is to be carried out (see 9.3.7.2), the three stages below shall be followed.

9.3.8.2 Stage 1: Obtain samples of product

Select 10 product items from the single batch of product. The ability of the product to support microbial growth should be taken into account in storing the single batch.

9.3.8.3 Stage 2: Perform confirmatory verification dose experiment

9.3.8.3.1 Irradiate these product items at VD_{max}^{25} as determined in 9.3.5.

The highest dose to product items shall not exceed VD_{max}^{25} by more than 10 %.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of VD_{max}^{25}.

Determine the dose delivered (see 5.5).

If the highest dose to product items exceeds VD_{max}^{25} by more than 10 %, the confirmatory verification dose experiment shall be repeated.

If the arithmetic mean of the highest and lowest doses to product items is less than 90 % of VD_{max}^{25}, the confirmatory verification dose experiment may be repeated. If this mean dose is less than 90 % of VD_{max}^{25} and, on performance of tests of sterility, acceptable results are observed (see 9.3.8.4.1), the confirmatory verification dose experiment need not be repeated.

9.3.8.3.2 Subject each irradiated product item individually to a test of sterility (see 5.4.1) and record the number of positive tests of sterility.

9.3.8.4 Stage 3: Interpretation of results

9.3.8.4.1 If there are no positive tests of sterility from the 10 tests carried out, giving a total of two positive tests of sterility obtained from the original and confirmatory verification dose experiments performed in carrying out substantiation of 25 kGy, accept confirmatory verification and thereby substantiate 25 kGy as the sterilization dose.

9.3.8.4.2 If any positive tests of sterility are obtained, do not accept confirmatory verification as the sterilization dose might be inadequate.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{25}, or a specific bioburden-related cause, implement corrective action and repeat the confirmatory verification dose experiment using a further 10 product items and the same VD_{max}^{25} as that used originally. Interpret the results of the repeat confirmatory verification dose experiment in accordance with 9.3.8.4.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{25}, or a specific bioburden-related cause, the selected sterilization dose of 25 kGy is not substantiated and an alternative method for establishing a 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 batch average bioburden of product is less than or equal to 1,5.

NOTE All three batch average bioburden values (see 9.4.3.2) shall be less than or equal to 1,5.

9.4.1.2 In applying Method VD_{max}^{15}, an entire product item (SIP equal to 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 a worked example, see 11.3.

9.4.2 Stage 1: Obtain samples of product

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

9.4.3 Stage 2: Determine average bioburden

9.4.3.1 Apply the 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 bioburden);

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

NOTE 1 Bioburden is generally determined on individual product items, but when the bioburden is low (e.g. for VD_{max}^{15} less than 1,5), it is permissible to pool the 10 product items for the determination of batch average bioburden.

NOTE 2 When no colonies are observed in the determination of bioburden, this is sometimes expressed as being below the limit of detection. Use of the limit of detection as a bioburden value in calculating average bioburden could lead to an overestimation. Overestimation could affect the validity of the verification dose experiment.

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

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

Obtain the value of SIP equal to 1,0 VD_{max}^{15} from Table 10 using one of the following as the average bioburden:

a) If one or more of the batch average bioburdens is two or more times greater than the overall average bioburden, use the highest batch average bioburden, or

b) If each of the batch average bioburdens is less than two times the overall average bioburden, use the overall average bioburden.

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

Table 10 — Values of VD_{max}^{15} for levels of average bioburden less than or equal to 1,5

Average bioburden	SIP equal to 1,0 VD_{max}^{15} (kGy)	Average bioburden	SIP equal to 1,0 VD_{max}^{15} (kGy)
≤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 SIP equal to 1,0 VD_{max}^{15} is equal to 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 on which a bioburden determination was carried out in Stage 2, or from a fourth batch manufactured under conditions which are representative of normal production (see 5.3).

9.4.5.2 Irradiate these product items at VD_{max}^{15} obtained from Table 10.

The highest dose to product items shall not exceed VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of VD_{max}^{15}.

Determine the dose delivered (see 5.5).

If the highest dose to product items exceeds VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater, the verification dose experiment shall be repeated.

If the arithmetic mean of the highest and lowest doses 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 tests of sterility, acceptable results are observed (see 9.4.6.1), the verification dose experiment need not be repeated.

9.4.5.3 Subject each irradiated product item individually to a test of sterility (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 If no more than one positive test of sterility is obtained from the 10 tests carried out, accept verification and thereby substantiate 15 kGy as the sterilization dose.

9.4.6.2 If two positive tests of sterility are obtained, perform a confirmatory verification dose experiment (see 9.4.7).

9.4.6.3 If three or more positive tests of sterility are obtained, do not accept verification as the sterilization dose might be inadequate.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{15}, or a specific bioburden-related cause, implement corrective action and repeat the verification dose experiment using a further 10 product items and the same VD_{max}^{15} as that used in the verification dose experiment that was not accepted. Interpret the results of the repeat verification dose experiment in accordance with 9.4.6.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{15}, or a specific bioburden-related cause, the selected sterilization dose of 15 kGy is not substantiated and an alternative method for establishing a sterilization dose shall be used (see Clause 6).

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 shall be followed.

9.4.7.2 Stage 1: Obtain samples of product

Select 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 the fourth batch used in Stage 4 (see 9.4.5) or from a batch manufactured under conditions which are representative of normal production (see 5.3).

9.4.7.3 Stage 2: Perform confirmatory verification dose experiment

9.4.7.3.1 Irradiate these product items at VD_{max}^{15} as determined in 9.4.4.

The highest dose to product items shall not exceed VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of VD_{max}^{15}.

Determine the dose delivered (see 5.5).

If the highest dose to product items exceeds VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater, the confirmatory verification dose experiment shall be repeated.

If the arithmetic mean of the highest and lowest doses to product items is less than 90 % of VD_{max}^{15}, the confirmatory verification dose experiment may be repeated. If this mean dose is less than 90 % of VD_{max}^{15} and, on performance of tests of sterility, acceptable results are observed (see 9.4.7.4.1), the confirmatory verification dose experiment need not be repeated.

9.4.7.3.2 Subject each irradiated product item individually to a test of sterility (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 If there are no positive tests of sterility from the 10 tests carried out, giving a total of two positive tests of sterility obtained from the original and confirmatory verification dose experiments performed in carrying out substantiation of 15 kGy, accept confirmatory verification and thereby substantiate 15 kGy as the sterilization dose.

9.4.7.4.2 If any positive tests of sterility are obtained, do not accept confirmatory verification as the sterilization dose might be inadequate.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{15}, or a specific bioburden-related cause, implement corrective action and repeat the confirmatory verification dose experiment using a further 10 product items and the same VD_{max}^{15} as that used originally. Interpret the results of the repeat confirmatory verification dose experiment in accordance with 9.4.7.4.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{15}, or a specific bioburden-related cause, the selected sterilization dose of 15 kGy is not substantiated and an alternative method for establishing a 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 less than or equal to 1,5.

9.5.2.2 In applying Method VD_{max}^{15}, an entire product item (SIP equal to 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 shall be followed.

9.5.3 Stage 1: Obtain samples of product

Select 10 product items from the single batch, in accordance with 5.1 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 1 Bioburden is generally determined on individual product items, but when the bioburden is low (e.g. for VD_{max}^{15} less than 1,5), it is permissible to pool the 10 product items for the determination of average bioburden.

NOTE 2 When no colonies are observed in the determination of bioburden, this is sometimes expressed as being below the limit of detection. Use of the limit of detection as a bioburden value in calculating average bioburden could lead to an overestimation. Overestimation could affect the validity of the verification dose experiment.

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

Obtain SIP equal to 1,0 VD_{max}^{15} from Table 10 using the average bioburden. If the average bioburden is not given in Table 10, use the closest tabulated value greater than the 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 these product items at VD_{max}^{15} obtained from Table 10.

The highest dose to product items shall not exceed VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of VD_{max}^{15}.

Determine the dose delivered (see 5.5).

If the highest dose to product items exceeds VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater, the verification dose experiment shall be repeated.

If the arithmetic mean of the highest and lowest doses 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 tests of sterility, acceptable results are observed (see 9.5.7.1), the verification dose experiment need not be repeated.

9.5.6.3 Subject each irradiated product item individually to a test of sterility (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 If no more than one positive test of sterility is obtained from the 10 tests carried out, accept verification and thereby substantiate 15 kGy as the sterilization dose.

9.5.7.2 If two positive tests of sterility are obtained, perform a confirmatory verification dose experiment (see 9.5.8).

9.5.7.3 If three or more positive tests of sterility are obtained, do not accept verification as the sterilization dose might be inadequate.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{15}, or a specific bioburden-related cause, implement corrective action and repeat the verification dose experiment using a further 10 product items and the same VD_{max}^{15} as that used in the verification dose experiment that was not accepted. Interpret the results of the repeat verification dose experiment in accordance with 9.5.7.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of the determination of bioburden, incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{15}, or a specific bioburden-related cause, the selected sterilization dose of 15 kGy is not substantiated and an alternative method for establishing a sterilization dose shall be used (see Clause 6).

9.5.8 Confirmatory verification dose experiment

9.5.8.1 General

If a confirmatory verification dose experiment is to be carried out (see 9.5.7.2), the three stages below shall be followed.

9.5.8.2 Stage 1: Obtain samples of product

Select 10 product items from the single batch of product. The ability of the product to support microbial growth should be taken into account in storing the single batch.

9.5.8.3 Stage 2: Perform confirmatory verification dose experiment

9.5.8.3.1 Irradiate these product items at VD_{max}^{15} as determined in 9.5.5.

The highest dose to product items shall not exceed VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of VD_{max}^{15}.

Determine the dose delivered (see 5.5).

If the highest dose to product items exceeds VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater, the confirmatory verification dose experiment shall be repeated.

If the arithmetic mean of the highest and lowest doses to product items is less than 90 % of VD_{max}^{15}, the confirmatory verification dose experiment may be repeated. If this mean dose is less than 90 % of VD_{max}^{15} and, on performance of tests of sterility, acceptable results are observed (see 9.5.8.4.1), the confirmatory verification dose experiment need not be repeated.

9.5.8.3.2 Subject each irradiated product item individually to a test of sterility (see 5.4.1) and record the number of positive tests of sterility.

9.5.8.4 Stage 3: Interpretation of results

9.5.8.4.1 If there are no positive tests of sterility from the 10 tests carried out, giving a total of two positive tests of sterility obtained from the original and confirmatory verification dose experiments performed in carrying out substantiation of 15 kGy, accept confirmatory verification and thereby substantiate 15 kGy as the sterilization dose.

9.5.8.4.2 If any positive tests of sterility are obtained, do not accept confirmatory verification as the sterilization dose might be inadequate.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{15}, or a specific bioburden-related cause, implement corrective action and repeat the confirmatory verification dose experiment using a further 10 product items and the same VD_{max}^{15} as that used originally. Interpret the results of the repeat confirmatory verification dose experiment in accordance with 9.5.8.4.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{15}, or a specific bioburden-related cause, the selected sterilization dose of 15 kGy is not substantiated and an alternative method for establishing a sterilization dose shall be used (see Clause 6).

10 Sterilization dose audit

10.1 Purpose and frequency

Once the sterilization dose has been established, periodic sterilization dose audits shall be carried out to confirm the continued appropriateness of the sterilization dose. All actions resulting from the sterilization dose audit shall apply to all product comprising the product family (see Clause 4).

The frequency at which sterilization dose audits are carried out shall be in accordance with ISO 11137-1:2006, 12.1. Sterilization 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, Method 2A or Method 2B

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 2A, use an SIP equivalent to that used originally in establishing the sterilization dose.

NOTE Method 2B requires the entire product to be utilized [see 8.3.1.1 a)].

10.2.1.2 In applying a sterilization dose audit, the four stages below shall be followed.

NOTE For worked examples, see 11.4 and 11.5.

10.2.2 Stage 1: Obtain samples of product

Select 110 product items from a single batch of product, in accordance with 5.1, 5.2 (if applicable) and 5.3.

10.2.3 Stage 2: Determine average bioburden

Determine the bioburden of each of 10 product items and calculate the average bioburden. If a correction factor (see ISO 11737-1) was used in establishing the original sterilization dose, apply the correction factor found from the most recent validation of the method of bioburden determination.

NOTE 1 Bioburden is generally determined on individual product items, unless the bioburden is low (e.g. less than 10), in which case it is possible to pool the 10 product items for the determination of batch average bioburden. This guidance does not apply to SIPs, which should not be pooled; instead, a larger SIP should be chosen (see 5.2.5).

NOTE 2 When no colonies are observed in the determination of bioburden, this is sometimes expressed as being below the limit of detection. Use of the limit of detection as a bioburden value in calculating average bioburden could lead to an overestimation. Overestimation could affect the validity of the verification dose experiment.

NOTE 3 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 most recent dose setting exercise or, if applicable, at the adjusted dose (see 10.2.6.4) obtained from the most recent sterilization dose audit that resulted in augmentation of the sterilization dose. When applicable, use the adjusted dose until the sterilization dose has been re-established.

On auditing a sterilization dose set by Method 1, the highest dose to product items shall not exceed the verification dose by more than 10 %. On auditing a sterilization dose set by Method 2A or 2B, the highest dose to product items shall not exceed D^{**} by more than 10 % or 1,0 kGy, whichever is greater.

On auditing a sterilization dose set by Method 1, the arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of the verification dose. On auditing a sterilization dose set by Method 2A or 2B, this mean dose should not be less than 90% of D^{**} or less than D^{**} minus 1,0 kGy, whichever dose is the lesser.

Determine the dose delivered (see 5.5).

If, on auditing a sterilization dose set by Method 1, the highest dose to product items exceeds the verification dose by more than 10 % or, on auditing a sterilization dose set by Method 2A or 2B, the highest dose to product items exceeds D^{**} by more than 10 % or 1,0 kGy, whichever is greater, the verification dose experiment shall be repeated.

If, on auditing a sterilization dose set by Method 1, the arithmetic mean of the highest and lowest doses to product items is less than 90 % of the verification dose or, on auditing a sterilization dose set by Method 2A or 2B, this mean dose is less than 90 % of D^{**} or less than D^{**} minus 1,0 kGy, whichever dose is lesser, the verification dose experiment may be repeated. If the conditions pertaining to a repeat of the verification dose experiment apply and, on performance of tests of sterility, acceptable results are observed (see 10.2.5.1), the verification dose experiment need not be repeated.

10.2.4.2 Subject each irradiated product item individually to a test of sterility (see 5.4.1) 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 no more than two positive tests of sterility are obtained from the 100 tests carried out, accept the sterilization dose audit.

10.2.5.2 If three or more positive tests of sterility are obtained, do not accept the sterilization dose audit as the sterilization dose might be inadequate.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose or D^{**}, or a specific bioburden-related cause, implement corrective action and repeat the verification dose experiment as soon as practicable using a further 100 product items and the same verification dose or D^{**} as that used in the sterilization dose audit that was not accepted. Interpret the results of the repeat verification dose experiment in accordance with 10.2.5.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of the verification dose or D^{**}, or a specific bioburden-related cause, the following shall apply:

a) If three or four positive tests of sterility are obtained, augment the sterilization dose immediately (see 10.2.6). Repeat the verification dose experiment as soon as practicable using a further 100 product items and the same verification dose or D^{**} as that used in the sterilization dose audit that was not accepted. Interpret the results of the repeat verification dose experiment in accordance with 10.2.5.3.

b) If five to 15 positive tests of sterility are obtained, augment the sterilization dose immediately (see 10.2.6) and re-establish the sterilization dose (see 10.4) as soon as practicable using the original dose setting method or another dose establishment method. Continue to use the augmented sterilization dose until re-establishment of the sterilization dose is completed.

c) If more than 15 positive tests of sterility are obtained, discontinue sterilization at the previously established sterilization dose (see 10.4). Do not augment the sterilization dose and do not resume sterilization until the sterilization dose is re-established using another method (see Clause 6).

10.2.5.3 Interpret the results of the repeat verification dose experiment, performed in accordance with 10.2.5.2 a), as follows:

a) If no more than two positive tests of sterility are obtained from the 100 tests carried out, and a review of environmental and manufacturing controls indicate no values outside established specifications, and the outcomes of bioburden determinations indicate no values outside specified bioburden limit, resume use of the previously established sterilization dose. If values exceed specifications, investigate and correct the cause and resume use of the previously established sterilization dose.

b) If three or four positive tests of sterility are obtained, re-establish the sterilization dose immediately (see 10.4) using the original dose setting method or another dose establishment method. Continue to use the augmented sterilization dose until re-establishment of the sterilization dose is completed (see Clause 6).

c) If five to 15 positive tests of sterility are obtained, re-establish the sterilization dose immediately (see 10.4) using another method (see Clause 6). Augment the sterilization dose using the findings from the repeat verification dose experiment and continue to use the augmented sterilization dose until re-establishment of the sterilization dose is completed.

d) If more than 15 positive tests of sterility are obtained, discontinue sterilization immediately and re-establish the sterilization dose (see 10.4) using another method (see Clause 6). Do not resume sterilization until re-establishment of the sterilization dose is completed.

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[13]. 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 as 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 as the "number of audit positives".

10.2.6.3 Stage 2: Determine extrapolation factor, E

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 less than or equal to 9, use Equation (13).

$$\text{extrapolation factor} = 2 + 0{,}2\,(E - 1) \quad (13)$$

If $(E - 1)$ is greater than 9, use Equation (14).

$$\text{extrapolation factor} = 0{,}4\,(E - 1) \quad (14)$$

If the calculation using Equation (13) or Equation (14) gives a value greater than 4,2 kGy, set the extrapolation factor to 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 Method VD_{max}^{25} or Method VD_{max}^{15}

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}^{25} or Method VD_{max}^{15}, use an SIP equivalent to that used originally in substantiating the sterilization dose.

NOTE Method VD_{max}^{15} requires the entire product item to be utilized (see 9.4.1.2 and 9.5.2.2).

10.3.1.2 In applying the sterilization dose audit, the four stages below shall be followed.

NOTE For a worked example, see 11.6.

10.3.2 Stage 1: Obtain samples of product

Select 20 product items from a single batch of product, in accordance with 5.1, 5.2 (if applicable) and 5.3.

10.3.3 Stage 2: Determine average bioburden

10.3.3.1 Apply the correction factor found from the most recent validation of the method of bioburden determination.

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

NOTE 1 Bioburden is generally determined on individual product items, unless the bioburden is low (e.g. for Method VD_{max}^{25} less than 10, for Method VD_{max}^{15} less than 1,5), in which case it is permissible to pool the 10 product items for the determination of average bioburden. This guidance does not apply to SIPs, which should not be pooled. Instead, a larger SIP should be chosen (see 5.2.5).

NOTE 2 When no colonies are observed in the determination of bioburden, this is sometimes expressed as being below the limit of detection. Use of the limit of detection as a bioburden value in calculating average bioburden could lead to an overestimation. Overestimation could affect the validity of the verification dose experiment.

NOTE 3 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 at VD_{max}^{25} or VD_{max}^{15} obtained from the original substantiation exercise, whichever is applicable.

The highest dose to product items shall not exceed VD_{max}^{25} by more than 10 % or VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater.

The arithmetic mean of the highest and lowest doses to product items should be less than 90 % of VD_{max}^{25} or VD_{max}^{15}.

Determine the dose delivered (see 5.5).

If the highest dose to product items exceeds VD_{max}^{25} by more than 10 % or VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater, the verification dose experiment shall be repeated.

If the arithmetic mean of the highest and lowest doses to product items is less than 90 % of VD_{max}^{25} or VD_{max}^{15}, the verification dose experiment may be repeated. If this mean dose is less than 90 % of VD_{max}^{25} or VD_{max}^{15} and, on performance of tests of sterility, acceptable results are observed (see 10.3.5.1), the verification dose experiment need not be repeated.

10.3.4.2 Subject each irradiated product item individually to a test of sterility (see 5.4.1) 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 If no more than one positive test of sterility is obtained from the 10 tests carried out, accept the sterilization dose audit.

10.3.5.2 If two positive tests of sterility are obtained, perform a confirmatory sterilization dose audit (see 10.3.6).

10.3.5.3 If three or more positive tests of sterility are obtained, do not accept the sterilization dose audit as the sterilization dose might be inadequate.

If the occurrence of these positive tests of sterility can be ascribed to incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{25} or VD_{max}^{15}, or a specific bioburden-related cause, implement corrective action and repeat the verification dose experiment as soon as practicable using a further 10 product items and the same VD_{max}^{25} or VD_{max}^{15} as that used in the sterilization dose audit that was not accepted. Interpret the results of the repeat verification dose experiment in accordance with 10.3.5.

If the occurrence of these positive tests of sterility cannot be ascribed to incorrect performance of tests of sterility or incorrect delivery of VD_{max}^{25} or VD_{max}^{15}, or a specific bioburden-related cause, the following shall apply:

a) If three to six positive tests of sterility are obtained, augment the sterilization dose immediately (see 10.3.7) and re-establish the sterilization dose (see 10.4) as soon as practicable using another method (see Clause 6). Continue to use the augmented sterilization dose until re-establishment of the sterilization dose is completed.

b) If seven or more positive tests of sterility are obtained, discontinue sterilization at the previously established sterilization dose (see 10.4). Do not augment the sterilization dose and do not resume sterilization until the sterilization dose is re-established using another method (see Clause 6).

10.3.6 Confirmatory sterilization dose audit

10.3.6.1 General

10.3.6.1.1 For the performance of a confirmatory sterilization dose audit for a sterilization dose established using Method VD_{max}^{25} or Method VD_{max}^{15}, use an SIP equivalent to that used originally in substantiating the sterilization dose.

NOTE Method VD_{max}^{15} requires the entire product item to be utilized (see 9.4.1.2 and 9.5.2.2).

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 10 product items from a single batch of product, in accordance with 5.1, 5.2 (if applicable) and 5.3. The 10 product items for the performance of confirmatory sterilization dose audit may be selected from either the batch used 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 (see 5.3).

10.3.6.3 Stage 2: Perform confirmatory verification dose experiment

10.3.6.3.1 Irradiate these product items at VD_{max}^{25} or VD_{max}^{15} obtained from the original substantiation exercise, whichever is applicable (see 9.2.4 or 9.4.4).

The highest dose to product items shall not exceed VD_{max}^{25} by more than 10 % or VD_{max}^{15} by more than 10 % or 0,1 kGy, whichever is greater.

The arithmetic mean of the highest and lowest doses to product items should not be less than 90 % of VD_{max}^{25} or VD_{max}^{15}.

Determine the dose delivered (see 5.5).

If the highest dose to product items exceeds VD_{max}^{25} by more than 10 % or VD_{max}^{15} by more than 10 %, or 0,1 kGy, whichever is greater, the confirmatory verification dose experiment shall be repeated.

If the arithmetic mean of the highest and lowest doses to product items is less than 90 % of VD_{max}^{25} or VD_{max}^{15}, the confirmatory verification dose experiment may be repeated. If this mean dose is less than 90 % of VD_{max}^{25} or VD_{max}^{15} and, on performance of tests of sterility, acceptable results are observed (see 10.3.6.4), the confirmatory verification dose experiment need not be repeated.

10.3.6.3.2 Subject each irradiated product item individually to a test of sterility (see 5.4.1) 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

Interpret the results of the confirmatory verification dose audit, performed in accordance with 10.3.5.2, as follows:

a) If there are no positive tests of sterility from the 10 tests carried out, giving a total of two positive tests of sterility obtained from the verification and confirmatory verification dose experiments performed in carrying out the sterilization dose audit, accept the sterilization dose audit.

b) If one to four positive tests of sterility are obtained, augment the sterilization dose immediately (see 10.3.7) and re-establish the sterilization dose (see 10.4) using another method (see Clause 6). Continue to use the augmented sterilization dose until re-establishment of the sterilization dose is completed.

c) If five or more positive tests of sterility are obtained, discontinue sterilization at the previously established sterilization dose (see 10.4). Do not augment the sterilization dose and do not resume sterilization 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 VD_{max}^{25}or VD_{max}^{15}, or a specific bioburden-related cause, implement corrective action and repeat the confirmatory sterilization dose audit (see 10.3.6.3) using a further 10 product items and the same VD_{max}^{25} or VD_{max}^{15} as that obtained from the original sterilization dose substantiation exercise. Interpret the results in accordance with a) to c) above.

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

10.3.7.1 Method VD_{max}^{25}

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 value greater than the average bioburden to obtain the dose augmentation value. Use this latter value in Equation (18) to calculate the augmented sterilization dose.

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

Table 11 — Method VD_{max}^{25} augmentation values for average bioburden less than or equal to 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

10.3.7.2 Method VD_{max}^{15}

From Table 12, 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 12, use the closest tabulated value greater than the average bioburden to obtain the dose augmentation value. Use this latter value in Equation (19) to calculate the augmented sterilization dose.

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

Table 12 — Method VD_{max}^{15} augmentation values for average bioburden less than or equal to 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)
≤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		

10.4 Failure of a sterilization dose audit

Following failure of a sterilization dose audit requiring the re-establishment of the sterilization dose, the cause of failure shall be investigated and correction and/or corrective action undertaken (see 4.4 of ISO 11137-1:2006). As part of the investigation, the effect of processing product at the sterilization dose that has failed sterilization dose audit on the achievement of the specified SAL for previously processed batches of product shall be considered and a risk assessment undertaken on their suitability for use. The investigation and subsequent actions shall be recorded (see 4.1.2 of ISO 11137-1:2006).

NOTE It might not be possible to determine the effect on attainment of this SAL until the sterilization dose has been re-established.

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 has a selected SAL of 10^{-3} and could be tested for verification using the entire product item (SIP equal to 1,0) (see Table 13). The second is for a product that has a selected SAL of 10^{-6} and was too large to be tested easily, so a portion of the product (SIP less than 1,0) was used (see Table 14). The third is for a product that has a selected SAL of 10^{-6} and could be tested for verification using the entire product item (SIP equal to 1,0) with a bioburden less than 1,0 (see Table 15).

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

Term	Value	Comment
Stage 1		
SAL	10^{-3}	For example, the product has a selected SAL of 10^{-3}.
SIP	1,0	The entire product was chosen for bioburden determination and the verification dose experiment.
Stage 2		
Average bioburden	382	Batch average bioburdens of 108, 80 and 382 were observed from the three batches tested. — The overall average bioburden was 190. — The highest batch average bioburden was 382. The highest batch average bioburden of 382 is more than two times the overall average bioburden; therefore, 382 was used to obtain the verification dose.
Stage 3		
Verification dose	9,7 kGy	As an average bioburden of 382 is not listed in Table 5, the next greater tabulated value of 400 was used.
Stage 4		
Verification dose experiment	9,4 kGy to 10,4 kGy	The dose to product items in the verification dose experiment ranged from 9,4 kGy to 10,4 kGy.
Stage 5		
Interpretation of results	1 positive	The highest dose to product items is less than the calculated upper limit (10,7 kGy), and the arithmetic mean of the highest and lowest doses, 9,9 kGy, is greater than 90 % of the verification dose (90 % of 9,7 kGy is 8,7 kGy). Doses are within the calculated limits and the results of tests of sterility were acceptable (i.e. less than or equal to two positives); therefore, verification was accepted.
Stage 6		
Sterilization dose for an SAL of 10^{-3}	12,9 kGy	The 10^{-3} sterilization dose for the highest batch average bioburden of 382 is 12,9 kGy from Table 5[a].

[a] As an average bioburden of 382 is not listed in Table 5, the next greater tabulated value of 400 was used.

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

Term	Value	Comment
Stage 1		
SAL	10^{-6}	For example, the product has a selected SAL of 10^{-6}.
SIP	0,05	The product was too large to be subjected to a test of sterility; a 1/20 portion was selected for dose setting.
Stage 2		
Average bioburden	59	The bioburden results from the SIPs for the three batches gave averages of 50, 62 and 65. Counts greater than or equal to 2 cfu per SIP on 85 % of the product items were obtained, demonstrating the adequacy of the SIP. — The overall SIP average bioburden was 59. — The highest batch SIP average bioburden was 65. The highest batch SIP average bioburden of 65 is less than two times the overall SIP average bioburden. Therefore, 59 was used to obtain the verification dose.
Stage 3		
Verification dose	7,3 kGy	As an average bioburden of 59 is not listed in Table 5, the next greater tabulated bioburden of 60 was used to obtain the verification dose.
Stage 4		
Verification dose experiment	6,5 kGy to 7,7 kGy	The dose to product items in the verification dose experiment ranged from 6,5 kGy to 7,7 kGy.
Stage 5		
Interpretation of results	2 positives	The highest dose to product items is less than the calculated upper limit (8,0 kGy) and the arithmetic mean of the highest and lowest doses, 7,1 kGy, is greater than 90 % of the verification dose (90 % of 7,3 kGy is 6,6 kGy). Doses are within the calculated limits and the results of tests of sterility were acceptable (i.e. less than or equal to two positives); therefore, verification was 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 an average bioburden of 1 180 is not listed in Table 5, the next greater tabulated value of 1 200 was used.

Table 15 — Determination of sterilization dose (Method 1, SIP equal to 1,0, bioburden less than 1,0)

Term	Value	Comment
Stage 1		
SAL	10^{-6}	For example, the product has a selected SAL of 10^{-6}.
SIP	1,0	For bioburden values less than 1,0, it is required to use the entire product item for bioburden determination and the verification dose experiment.
Stage 2		
Average bioburden	0,63	The bioburden results from the three batches gave averages of 0,6, 0,6, and 0,7. — The overall average bioburden of 0,63. — The highest batch average bioburden was 0,7. The highest batch average bioburden of 0,7 was less than two times the overall average bioburden of 0,63. Therefore 0,63 was used to obtain the verification dose.
Stage 3		
Verification dose	2,7 kGy	The average bioburden of 0,63 is not listed in Table 6; the next greater tabulated value of 0,70 was used to obtain the verification dose.
Stage 4		
Verification dose experiment	2,0 kGy to 2,6 kGy	The dose to product items in the verification dose experiment ranged from 2,0 kGy to 2,6 kGy.
Stage 5		
Interpretation of results	2 positives	The highest dose to product items is less than the calculated upper limit (3,0 kGy) and the arithmetic mean of the highest and lowest doses, 2,3 kGy, is less than 90 % of the verification dose (90 % of 2,7 kGy is 2,4 kGy). Although this mean is less than the calculated lower limit, the results of tests of sterility were acceptable (i.e. less than or equal to two positives); therefore, verification was accepted.
Stage 6		
Sterilization dose for an SAL of 10^{-6}	13,7 kGy	The 10^{-6} sterilization dose for an average bioburden of 0,63 is 13,7 kGy from Table 6 [a].

[a] As an average bioburden of 0,63 is not listed in Table 6, the next greater tabulated value of 0,70 was 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 item (SIP equal to 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 less than 1,0), given in Tables 21, 22, 23, 24 and 25. One worked example is given for Method 2B, with the requirement that the entire product be used; see Tables 26, 27, 28, 29 and 30.

In the following examples, notation is lower case when text 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 equal to 1,0)

11.2.2.1 Stage 1: Select SAL and obtain samples of product

11.2.2.1.1 The product has a selected SAL of 10^{-6}. The entire product was used for dose setting (SIP equal to 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. 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 incremental dose (kGy)								
		2	4	6	8	10	12	14	16	18
1	Highest 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	Highest 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	Highest 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 The arithmetic mean of the highest and lowest doses at each incremental dose is greater than the specified lower limit.										

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 obtain A. For example, the number of positive tests of sterility at median ffp (2,6 kGy) was 11, so A is 0,65 kGy.
FFP	1,95 kGy	FFP is the median of the three batch ffps minus A. For 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 one 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.

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.
Verification dose experiment	7,0 kGy to 8,0 kGy	The dose to product items in the verification dose experiment ranged from 7,0 kGy to 8,0 kGy.
DD^*	8,0 kGy	DD^* is the highest dose delivered in the verification dose experiment. Actions with regard to upper and lower dose limits are dependent on CD^* (see 8.2.4). For example: DD^* does not exceed the upper limit (10,0 kGy). The arithmetic mean of DD^* and the lowest dose to product (7,5 kGy) is less than the lower limit (8,0 kGy). NOTE In such circumstances, had CD^* been greater than 15, the verification dose experiment may have been repeated.
CD^*	2	CD^* is the number of positive tests of sterility observed in the verification dose experiment. For example: Two positive tests of sterility were observed and this number is acceptable.
FNP	8,0 kGy	For example: CD^* is two and, therefore, FNP is equal to DD^* which is equal to 8,0 kGy.