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Technical Information Report

AAMI/ISO TIR 13409:1996

Sterilization of health care products—Radiation Sterilization—Substantiation of 25 kGy as a sterilization dose for small or infrequent production batches

AAMI
Association for the Advancement of Medical Instrumentation

List of Titles

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TIR 13409 Substantiation of 25 kGy as a Sterilization Dose

AAMI/ISO TIR No. 13409—1996

Sterilization of Health Care Products—Radiation Sterilization—Substantiation of 25 kGy as a Sterilization Dose for Small or Infrequent Production Batches

Approved 9 May 1996

COMMITTEE REPRESENTATION

This technical information report was developed by the Radiation Sterilization Working Group of the AAMI Sterilization Standards Committee.

At the current time, the **Sterilization Standards Committee** has the following members:

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

BACKGROUND OF AAMI ADOPTION OF ISO TR 13409:1996

Sterilization of health care products—Radiation sterilization—Substantiation of 25 kGy for radiation

sterilization of small or infrequent production batches

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

ISO TR 13409:1996 (Technical Report, type 2) was developed by ISO Technical Committee 198, *Sterilization of health care products*, to fill a need for a technical report for radiation sterilization of health care products that are produced in small or infrequent batches. TC 198 approved and published the technical report in 1996. U.S. participation in ISO/TC 198 is organized through the U.S. Technical Advisory Group for ISO/TC 198, administered by the Association for the Advancement of Medical Instrumentation (AAMI). The United States made a considerable contribution to this report.

This document will provide a method of substantiating the suitability of 25 kGy as a sterilization dose, which may be used for radiation sterilization of products that are manufactured in small quantities (that is, less than 1,000 product units) either as a single batch or for production of a number of small batches. This Method originated in the United States and is analogous to Method 3 contained in ANSI/AAMI ST32—1991*, *Guideline for Gamma Radiation Sterilization,* upon which the ISO report is based.

AAMI encourages its committees to harmonize their work with international documents as much as possible. The AAMI Radiation Sterilization Working Group and the AAMI Sterilization Standards Committee reviewed this technical report to determine to what extent the document could be harmonized. During this review, the Working Group decided to adopt ISO TR 13409:1996 as an AAMI Technical Information Report (TIR).

Procedures require that AAMI consult with the technical committee about 5 years after the publication date (and periodically thereafter) for guidance on whether the document is still useful and that the information is relevant or of historical value. In the event that the information is not useful, the TIR is removed from circulation.

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

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*Superseded by ANSI/AAMI/ISO 11137—1994

NOTE—Beginning with the ISO foreword on page x, this AAMI TIR is identical to ISO TR 13409:1996.

FOREWORD

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

The main task of technical committees is to prepare International Standards, but in exceptional circumstances a technical committee may propose the publication of a technical report of one of the following types:

— type 1, when the required support cannot be obtained for the publication of an International Standard, despite repeated efforts;

— type 2, when the subject is still under technical development or where for any other reason there is the future but not immediate possibility of an agreement on an International Standard;

— type 3, when a technical committee has collected data of a different kind from that which is normally published as an International Standard (“state of the art”, for example).

Technical Reports of types 1 and 2 are subject to review within 3 years of publication, to decide whether they can be transformed into International Standards. Technical Reports of type 3 do not necessarily have to be reviewed until the data they provide are considered to be no longer valid or useful.

ISO/TR 13409:1996, which is a Technical Report of type 2, was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products.*

This document is being issued in the Technical Report (type 2) series of publications (according to subclause G.3.2.2 of part 1 of the ISO/IEC Directives, 1995) as a "prospective standard for provisional application" in the field of radiation sterilization because there is an urgent need for guidance on how standards in this field should be used to meet an identified need.

This document is not to be regarded as an "International Standard." It is proposed for provisional application so that information and experience of its use in practice may be gathered. Comments on the content of this document should be sent to the ISO Central Secretariat.

A review of this Technical Report (type 2) will be carried out not later than 3 years after its publication with the options of: extension for another 3 years; conversion into an International Standard; or withdrawal.

Annex A of this Technical Report is for information only.

INTRODUCTION

The International Standard ISO 11137, *Sterilization of health care products—Requirements for validation and routine control—Radiation sterilization*, specifies the requirements for assuring that the activities associated with the process of radiation sterilization are performed properly. One of the activities encompassed within the standard is the selection of the dose of radiation to be applied to healthcare products to render them sterile (the sterilization dose). ISO 11137 specifies that one of two approaches is to be used to select the sterilization dose; either (i) the selection of a product specific sterilization dose; or (ii) the application of a minimum dose of 25 kGy following selection of the appropriateness of this dose.

An informative annex to ISO 11137 (Annex B) describes two methods of selecting a sterilization dose. These Methods are designated Method 1 and Method 2. The basis for these Methods owes much to the ideas first propounded by Tallentire (Tallentire, 1973 ; Tallentire, Dwyer, and Ley, 1971 ; Tallentire and Khan, 1978) . Subsequently, standardized methods were developed (Davis et al, 1981 ; Davis, Strawderman, and Whitby, 1984 ; Whitby and Gelda, 1979) which formed the basis of the dose substantiation procedures put forward in the Association for the Advancement of Medical Instrumentation (AAMI) recommended practice for sterilization by gamma irradiation, *Guideline for gamma radiation sterilization* (AAMI, 1984).

These Methods of selection of sterilization dose use data derived from the inactivation of the microbial population in its natural state, and 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 species has its own unique D_{10} value. In the model, the probability that a particular item will be sterile after exposure to a given dose of radiation is defined in terms of the initial number of organisms on the item prior to irradiation and their D_{10} values.

The application of Methods 1 and 2 as described in Annex B of ISO 11137 requires that a relatively large number of product items, drawn from a variety of separate production batches, are used to establish the sterilization dose. This is not always practicable. Health care manufacturers regularly produce new products, and they are also, on occasion, required to manufacture a single batch of a product for a special order, field trial or clinical investigation. In addition, batches of many health care products are small and might be produced infrequently (that is, less than once every 3 months). For products manufactured in all these situations, determination and maintenance of a validated sterilization dose is as important as for large production batches. The method described in this report provides guidance on how to allow substantiation of 25 kGy as an

appropriate sterilization dose within the limitations stipulated in the method.

The present method is based on Method 1, described in ISO 11137 , Annex B, paragraphs B.3.4.1–B.3.4.1.3. Method 1 depends upon experimental verification that the response to radiation of bioburden is greater than that of a microbial population having a standard distribution of resistances. In practice, an estimate is made of the average bioburden prior to irradiation. For this bioburden, the dose that gives an SAL of 10^{-2} for the standard distribution of resistances is obtained. This dose is designated the verification dose, and it represents the dose that will reduce a microbial population with a standard distribution of resistances to a level that gives on average a one in 100 probability of a nonsterile product unit. A sample of 100 product units or portion thereof (SIP) is then exposed to the verification dose, and each product unit is tested individually for sterility. If there are not more than two positives out of the 100 tests, the sterilization dose is selected for any desired SAL at the estimated level of bioburden.

With the present method, if the verification dose experiment is passed, the product is sterilized using a sterilization dose of 25 kGy on the assumption that microorganisms having a standard distribution of resistances represent a more severe challenge to the sterilizing dose than organisms occurring on products.

It was decided to publish the present method as a Technical Report Type 2 because, unlike Methods 1 and 2 which had been used extensively since 1984, there was little practical experience in the application of this Method. Users of this Method are urged to submit any comments on the application and content of this document so that this experience can be taken into account when ISO 11137 is next revised.

Manufacturers of health care products who intend to use the protocols contained in this Technical Report are reminded that the requirements for all users of Radiation Sterilization contained in ISO 11137 equally apply to the manufacture and control of products for which a sterilization dose of 25 kGy is intended to be substantiated by this Method. In particular, there is a requirement that products be manufactured in circumstances such that the bioburden is controlled. Compliance with the requirements for proper control of the quality of raw materials, for the manufacturing environment and for the establishment of the basic properties of the packaging material are all essential.

Sterilization of health care products—Radiation sterilization—Substantiation of 25 kGy as a sterilization dose for small or infrequent production batches

1 Scope

This technical information report (TIR) describes a method of substantiating the suitability of 25 kGy as a sterilization dose for radiation sterilization of products with an average bioburden of less than 1,000 colony forming units (cfu) that are manufactured in small quantities (less than 1,000 product units).

This Method may be used to substantiate a sterilization dose of 25 kGy for any of the following situations:

a) a single batch of product units;

b) initial production of a new product while the sterilization dose is being established by another method;

c) routine production of small batches.

NOTE 1 Information collected in applying the method of dose substantiation described in this TIR may be used in meeting the product qualification requirements for sterilization dose selection of ISO 11137 (see 6.2.2).

NOTE 2 This TIR is considered "informative," and use of the terms "shall," "should," etc. should be considered within the context of this technical information report only. That is, if the decision is made to use this Method of

dose substantiation, then the method should be followed in adherence with the requirements ("shall") and recommendations ("should") as set forth in this TIR.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this TIR (International Technical Report, Type 2). At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this TIR are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

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

ISO 11737-1:1995, *Sterilization of medical devices—Microbiological methods—Part 1: Estimation of the population of microorganisms on products*

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

3 Definitions, symbols and abbreviations

For the purposes of this TIR, the following definitions apply and are presented in the order in which they occur in the text.

* To be published.

3.1 sterilization dose: Minimum absorbed dose required to achieve the specified sterility assurance level.

3.2 batch: Defined quantity of bulk, intermediate, or finished product that is intended or purported to be uniform in character and quality, and which has been produced during a defined cycle of manufacture.

3.3 bioburden: Population of viable microorganisms on a product unit.

NOTE 3 In the context of radiation sterilization, bioburden is determined immediately prior to sterilization.

3.4 fraction positive: Quotient derived from the number of positive tests of sterility divided by the total number of tests of sterility performed (number of positive tests of sterility plus number of negative tests of sterility).

3.5 verification dose: Dose of radiation to which product units, or portions thereof, are nominally exposed in the verification dose experiment with the intention of achieving a predetermined sterility assurance level (SAL).

NOTE 4 For this Method, the verification dose is selected to achieve a predetermined SAL ranging from 10^{-1} to $10^{-1,95}$, the actual value depending upon the number of product units, or portions thereof, used in the verification dose experiment.

3.6 product unit: Health care product, collection of products, or components within a primary package.

3.7 sterility assurance level (SAL): The probability of a viable microorganism being present on a product unit after sterilization.

NOTE 5 SAL is normally expressed as 10^{-n}.

NOTE 6 In the context of validation, the SAL may take levels other than that achieved by sterilization.

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

3.9 test of sterility: Test performed to establish the presence or absence of viable microorganisms on product units, or portions thereof, when subjected to defined culture conditions.

3.10 false positive: Result of a test of sterility in which a true negative is interpreted as a positive.

3.11 sterilization dose audit: Action taken to detect whether or not a change in sterilization dose is needed.

3.12 D_{10}: Radiation dose required to kill 90 percent of a homogeneous microbial population where it is assumed that the death of microbes follows first order kinetics.

NOTE 7 In this context, the unit of D_{10} is kGy.

3.13 false negative: Result of a test of sterility in which a true positive is interpreted as a negative.

3.14 negative test of sterility: A test of sterility which does not exhibit detectable microbial growth after incubation.

3.15 positive test of sterility: A test of sterility which exhibits detectable microbial growth after incubation.

4 Selection and testing of product

4.1 Selection

4.1.1 Method of selection

The method of selecting product units for subsequent testing can influence the test result observed. It is preferred to select product units at random. When selecting product units from small batches or from a batch of product which is only manufactured intermittently, it is particularly important that the product units be representative of processing procedures and conditions. Product units for testing may be selected from items rejected during the manufacturing process provided that they have been subjected to the same processing and conditions as the remainder of the batch.

4.1.2 Sample item portion (SIP)

Whenever practicable, an entire product unit should be used for testing, but it is recognized that this is not always possible. In such situations, a selected portion of a product unit (sample item portion, SIP), which is convenient to handle during testing, may be substituted. The SIP should be as large a portion of the product unit as is possible to manipulate readily in the laboratory. SIP can be calculated on the basis of length, weight, volume, or surface area of the product unit to be tested, as appropriate.

The SIP has to represent validly the microbial challenge presented to the sterilization process and the diverse elements of complex product units. The distribution of viable microorganisms on the product unit shall be considered and, if it can be demonstrated that these microorganisms are evenly distributed, the SIP may be selected from any single location on the product unit. In the absence of such a demonstration, the SIP shall be constituted from several portions of a product unit selected at random.

Twenty SIPs should be prepared and a test of sterility performed in accordance with ISO 11737-2. At least 17 of these tests shall be positive. If this criteria is not achieved, a larger SIP is required.

NOTE 8 The occurrence of 17 positives out of 20 tests of sterility indicates that there is an average of 2 cfu/SIP.

NOTE 9 If the entire product unit is tested, no minimum number of positives is specified for non-irradiated samples.

If a product unit or SIP cannot be tested in available laboratory glassware, it may be divided into two or more containers and these containers scored together as one unit; if in the performance of a test of sterility one container yields a positive result, the entire unit is considered positive.

If the product unit has a label claim of sterility of the fluid path only, testing of the fluid path should be considered as the entire product unit (i.e., SIP = 1.0).

The preparation and packaging of an SIP shall be conducted under conditions chosen to minimize alterations in the bioburden.

NOTE 10 Environmentally controlled conditions should be used for preparation of SIPs.

NOTE 11 Packaging materials should be equivalent to those used for the finished product.

Packaging shall be capable of withstanding the radiation doses to be delivered. Packaging for products, or portions thereof, for irradiation shall be chosen in order to minimize contamination during post-irradiation handling.

4.1.3 Sample item portion for kits

A kit is considered to be a product unit containing more than one health care product; these may be a) multiple units of identical items, or b) a variety of procedure-related items.

a) **Kits containing multiples of the same health care product.** The SIP for such kits shall be based upon a single health care product and not the summation of all the products in the kit. For example, for a kit containing 5 syringes and one syringe is tested in its entirety, then the syringe SIP = 1.0.

b) **Kits containing different health care products.** The SIP for such kits shall be based upon each type of health care product and a separate SIP established for each product in the kit. For example, if a kit contains two gowns, two towels, two pairs of gloves, and a drape, then individual SIPs must be determined for each type of health care product independent of the other product in the kit.

4.2 Microbiological testing

Bioburden determinations and tests of sterility undertaken as part of this Method shall be conducted using acceptable laboratory practices and in accordance with ISO 11737-1 and ISO 11737-2 respectively.

NOTE 12 ISO 11737-2 is currently in preparation. Until this International Standard is published, information on the test of sterility can also be found, for example, in AAMI 1991.

The method described hereafter uses a single culture medium for the performance of the test of sterility. The use of a single medium assumes that the medium will be optimal for the culture of aerobic and facultative organisms which could survive. When this assumption is not valid, this Method shall be conducted using other appropriate media and incubation conditions.

NOTE 13 Soybean Casein Digest Broth, with an incubation temperature of (30 $\pm$ 2)° C and an incubation period of 14 days, is generally recommended when a single medium is used.

4.3 Product irradiation

The irradiation of product, or SIPs, shall be in compliance with ISO 11137, Annex C1.5.4.

It is preferred that the product be irradiated in its original form and package. However, to minimize and/or simplify the manipulations during testing and reduce the possibility of false positives in the performance of tests of sterility, the product may be disassembled and repackaged prior to exposure to the verification dose.

NOTE 14 Manipulations prior to irradiation are not always acceptable. In certain instances, such manipulations can change the response of the microorganisms to irradiation. For example, manipulations can alter the chemical environment (typically oxygen tension) in the vicinity of the microorganisms.

5 Method of substantiation of 25 kGy

5.1 Rationale

This Method is an adaptation of Method 1 described in ISO 11137.

Method 1 depends upon experimental verification that the response to radiation of the product bioburden is greater than that of a microbial population having a standard distribution of resistances; this is achieved by performance of a verification dose experiment employing 100 product units, or portions thereof, and a requirement to meet the defined acceptance criteria that demonstrate an SAL of 10^{-2}.

The present method is intended for products manufactured in batches of less than 1,000 product units; consequently, the total number of product units taken for bioburden determination is less than the minimum required with Method 1 and the number taken for the verification dose experiment is less than the 100 required when using Method 1.

As fewer product units are tested in the verification dose experiment, an SAL of 10^{-2} cannot be the basis of acceptance, but rather a higher SAL value has to be employed. This higher SAL value is derived from the reciprocal of the number of product units tested in the verification dose experiment. Inevitably, the use of a higher SAL value means that the ability of the method to detect bioburden with a higher resistance to radiation than that corresponding to the standard distribution of resistances is diminished. Consequently, an upper limit of SAL of 10^{-1}, corresponding to a minimum of 10 product units for the verification dose experiment, is imposed for the present method of dose substantiation.

Test sample sizes for the performance of bioburden determination and verification dose experiment are given in Table 1. These sample sizes are based on Tables I and II-A of ISO 2859-1 ISO, Inspection Level II, using the relationship between the production batch size and sample size. This relationship is approximated by a straight line on log-log scales (that is, plotting log of sample size versus log of geometric mean of the limits of each batch size interval) (Hald, 1981). This relationship is fit by the following equation:

$$\text{Sample Size} = 0.58 \times (\text{Production Batch Size})^{0.74} \qquad \text{Equation 1}$$

Table 1—Test sample sizes for performance of bioburden determination and verification dose experiment

Production batch size	Test sample size	
	Bioburden determination	Verification dose
831 - 999	10	90
702 - 830	10	80
578 - 701	10	70
462 - 577	10	60
352 - 461	10	50
251 - 351	10	40
160 - 250	10	30
80 - 159	10	20
20 - 79	10	10

ISO 2859-1, Inspection level II also takes account of the economics of withdrawing large numbers of product units from small production batches. However, a lower limit of 10 is imposed for the number of product units for the verification dose experiment. The rationale for this is that the distribution of the microorganisms on

product units, produced in small batches, may not allow representative product units to be withdrawn as a sample size less than 10.

In practice, a determination is made of the average bioburden and this determination is used to calculate the verification dose at the predetermined value of SAL.

For Method 1, the verification dose for a 10^{-2} SAL may be read from a table, with values derived from the inactivation of a microbial population having a standard distribution of resistances. For the present method, however, a variety of SAL values are employed in the verification dose experiment depending upon the production batch size. An alternative approach has been employed in which the non-linear relationship between verification dose in kGy at a given SAL and log average bioburden is approximated to a series of linear relationships corresponding to different ten-fold domains of average bioburden within the limits of 1 and 1,000. Each of these linear relationships is characterized by 2 constants, the intercept (I) and slope (S) given in Table 2; thus

Verification dose at a given SAL = I + (S x log(Average SIP Bioburden)) Equation 2

Table 2—(I) and (S) values for calculation of verification dose for test sample size and bioburden level

Test sample size	Bioburden 1 to 10		Bioburden 11 to 100		Bioburden 101 to 1,000	
	I	S	I	S	I	S
10	1.25	1.65	0.67	2.23	-0.26	2.71
20	1.71	1.82	1.14	2.4	0.35	2.81
30	2.00	1.93	1.46	2.49	0.71	2.87
40	2.21	2.01	1.69	2.55	1.00	2.90
60	2.52	2.12	2.03	2.63	1.40	2.95
70	2.65	2.16	2.16	2.66	1.55	2.97
80	2.76	2.19	2.30	2.67	1.67	2.99
90	2.86	2.22	2.39	2.70	1.80	3.00

NOTE 15 In entering Table 2, the bioburden value should be that obtained for the SIP used in the verification dose experiment.

NOTE 16 The verification dose (kGy) may be rounded (using standard rounding procedures) to one place of decimals.

In practice, the requisite number of product units, or portions thereof, are exposed to the calculated verification dose and each product unit is individually subjected to a test of sterility. If the results of tests of sterility meet the requirements defined in the procedure, then a 25 kGy dose is used for sterilization.

5.2 Limitations of the method

NOTE 17 Although the basis of this Method is Method 1 of ISO 11137 , changes have been made to the procedures. The result of these changes is an increased probability of failing the verification dose experiment. The increased probability is principally seen with product of low bioburden in combination with small sample sizes. It is important to recognize that failure means that the use of a sterilization dose of 25 kGy cannot be substantiated using this Method. When substantiation of 25 kGy is not achieved with this Method, the primary manufacturer may choose to develop information on the radiation resistance of selected isolates recovered from product after exposure to a screening radiation dose, on the assumption that the isolates' responses to radiation are typical of that of resistant organisms occurring on product. In designing the experiments, due consideration should be given to the nature of the test pieces used and the relevance to the conditions under which the

organisms exist naturally on the product. Conditions of growth of isolates prior to inclusion on test pieces, their storage on test pieces and their recovery from the test pieces should also be taken into account.

This Method shall only be used to substantiate a sterilization dose of 25 kGy for product which have been produced in accordance with a Quality System (ISO 13485 and ISO 13488).

In addition, this Method shall only be used for production batch sizes of less than 1,000 product units where the average product bioburden is less than 1,000 colony forming units (cfu). If either of these conditions is not met, another method shall be used, such as Method 1 or 2 detailed in ISO 11137 .

For products which support microbial growth, this Method shall only be used when evidence is available to demonstrate that the bioburden does not change during the interval between determination of bioburden and the verification dose experiment.

NOTE 18 With this Method, it is necessary to hold product units for the verification dose experiment until determination of the average bioburden is completed. The ability of the product to support microbial growth should be taken into account in selecting storage conditions for the product units while bioburden is being determined.

5.3 Technical requirement

When substantiating the selection of 25 kGy, the party performing the substantiation shall:

a) have access to competent microbiological laboratory services;

b) perform microbiological testing in accordance with ISO 11737-1 and ISO 11737-2 ; and

NOTE 19 ISO 11737-2 is currently in the course of preparation. Until this International Standard is published, information on the test of sterility can be found, for example, in AAMI 1991 .

c) have access to a radiation source capable of delivering accurate and precise doses ranging from 1 kGy upward with either:

1) a Co 60 or Cs 137 radiation source; or

2) an electron beam or x-ray irradiator operated at an energy level and dose rate similar to those used in processing.

5.4 Procedure

The following six procedural stages shall be carried out:

NOTE 20 A worked example appears in clause 6 .

a) **Stage 1: Establish test sample sizes.** From a knowledge of the size of the production batch, use Table 1 to establish the number of product units required for determination of bioburden and the verification dose experiment.

b) **Stage 2: Obtain samples of product units**. Take the appropriate number of product units (chosen at random) from the batch in order to perform the bioburden determination and the verification dose experiment.

c) **Stage 3: Determine average bioburden**. Determine the bioburden on each of the 10 product units, or portions thereof, and calculate the average bioburden.

d) **Stage 4: Establish verification dose**. From a knowledge of the average bioburden and of the number of product units, or portions thereof, to be used in the verification dose experiment, calculate the verification dose using equation 2 and the appropriate values of I and S derived from Table 2.

e) **Stage 5: Perform verification dose experiment**.

NOTE 21 Use of the verification dose experiment without determination of bioburden is not valid.

Irradiate the product units, or portions thereof, obtained in stage 2 at the verification dose calculated in stage 4. The actual dose may vary from the calculated verification dose by not more than + 10 percent.

NOTE 22 In this context the “actual dose” refers to the maximum dose received by the group of product units.

If the delivered dose is less than 90 percent of the calculated verification dose, the test may be repeated.

NOTE 23 In this context the "delivered dose" refers to the arithmetic mean of the maximum and minimum doses.

Individually subject the irradiated product units, or parts thereof, to a test of sterility using Soybean Casein Digest Broth, incubated at (30 ± 2)° C for 14 days (in accordance with ISO 11737-2). Record the number of positive tests of sterility.

NOTE 24 Other media and incubation conditions may be employed as appropriate (see 4.2).

f) **Stage 6: Interpretation of results**. For a verification dose experiment performed with 10 or 20 product units, or portions thereof, statistical verification is accepted if there is no more than one positive test of sterility observed. For a verification dose experiment performed with 30 or more product units, or portions thereof, statistical verification is accepted if there are no more than two positive tests of sterility observed. If the verification procedure is passed (that is, statistical verification is accepted), the sterilization dose of 25 kGy is substantiated.

If statistical verification is not accepted, and the number of positive tests of sterility cannot be ascribed to incorrect performance of the bioburden determination, the performance of tests of sterility, or the delivery of the verification dose (e.g. the delivered dose was less than 90 percent of the calculated verification dose), this Method of dose substantiation is not applicable and cannot be pursued without identification of the cause of the failure and implementation of corrective actions.

Results of environmental and manufacturing controls and product bioburden determination should be reviewed in conjunction with evaluation of the sterilization dose audit results. If the review indicates lack of control, appropriate action should be taken.

5.5 Sterilization dose audit

Sterilization dose audits shall be carried out following the six procedural stages described in clause 5.4 .

5.6 Routine production

Following successful substantiation in accordance with clause 5.4 on three separate occasions, a dose of 25 kGy may be used for routine production of a number of small production batches at a frequency of more than one batch every 3 months, without substantiation on a batch by batch basis. In this situation, sterilization dose audits shall be performed at 3 month intervals to detect any changes in the bioburden and/or bioburden resistance that could invalidate the sterilization dose.

NOTE 25 Sterilization dose audits are not applicable for either production of a single batch of product or for a production of a number of small batches at a frequency of less than one batch every 3 months. For these instances, substantiation of 25 kGy for each production batch is required.

6 Worked examples

The first example is for a product that is too large to be tested in its entirety, so a portion of the product (SIP < 1) was used. This example is for a single production batch.

The second example is for a product unit that can be tested using the whole unit (SIP = 1). This second example

is for a production batch chosen for sterilization dose audit. The production frequency is greater than one batch per quarter and the sterilization dose of 25 kGy was previously substantiated on three consecutive production batches.

Example 1—Substantiation of 25 kGy as the sterilization dose (SIP < 1.0)

Term	Value	Comment
Stage 1		
Production batch size	70	The production batch size was 70.
Test sample size for bioburden determination	10	The test sample size from Table 1 is 10.
Test sample size for verification dose experiment	10	The test sample size from Table 1 is 10.
Stage 2		
Obtain samples	20	A random sample of 20 product units was obtained from the production batch (10 for bioburden determination and 10 for the verification dose experiment).
Stage 3		
SIP	0.50	As the product was too large to perform a test of sterility easily, a 1/2 portion was selected for testing.
SIP bioburden	45	SIP bioburden results of 50, 66, 38, 46, 58, 32, 34, 44, 56 and 26 were observed from the 10 SIPs tested, for an average SIP bioburden of 45.
Average bioburden	90	The average bioburden for the product tested was calculated as follows: 45/0.5 = 90. The bioburden was less than 1,000 organisms, therefore, this Method may be used.
Stage 4		
Verification dose calculation	4.4 kGy	The I and S values in Table 2 for an SIP bioburden of 45 are 0.67 and 2.23, respectively. The verification dose = I + [S x log (average SIP bioburden)] kGy = 0.67 + [2.23 x log (45)] kGy = 4.36 kGy = 4.4 kGy
NOTE The verification dose may be rounded (using standard procedures) to one place of decimals.		
Stage 5		
Verification dose experiment	3.2 to 4.4 kGy	The dose ranged from 3.2 to 4.4 kGy with a mean of 3.8 kGy. The actual dose was below the upper limit of 4.8 kGy. The delivered dose fell below 90% of the calculated verification dose.
	1 positive /10 tested	The test of sterility yielded 1 positive from the 10 SIPs tested. Therefore, the verification experiment was successful and no further action was necessary. If this experiment had failed, the verification experiment could have been repeated.
Stage 6		
Interpretation of results	25.0kGy	The test of sterility result was acceptable (that is ≤ 1 positive per 10 SIPs tested); therefore, even though the mean dose fell below 90% of the calculated verification dose, the sterilization dose of 25 kGy was confirmed.

Example 2—Sterilization dose audit (SIP = 1.0)

Term	Value	Comment
Stage 1		
Production batch size	650	The production batch size was 650.
Test sample size for bioburden determination	10	The test sample size from Table 1 was 10.
Test sample size for verification dose experiment	70	The test sample size from Table 1 was 70.
Stage 2		
Obtain samples	80	A random sample of 80 product units was obtained from the production batch (10 for bioburden determination and 70 for the verification dose experiment).
Stage 3		
SIP	1.0	The entire product is to be used for the test of sterility.
Bioburden	38	Bioburden results of 50, 43, 22, 25, 36, 47, 34, 49, 29 and 45 were observed from the 10 product units tested, for an average bioburden of 38.
Average bioburden	38	The average bioburden for the product tested was 38 since an SIP of 1.0 was used. The bioburden was less than 1,000 organisms; therefore, this Method may be used.
Stage 4		
Verification dose calculation	6.4 kGy	The I and S values in Table 2 for an average bioburden of 38 are 2.16 and 2.66, respectively. The verification dose = I + [S x log (average SIP bioburden)] kGy = 2.16 + [2.66 x log (38)] kGy = 6.36 kGy = 6.4 kGy NOTE The verification dose was rounded (using standard procedures) to one place of decimals.
Stage 5		
Verification dose experiment	6.0 to 6.9 kGy 0 positive/ 70 tested	The dose ranged from 6.0 to 6.9 kGy with a mean of 6.5 kGy. The maximum dose was below the upper limit of 7.0 kGy. The mean dose was greater than 90% of the calculated verification dose. The test of sterility yielded 0 positives from the 70 product units tested.
Stage 6		
Interpretation of results	25.0 kGy	The test of sterility result was acceptable (that is, ≤ 2 positives per 70 product units tested); therefore, the sterilization dose of 25 kGy was confirmed.

Annex A
(informative)

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