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Sterilization of health care products — Biological indicators —

Part 8: Method for validation of a reduced incubation time for a biological indicator

Stérilisation des produits de santé — Indicateurs biologiques —

Partie 8: Méthode pour la validation d'un temps d'incubation réduit pour un indicateur biologique

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Foreword

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

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

A list of all parts in the ISO 11138 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

A biological indicator incubation time is the minimum period of cultivation required before making a final determination that a biological indicator is negative (shows no growth). The reference incubation time for biological indicators for established sterilization processes such as moist heat and ethylene oxide is 7 d (see ISO 11138-1:2017). In some instances where biological indicator results are needed as part of the product release process, a 7-day incubation time might not be practical. This is especially the case where biological indicators are used to monitor sterilization processes in hospitals or other health care facilities such as dental or general practitioner offices.

The purpose of a reduced incubation time procedure is to demonstrate recovery of the surviving test organisms within the specified reduced incubation time period. The reduced incubation time is a function of the test method and conditions used to establish the incubation time and is independent of the process parameters for the sterilization method used to deliver the lethality.

Biological indicators with an incubation time of less than 7 d (a Reduced Incubation Time, or RIT) have been in use since the 1970s. The methodology to determine the RIT was originally created by the biological indicator manufacturers. Later, the United States Food and Drug Administration published guidance for manufacturers seeking regulatory clearance to market biological indicators to health care facilities in the United States (see Reference [1]). This guidance contained a protocol for validating an incubation time that was less than 7 d. This document was specific to regulations for commercial practices in a single country and did not address requirements for RIT methodology outside of that application. The purpose of this document is to describe an internationally agreed approach to the validation of the reduced incubation time of a biological indicator.

Sterilization of health care products — Biological indicators —

Part 8: Method for validation of a reduced incubation time for a biological indicator

1 Scope

1.1 This document specifies the requirements for a test method to be utilized to establish or confirm a reduced incubation time (RIT) that is shorter than the 7-day reference incubation time specified in 7.3.2 of ISO 11138-1:2017 for biological indicators used to monitor moist heat sterilization processes or ethylene oxide (EO) sterilization processes.

NOTE For biological indicators used for EO sterilization, the stated RIT is applicable to 100 % EO processes or processes that use EO blends, regardless of the product load.

1.2 This document is applicable to manufacturers of biological indicators (BIs) and to end users of BIs who intend to, if required by their quality system, establish, validate or confirm a RIT.

1.3 This document does not apply to biological indicators used to monitor dry heat, low temperature steam formaldehyde (LTSF) or vaporized hydrogen peroxide (VH2O2) sterilization processes.

NOTE The method described in this document to establish a RIT for biological indicators used to monitor moist heat or EO sterilization processes has been used extensively for many years. However, there is limited experience in use of this method to establish a RIT for biological indicators used to monitor dry heat, low temperature steam formaldehyde or vaporized hydrogen peroxide sterilization processes. This document, therefore, does not include these sterilization processes.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 18472, *Sterilization of health care products — Biological and chemical indicators — Test equipment*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
biological indicator
test system containing viable microorganisms providing a specified resistance to a specified sterilization process

[SOURCE: ISO 11139:2018, 3.29]

3.2
carrier
supporting material on or in which test microorganisms are deposited

[SOURCE: ISO 11139:2018, 3.33]

3.3
culture condition
combination of growth media and manner of incubation used to promote germination, growth and/or multiplication of microorganisms

Note 1 to entry: The manner of incubation can include the temperature, time and any other conditions specified for incubation.

[SOURCE: ISO 11139:2018, 3.70]

3.4
fractional cycle
operating cycle in which the exposure phase is reduced compared with that specified for the sterilization cycle

[SOURCE: ISO 11139:2018, 3.123]

3.5
inoculated carrier
supporting material on or in which a specified number of viable test organisms have been deposited

[SOURCE: ISO 11139:2018, 3.144]

3.6
resistometer
test equipment designed to create specified combinations of the physical and/or chemical parameters of a sterilization process

[SOURCE: ISO 11139:2018, 3.233]

3.7
self-contained biological indicator
biological indicator (3.1) presented such that the primary package, intended for incubation, contains the incubation medium required for incubation and recovery of the test organism

[SOURCE: ISO 11139:2018, 3.248]

4 General

4.1 When establishing, validating, or confirming a RIT (i.e. an incubation time of less than 7 d), the exposure shall be designed to achieve a fractional response in either:

a) a resistometer meeting the requirements of ISO 18472, or

b) a sterilizer process where all parameters are specified, controlled, and repeatable.

4.2 After successful RIT validation of the BI per this document, if ongoing lot-to-lot assessment of RIT is desired by the BI manufacturer as part of a quality control program, this testing may follow other statistically valid sampling plans as determined by the BI manufacturer's Quality Systems requirements.

4.3 The method described in this document shall be used to establish the RIT for a biological indicator design whose primary components include a spore carrier and a specific recovery medium (e.g. a spore strip supplied with recovery medium as a kit, or a self-contained biological indicator).

4.4 The RIT validation for a spore strip that includes a recovery medium as a kit or a self-contained BI does not have to be repeated by the end user as long as the end user uses the BI with the same sterilizing agent and BI incubation temperature as that used in the validation, e.g. design validated and used in ethylene oxide. See ISO 11138-7:2019, 12.3.3.

If an end user intends to use a RIT with a spore carrier/recovery medium combination or incubation conditions that have not been tested by a BI manufacturer (e.g. a spore strip used with a recovery medium not supplied as part of a kit), the end user shall establish the RIT for that system using the method provided in this standard.

4.5 A requirement for end user verification of a RIT is determined by the quality system requirements of the end user. If an end user intends to confirm a RIT provided by the BI manufacturer, the end user shall follow the test method used by the BI manufacturer.

5 Selection and preparation of samples

5.1 Biological indicators used for testing by this method shall be chosen from pre-production or production lots of BIs.

5.2 Biological indicator sample holders should provide for adequate separation of the exposed BIs to avoid a shielding effect. The holder should be constructed and prepared for use (e.g. pre-warmed) in such a way that it does not influence exposure conditions.

NOTE Some examples of properties of the holder include:

a) chemically non-reactive with the sterilizing agent;

b) a good heat conductor;

c) the mass ratio of the holder to the exposed samples is kept to a minimum;

d) made in the form of mesh, as opposed to solid materials.

6 Exposure and culturing

6.1 Identify the fractional cycle exposure conditions in which 30 % to 95 % of the BIs exposed are expected to be positive after 7 d of incubation.

NOTE 1 Other process variables (e.g. time, temperature, or concentration) can be modified to achieve the fractional response.

NOTE 2 Different fractional cycles can be used provided each set achieves 30 % to 95 % positives.

6.2 Obtain a minimum of 100 BIs from each of 3 different lots (for a minimum total sample size of 300 biological indicators). Expose the BIs to the fractional cycles determined in 6.1.

6.3 Place the BI samples in the chamber such that all the samples are equally exposed to the sterilization conditions.

6.4 The time interval between the conclusion of the process and initiation of exposure of the spore carrier to the recovery medium shall be documented and consistent for all tests.

6.5 Incubate the BIs for 7 d at the temperature identified in the BI instructions for use. Record positive and negative BI results for each BI at predetermined intervals. The predetermined intervals will specify the resolution of the RIT, therefore the defined interval shall be representative of the anticipated outcome (e.g. days, hours, etc.).

NOTE In some cases the BI design and/or media volume is not conducive to the required 7-day incubation period. In these instances, it can be necessary to take steps to prevent media evaporation (e.g. adding film wrap or tape around the cap of a self-contained BI, or increasing the relative humidity of the incubation process) particularly when incubating at ≥ 55 °C. It is important that these steps are performed consistently from lot to lot.

7 Determination of reduced incubation time

7.1 For each fractional cycle, determine the number of positive BIs in each sample set for each lot after 7 d of incubation. Determine the percentage of positive BIs (see 7.1.1).

7.1.1 The 30 % to 95 % positive BI window is defined by Formula (1):

$$N_F / N_T \times 100 \quad (1)$$

where

N_F is the number of positive BIs at 7-day incubation per fractional cycle and BI lot number;

N_T is the total number of BIs tested in the fractional cycle for each BI lot.

The value shall be ≥ 30 % and ≤ 95 % for the results to be used in the RIT calculation.

7.1.2 Any sample set for each fractional cycle for each lot where the percentage of positive BIs from the fractional cycle after 7-day incubation is outside of the 30 % to 95 % positive BI window shall not be used for the RIT determination. An example calculation is as follows:

EXAMPLE

100 BIs from a single lot are exposed to a factional cycle (N_T = 100). 20 positive BIs out of the 100 tested are observed at 7 d of incubation (N_F = 20).

$N_F / N_T \times 100 = (20/100) \times 100 = 20$ %

Since 20 % is not within the acceptable range of 30 % to 95 %, these results cannot be used toward establishing the RIT for that lot.

7.1.3 The exposures for these sample sets shall be repeated using unexposed BIs until every fractional cycle sample set for each lot is within the 30 % to 95 % positive BIs window (see 7.1.1 for definition of the window) and the total number of BIs exposed for each lot is 100 at minimum.

An example calculation is as follows:

EXAMPLE

100 BIs from a single lot are exposed to a fractional cycle (N_T = 100). 60 positive BIs out of the 100 tested are observed at 7-day incubation (N_F = 60):

$N_F / N_T \times 100 = (60/100) \times 100 = 60$ %

Since 60 % is within the acceptable range of 30 % to 95 % the RIT results for this fractional cycle can be used in the RIT calculation for this lot.

7.2 For each fractional cycle of the 3 lots tested, determine the shortest time interval where the number of positive BIs at that time interval have a greater than or equal to 97 % correlation to the number of BIs positive at 7 d.

Formula (2) to determine the greater than or equal to 97 % correlation to 7-day incubation for each fractional cycle for each lot is defined by the following (correlation calculation to 7-day incubation):

$$N_x / N_y \times 100 \geq 97\ \% \tag{2}$$

where

x is the RIT time interval selected;

N_x is the number of positive BIs at the time interval x for the individual fractional cycle for each lot;

N_y is the number positive BIs positive at 7-day incubation for the individual fraction cycle for each lot.

Only use Formula (2) after the individual fraction cycle for each lot has met the criteria in 7.1. Reference Table 1 sample calculations to determine the most conservative time interval.

NOTE Minimum correlation of ≥ 97 % is obtained without data rounding.

7.3 Using data from all fractional cycles for the 3 lots tested determine the longest time incubation interval where the criteria in 7.1 and 7.2 are met as the most conservative time. This is the RIT. Reference Table 1 sample calculations.