INTERNATIONAL STANDARD

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Third edition
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Sterilization of health care products — Chemical indicators —

Part 1: General requirements

Stérilisation des produits de santé — Indicateurs chimiques —

Partie 1: Exigences générales

Reference number
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Foreword

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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. www.iso.org/directives

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 198, *Sterilization of health care products*.

This third edition cancels and replaces the second edition (ISO 11140-1:2005), which has been technically revised.

ISO 11140 consists of the following parts, under the general title *Sterilization of health care products — Chemical indicators*:

— *Part 1: General requirements*

— *Part 3: Class 2 indicator systems for use in the Bowie and Dick-type steam penetration test*

— *Part 4: Class 2 indicators as an alternative to the Bowie and Dick-type test for detection of steam penetration*

— *Part 5: Class 2 indicators for Bowie and Dick-type air removal tests*

ISO 11140-2 has been withdrawn and replaced by ISO 18472.

Introduction

This part of ISO 11140 specifies performance requirements and/or test methods for chemical indicators intended for use with sterilization processes employing steam, dry heat, ethylene oxide, γ or β radiation, low temperature steam and formaldehyde or vaporized hydrogen peroxide.

Additional requirements for indicators intended for use with other sterilization methods (e.g. other forms of moist heat sterilization) are not specifically provided in this part of ISO 11140; however, the general requirements will apply.

The requirements for specific test indicators (e.g. Bowie-Dick test indicators and indicator systems) are covered in other parts of ISO 11140.

Standards for sterilizers and for the validation and process control of sterilization describe performance tests for sterilizers and methods of validation and routine control, respectively.

This part of ISO 11140 is intended for manufacturers of chemical indicators and specifies the general requirements for chemical indicators. The categorization structure for chemical indicators is used solely to denote the characteristics and intended use of each type of indicator when used as specified by the manufacturer. This categorization has no hierarchical significance. The chemical indicators described in this part of ISO 11140 are categorized into six types. The chemical indicators within each of these categorizations are further subdivided by the sterilization process for which they are designed to be used. This part of ISO 11140 defines the requirements for Type 1 and Types 3 to 6. In subsequent parts of ISO 11140, the requirements for Type 2 indicators are categorized by their intended use. The use of the indicators and indicator systems, specified in this part of ISO 11140, is described in for example the ISO 11135, the ISO 17665- series, ISO 15882, EN 285, and EN 13060.

Resistometers are used to characterize the performance of the chemical indicators described in this part of ISO 11140, with the exception of Type 2 indicators. Requirements for resistometers are specified in ISO 18472. Resistometers differ from sterilizers. As sterilizers cannot duplicate resistometer conditions they should not be used to test the performance of chemical indicators. Sterilizers from different manufacturers and of different ages have significantly different cycle profiles; for example, prolonged preconditioning phases. Resistometers allow for precise control of the specific test cycle sequences in order to study the effect of process parameters on indicator performance under controlled, repeatable conditions. Guidance on the selection, use and interpretation of the results of chemical indicators is given in ISO 15882. Users of chemical indicators are expected to make reference to this part of ISO 11140.

Sterilization of health care products — Chemical indicators —

Part 1: General requirements

WARNING — The use of this part of ISO 11140 can involve hazardous materials, operations and equipment. This part of ISO 11140 does not purport to address all of the safety problems associated with their use. It is the responsibility of the user of this part of ISO 11140 to determine the applicability of national or regional regulatory requirements and to establish appropriate occupational health and safety practices prior to use of any hazardous materials, operations and/or equipment.

1 Scope

This part of ISO 11140 specifies general requirements and test methods for indicators that show exposure to sterilization processes by means of physical and/or chemical change of substances, and which are used to monitor the attainment of one or more of the process parameter(s) specified for a sterilization process. They are not dependent for their action on the presence or absence of a living organism.

NOTE 1 Biological test systems are regarded as those test systems which are dependent for their interpretation on the demonstration of the viability of an organism. Test systems of this type are considered in the ISO 11138-series for biological indicators (BIs).

The requirements and test methods of this part of ISO 11140 apply to all indicators specified in subsequent parts of ISO 11140, unless the requirement is modified or added to by a subsequent part, in which case the requirement of that particular part will apply.

Relevant test equipment is described in ISO 18472.

NOTE 2 Additional requirements for specific test indicators/indicator systems (Type 2 indicators) are given in ISO 11140-3, ISO 11140-4 and ISO 11140-5.

2 Normative references

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

ISO 8601:2004, *Data elements and interchange formats — Information interchange — Representation of dates and times*

ISO 11135:2014, *Sterilization of health-care products — Ethylene oxide — Requirements for the development, validation and routine control of a sterilization process for medical devices*

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

ISO 11137-2:2013, *Sterilization of health care products — Radiation — Part 2: Establishing the sterilization dose*

ISO 11137-3:2006, *Sterilization of health care products — Radiation — Part 3: Guidance on dosimetric aspects*

ISO 11138-1:2006, *Sterilization of health care products — Biological indicators — Part 1: General requirements*

ISO 11138-2:2006, *Sterilization of health care products — Biological indicators — Part 2: Biological indicators for ethylene oxide sterilization processes*

ISO 11138-3:2006, *Sterilization of health care products — Biological indicators — Part 3: Biological indicators for moist heat sterilization processes*

ISO 11138-4:2006, *Sterilization of health care products — Biological indicators — Part 4: Biological indicators for dry heat sterilization processes*

ISO 11138-5:2006, *Sterilization of health care products — Biological indicators — Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization processes*

ISO 11140-3:2007, *Sterilization of health care products — Chemical indicators — Part 3: Class 2 indicator systems for use in the Bowie and Dick-type steam penetration test*

ISO 11140-4:2007, *Sterilization of health care products — Chemical indicators — Part 4: Class 2 indicators as an alternative to the Bowie and Dick-type test for detection of steam penetration*

ISO 11140-5:2007, *Sterilization of health care products — Chemical indicators — Part 5: Class 2 indicators for Bowie and Dick-type air removal tests*

ISO 17665-1:2006, *Sterilization of health care products — Moist heat — Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices*

ISO/TS 17665-2:2009, *Sterilization of health care products — Moist heat — Part 2: Guidance on the application of ISO 17665-1*

ISO/TS 17665-3:2013, *Sterilization of health care products — Moist heat — Part 3: Guidance on the designation of a medical device to a product family and processing category for steam sterilization*

ISO 18472:2006, *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.

3.1
bleed
unintentional lateral migration of the indicator agent beyond the margins within which the indicator agent was applied

3.2
critical process variable
variable identified as being essential to the attainment of sterilization and monitored by the chemical indicator

3.3
endpoint
point of the observed change defined by the manufacturer, occurring after the indicator has been exposed to specified stated values

3.4
exposure period
time from the attainment of the specified exposure conditions to its termination

3.5
graduated response
progressive observable change occurring on exposure to one or more critical process variables allowing assessment of the level achieved

3.6
indicator
combination of the indicator agent and its substrate that reveals change in one or more process variables based on a chemical or physical change resulting from exposure to a process

Note 1 to entry: An indicator intended to be used only in combination with a specific test load is also termed an indicator (both together becoming an indicator system).

Note 2 to entry: See Annex E.

3.7
indicator agent
active substance(s) or combination of substances

Note 1 to entry: See Annex E.

3.8
indicator system
combination of an indicator and a specific test load

3.9
off-set
transfer of indicator agent to a material in intimate contact with the surface of the indicator

3.10
process parameter
specified value for a critical process variable

Note 1 to entry: The specification for a sterilization process includes the process parameters and their tolerances.

[SOURCE: ISO/TS 11139:2006, 2.34]

3.11
saturated steam
water vapour in a state of equilibrium between condensation and evaporation

3.12
stated value
SV
value or values of a critical process variable at which the indicator is designed to reach its endpoint as defined by the manufacturer

3.13
substrate
carrier or support material on to which the indicator agent is applied

Note 1 to entry: See Annex E.

3.14
visible change
change defined by the manufacturer, which can be seen in Type 1 indicators after exposure to one or more critical process variables of the process

4 Categorization

4.1 General

The chemical indicators or indicator systems described in this part of ISO 11140 are for use in three main applications:

a) to allow differentiation between unprocessed and processed items;

b) in specific tests and/or procedures, e.g. the Bowie-Dick test;

c) placement inside individual load items in order to assess attainment of the process parameter(s) and attainment of the respective parameter(s) at the point of placement.

The six indicator types described in the main body of this part of ISO 11140 are categorized according to their performance requirements. Table 1 describes three categories according to their intended use. The chemical indicators within each of these categorizations are further subdivided by the sterilization process for which they are designed to be used. These categorizations have no hierarchical significance. Attainment of the chemical indicator's end point should not be regarded as an indication of attainment of an acceptable sterility assurance level, but rather one of many factors which should be taken into consideration when judging the acceptability of a sterilization process.

Table 1 — Categories according to intended use

Intended use		Type	Category	Description (intended use)
Indicate exposure to a process to allow differentiation between unprocessed and processed items, and/or indicate gross failure of a sterilization process.		1	e1	"Exposure" or process indicator Requirements according to Type 1
Indicators for use in special applications, e.g. Bowie and Dick-type test.		2	s2	"Special" indicator (e.g. Bowie-Dick) Requirements in accordance with ISO 11140-3, ISO 11140–4, and ISO 11140–5.
Indicators to be placed inside individual load items and to assess attainment of the critical process variables at the point of placement.	This indicator only reacts to one critical process variable.	3	i3	"Internal" indicator Single variable indicator Requirements according to Type 3
	This indicator reacts to more than one critical process variable.	4	i4	"Internal" indicator Multivariable indicator Requirements according to Type 4
	This indicator reacts to all critical process variables.	5	i5	"Internal" indicator Integrating indicator Requirements according to Type 5
	This indicator reacts to all critical process variables.	6	i6	"Internal" indicator Emulating indicator Requirements according to Type 6

4.2 Type 1: process indicators

Process indicators shall be designed for use with individual items (e.g. packs, containers) to show that the unit has been directly exposed to the sterilization process and to distinguish between processed and unprocessed items.

4.3 Type 2: indicators for use in specific tests

Type 2 indicators are intended for use in specific test procedures as defined in relevant sterilizer/sterilization standards. The requirements for specific test indicators and indicator systems (Type 2 indicators) are provided in ISO 11140-3, ISO 11140-4, and ISO 11140-5.

4.4 Type 3: single critical process variable indicators

A single critical process variable indicator shall be designed to react to one of the critical process variables (see 5.2) and is intended to indicate exposure to a sterilization process at a stated value (SV) of the chosen critical process variable (see 5.7 and 5.8).

4.5 Type 4: multicritical process variable indicators

A multicritical process variable indicator shall be designed to react to two or more of the critical process variables (see 5.2) and is intended to indicate exposure to a sterilization process at SVs of the chosen critical process variables (see 5.7 and 5.8).

4.6 Type 5: integrating indicators

An integrating indicator shall be designed to react to all critical process variables (see 5.2). The SVs are generated to be equivalent to, or exceed, the performance requirements given in the ISO 11138- series for BIs. The minimum SV shall be related to the minimum values required to achieve sterilization as specified in International Standards ISO 11135, ISO 11137 (all parts), ISO 17665 (all parts), or by local regulatory agencies (see Clauses 11 and 12).

NOTE The SVs demonstrate how the indicator integrates over the temperature range.

4.7 Type 6: emulating indicators

An emulating indicator shall be designed to react to all critical process variables for specified sterilization processes. The SVs are generated from process variables of sterilization processes as specified in International Standards ISO 11135, ISO 11137 (all parts) and ISO 17665 (all parts), or by regulatory agencies (see Clause 13).

5 General requirements

5.1 The requirements given in this clause shall apply to all chemical indicators (CI) unless specifically excluded or amended in a subsequent clause or part of ISO 11140.

NOTE For ease of reading, only the term “indicator” is used hereinafter, although requirements do also apply to indicator systems.

5.2 For the different sterilization processes, the following critical process variables are defined as being critical:

STEAM	Time, temperature, moisture
DRY HEAT	Time and temperature
ETHYLENE OXIDE	Time, temperature, relative humidity and ethylene oxide (EO) concentration
RADIATION	Total absorbed dose
LOW TEMPERATURE STEAM AND FORMALDEHYDE	Time, temperature, moisture and formaldehyde concentration
VAPORIZED HYDROGEN PEROXIDE	Time, temperature, hydrogen peroxide concentration

5.3 The manufacturer shall establish, document and maintain a formal quality system to cover all operations required by this part of ISO 11140.

NOTE ISO 9001 and ISO 13485 describe requirements for quality systems for design, manufacture and testing.

5.4 Each indicator shall be clearly marked with the type of process for which it is intended to be used (see 5.6 and 5.7), and either

a) with a number indicating the type of indicator, i.e. 1 to 6, or

b) with a combination of a letter plus a number to indicate a category, i.e. e1, s2, i3, i4, i5, or i6.

For Type 3, 4, 5 and 6 indicators, each indicator shall be clearly marked with the SVs.

NOTE Some indicator manufacturers might use the category notation to provide additional guidance for the intended use of the chemical indicator.

Where the size or format of the indicator does not permit this information to be stated in a font of six characters per centimetre or larger, the information shall be provided on the label and/or instructions for use.

5.5 The indicator shall comply with the requirements of this part of ISO 11140 for the duration of the shelf-life as specified by the manufacturer (see Annex A).

5.6 Abbreviated descriptions of the process shall be in accordance with the following symbols:

STEAM

— all steam sterilization processes

DRY

— all dry heat sterilization processes

EO

— all ethylene oxide sterilization processes

IRRAD

— all ionizing radiation sterilization processes

FORM

— all low temperature steam and formaldehyde sterilization processes

VH202

— all vaporized hydrogen peroxide sterilization processes

These descriptions are symbols and shall not be translated.

5.7 If the indicator is designed for use in a specific sterilization process, this information shall be stated or coded on the indicator, or within the technical information leaflet.

For example, if the indicator is designated for use in steam at 121 °C for 15 min, it would appear as follows:

STEAM

121 °C 15 min

(See 5.6.)

5.8 Each package of indicators or the technical information leaflet supplied with the package shall provide the following information:

a) the type or category (see Clause 4), process (see 5.6) and intended use (see 5.7) for which the indicator is designed;

b) the critical process variable(s) to which the indicator will respond, and where applicable, their SVs;

c) the change that is intended to occur; and for colour change indicators where the colour change cannot be adequately described, examples of the expected colour range for both changed and unchanged indicators;

d) instructions for use essential to ensure proper functioning of the indicator;

e) the storage conditions, before and after use;

f) the nature of any change and the time period over which it can occur when completely/incompletely changed indicators are stored according to the manufacturer's instructions;

g) any interfering substances that are likely to be encountered, or conditions that are likely to occur, during the intended use of the indicator and which are known to affect adversely the performance of the indicator;

h) any safety precautions required during and/or after use;

i) the manufacturer's or supplier's name and address; where national or regional regulations require, for example in the EU, the EU's authorized representative's name and address;

j) the expiry date, or the manufacturing date plus shelf-life, under the specified storage conditions, expressed in accordance with ISO 8601 (e.g. YYYY-MM);

k) a unique code (e.g. lot number) to provide traceability.

NOTE National or regional regulations could contain additional or different requirements.

5.9 The manufacturer shall retain documentary evidence that the indicator, when used as intended by the manufacturer, does not release any substance known to be toxic in sufficient quantities to cause either a health hazard, or a hazard to the intended properties of the product being sterilized before, during or after the sterilization process for which it is designated.

5.10 If the indicator is designed for use with a specific test load only, this information shall be stated or coded on the indicator, the package of indicators and the technical information leaflet supplied with the package, together with the symbol (see Figure 1). If the size or format of the indicator does not permit affixing of the symbol at a size 5 mm or greater, it is permissible to provide this information only on the package of indicators and the technical leaflet.

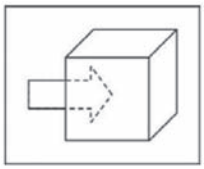


Figure 1 — Symbol for designation for use with a specific test load only

6 Performance requirements

6.1 General

6.1.1 Resistometers (see ISO 18472) are used to characterize the performance of the chemical indicators described in this part of ISO 11140 with the exception of Type 2 indicators (see 4.3). Resistometers allow for precise specification and control of the specific test conditions and cycle sequences in order to produce controlled, repeatable studies of the effect of process parameters on indicators. Resistometers differ from conventional sterilizers; therefore, if conventional sterilizers are used to attempt to duplicate resistometer conditions, erroneous and/or misleading results can occur.

6.1.2 The condition of the indicator after exposure to a sterilization process, during which all the critical process variables met or exceeded the specified level to produce a visible change, graduated response or end point, shall remain unchanged for a period of not less than six months from the date of use, when stored under the conditions specified by the indicator manufacturer.

6.1.3 If incompletely changed indicators deteriorate on storage, either returning to the unchanged condition or slowly completing the change reaction, this information shall be stated in the technical information supplied by the manufacturer [see 5.8 f)].

6.1.4 Indicators for steam processes shall be tested according to the method in 7.4 and the specified values in 11.7. The visible change or end point shall not be reached.

NOTE The dry heat test is designed to ensure that indicators for steam require the presence of steam in order to respond.

6.2 Type 1 indicators

6.2.1 After exposure of the indicator, the visible change shall be clearly observable (see Clause 8).

6.2.2 Migration of the indicator agent through the substrate to the surface opposite the one to which the indicator agent was applied shall not occur before, during or after the sterilization process for which it is designed, when tested according to the method given in 7.2 (see also 5.9).

6.3 Type 2 indicators

Specific requirements for Type 2 indicators and indicator systems are given in ISO 11140-3, ISO 11140-4, and ISO 11140-5.

6.4 Types 3, 4, 5 and 6 indicators

6.4.1 After exposure of the indicator to the SVs of critical process variables, the end point shall be clearly observable.

6.4.2 In use, the indicator agent shall not offset or bleed, penetrate the substrate to which it is applied, or materials in which it is in contact, unless this is a specific design attribute, before, during, or after the sterilization process for which it is designed when tested in accordance with the method in 7.2 (see also 5.9).

7 Test methods

7.1 General

Where appropriate, tests for compliance with the requirements for specific indicator types cited in Clauses 6 to 13 shall be carried out by exposing the indicators to the conditions specified and using equipment complying with ISO 18472, then examining the indicator for compliance.

Specific test methods for radiation indicators are not given here. Performance requirements for radiation indicators are given in 8.5.

NOTE Test equipment and methods for Type 2 indicators are contained in ISO 11140-3, ISO 11140-4 and ISO 11140-5.

7.2 Off-set (transference)

Place a second layer of a similar substrate to that of the indicator in intimate contact with the chemical indicator. Process the indicator in the sterilization process, as stated by the indicator manufacturer. Visually inspect the indicator, its substrate and the second layer of substrate, before and after processing, for compliance with 6.2.2 or 6.4.2.

7.3 Procedure — Steam indicators

7.3.1 Load the indicator on to a suitable sample holder. The sample holder shall not affect the performance of the indicator or impede exposure to critical process variables.

The sample holder shall allow the indicator or indicator system to be directly exposed to the test conditions. Different indicators might require different sample holder designs. Third parties should consult the indicator manufacturer for guidance.

7.3.2 Before initiating a test cycle, the inner surface of the resistometer shall be heated to the required temperature.

7.3.3 With the loaded sample holder in the resistometer, carry out the following sequence of operations:

a) Evacuate the resistometer to 4,5 kPa ± 0,5 kPa within 60 s.

b) Admit steam to the resistometer to obtain the required test temperature in 10 s or less.

c) Maintain the conditions for the required exposure time.

d) At the end of the exposure period, evacuate the resistometer to 10 kPa or less within 60 s, then admit air to ambient pressure.

7.3.4 Immediately remove the indicator from the resistometer to avoid prolonged exposure to critical process variables during testing. Visually examine for compliance and record the result.

7.4 Procedure — Dry heat indicators

7.4.1 Load the indicator on to a suitable sample holder. The sample holder shall not affect the performance of the indicator or impede exposure to critical process variables.

The sample holder shall allow the indicator or indicator system to be directly exposed to the test conditions. Different indicators might require different sample holder designs. Third parties should consult the indicator manufacturer for guidance.

7.4.2 Preheat the resistometer to the required test temperature.

7.4.3 Place the loaded sample holder in the resistometer, close the access port and initiate the process cycle. The time necessary to achieve the required temperature at the surface of the indicator within the resistometer shall not exceed 1 min.

7.4.4 Maintain the conditions for the required exposure time.

7.4.5 At the end of the exposure period, immediately remove the indicators from the resistometer and cool to 100 °C or less in a period not exceeding 1 min.

7.4.6 Visually examine for compliance and record the result.

7.5 Procedure — EO indicators

7.5.1 Load the indicator on to a suitable sample holder. The sample holder shall not affect the performance of the indicator or impede exposure to critical process variables.

The sample holder shall allow the indicator or indicator system to be directly exposed to the test conditions. Different indicators might require different sample holder designs. Third parties should consult the indicator manufacturer for guidance.

7.5.2 Before initiating a test cycle, the sample, sample holder and the inner surface of the resistometer shall be equilibrated to the required temperature.

7.5.3 With the loaded sample holder in the resistometer, carry out the following sequence of operations:

a) Evacuate the resistometer to 10 kPa ± 0,5 kPa.

b) Admit sufficient water vapour to raise the RH in the resistometer to the required level.

c) Admit ethylene oxide to the required ethylene oxide concentration in 1 min or less. For the zero gas exposure cycle, no ethylene oxide shall be admitted. If applicable, the diluent gas shall be admitted

to the working pressure. For the zero gas exposure cycle, the test shall not be carried out in a vessel where traces of ethylene oxide can be present.

d) Maintain these conditions for the required exposure time.

e) Within 1,5 min of the end of the exposure period, reduce the EO concentration surrounding the indicator to a level that will no longer affect the indicator.

7.5.4 Immediately remove the indicator from the resistometer to avoid prolonged exposure to process critical variables during testing. Visually examine for compliance and record the result.

7.6 Procedure — Low temperature steam and formaldehyde indicators

NOTE See Annex D.

7.6.1 Prepare an aqueous solution of formaldehyde at a concentration of 1 mol/l ± 0,01 mol/l. The formaldehyde concentration of this solution shall be established by the use of a validated analytical method.

7.6.2 Preheat the formaldehyde solution to 60 °C ± 0,5 °C.

7.6.3 Load the indicator on to a suitable sample holder. The sample holder shall not affect the performance of the indicator or impede exposure to critical process variables.

The sample holder shall allow the indicator or indicator system to be directly exposed to the test conditions. Different indicators might require different sample holder designs. Third parties should consult the indicator manufacturer for guidance.

7.6.4 Immerse the indicator, loaded on to the sample holder, in the formaldehyde solution.

Ensure that the indicators are completely immersed in the formaldehyde solution and do not float to the surface.

7.6.5 Maintain these conditions for the required exposure time.

7.6.6 At the end of the exposure period, reduce the formaldehyde concentration surrounding the indicator to a level that will no longer affect the indicator, within 1,5 min.

7.6.7 Immediately remove the indicator from the resistometer and visually examine for compliance. Record the result.

7.7 Procedure — Vaporized hydrogen peroxide indicators

7.7.1 Load the indicator on to a suitable sample holder. The sample holder shall not affect the performance of the indicator or impede exposure to critical process variables.

The sample holder shall allow the indicator or indicator system to be directly exposed to the test conditions. Different indicators might require different sample holder designs. Third parties should consult the indicator manufacturer for guidance.

7.7.2 Before initiating a test cycle, the sample, sample holder and the inner surface of the resistometer shall be equilibrated to the required temperature.

7.7.3 With the loaded sample holder in the resistometer, carry out the following sequence of operations:

a) If required, admit sufficient water vapour to raise the RH in the resistometer to the required level.

b) Admit vaporized hydrogen peroxide to the required test condition concentration within less than 2 s (for an exposure time of 0 min, no hydrogen peroxide shall be admitted).

c) Maintain these conditions for the required exposure time.

d) At the end of the exposure period, reduce the hydrogen peroxide concentration surrounding the indicator to a level that will no longer affect the indicator.

7.7.4 Immediately remove the indicator from the resistometer to avoid prolonged exposure to process critical variables during testing. Visually examine for compliance and record the result.

8 Additional requirements for process (Type 1) indicators

8.1 Process indicators printed or applied on to packaging material

Process indicators may be printed on packaging material or presented as self-adhesive labels, pouches, packaging tapes, tags, insert labels, etc.

8.2 Process indicators for steam sterilization processes

Following exposure to the specified test conditions, the process indicator shall perform as shown in Table 2. If the indicator is intended to be used only at 121 °C or 134 °C, then testing may only be conducted at that temperature (see 5.7).

Table 2 — Test and performance requirements for Type 1 process indicators for STEAM

Test environment	Test time	Test temperature	No change or a change that is markedly different from the visible change as specified by the manufacturer	Visible change as specified by the manufacturer
Saturated steam	2,0 min ± 5 s	121 °C (+3/0 °C)	Acceptable result	Unacceptable result
Saturated steam	10,0 min ± 5 s	121 °C (+3/0 °C)	Unacceptable result	Acceptable result
Saturated steam	0,3 min ± 5 s	134 °C (+3/0 °C)	Acceptable result	Unacceptable result
Saturated steam	2 min ± 5 s	134 °C (+3/0 °C)	Unacceptable result	Acceptable result
Dry heat	30 min ± 1 min	140 °C (+2/0 °C)	Acceptable result	Unacceptable result
NOTE The dry heat test is designed to ensure that process indicators for steam require the presence of steam in order to respond (see 6.1.4).				

8.3 Process indicators for dry heat sterilization processes

Following exposure to the specified test conditions, the process indicator shall perform as shown in Table 3.

Table 3 — Test and performance requirements for Type 1 process indicators for DRY

Test environment	Test time	Test temperature	No change or a change that is markedly different from the visible change as specified by the manufacturer	Visible change as specified by the manufacturer
Dry heat	20 min ± 1 min	160 °C +5/0 °C	Acceptable result	Unacceptable result
Dry heat	40 min ± 1 min	160 °C +5/0 °C	Unacceptable result	Acceptable result

8.4 Process indicators for ethylene oxide sterilization processes

Following exposure to the specified test conditions, the process indicator shall perform as shown in Table 4. If the indicator is intended to be used only at 37 °C or 54 °C, then testing may only be conducted at that temperature (see 5.7).

The absence of EO gas test shall be carried out in the absence of EO gas. If a colour change occurs without the apparent presence of ethylene oxide, the complete absence of EO gas might need to be verified.

Table 4 — Test and performance requirements for Type 1 process indicators for EO

Test environment	Test time	Test temperature	Relative humidity (RH)	Gas concentration	No change or a change that is markedly different from the visible change as specified by the manufacturer	Visible change as specified by the manufacturer
Absence of EO gas	90 min ± 1 min	60 °C ± 2 °C	≥85 %	none	Acceptable result	Unacceptable result
EO gas test at:	3 min ± 15 s	37 °C ± 1 °C	60 % ± 10 % RH	600 mg/l ± 30 mg/l	Acceptable result	Unacceptable result
	2 min ± 15 s	54 °C ± 1 °C				
EO gas test at:	25 min ± 15 s	37 °C ± 1 °C	60 % ± 10 % RH	600 mg/l ± 30 mg/l	Unacceptable result	Acceptable result
	20 min ± 15 s	54 °C ± 1 °C				
NOTE The reaction of some ethylene oxide indicators can be impaired by the presence of carbon dioxide or other gas. Where the formulation is such that this could occur, the indicator should be tested in a system employing not less than 80 % carbon dioxide or other gas in admixture with ethylene oxide [see 5.8 g)].						

8.5 Process indicators for radiation sterilization processes

Following exposure to the specified test conditions, the process indicator shall perform as shown in Table 5.

Table 5 — Test and performance requirements for Type 1 process indicators for IRRAD

Test environment	Intensity	Peak wavelength	Absorbed dose	Test time	No change or a change that is markedly different from the visible change as specified by the manufacturer	Visible change as specified by the manufacturer
Ultraviolet radiation	≥ 3,3 W/m^2	254 nm	N/A	120 min ± 5 min	Acceptable result	Unacceptable result
Ionizing radiation	N/A	N/A	1 kGy ± 0,1 kGy	N/A	Acceptable result	Unacceptable result
Ionizing radiation	N/A	N/A	10 kGy ± 1 kGy	N/A	Unacceptable result	Acceptable result
NOTE The ultraviolet radiation test is designed to ensure that the indicator will not respond to non-ionizing radiation such as inadvertent exposure to sunlight. A mercury vapour lamp has been shown to deliver the suitable peak wavelength.						

8.6 Process indicators for low temperature steam and formaldehyde sterilization processes

8.6.1 Following exposure to the specified test conditions, the process indicator shall perform as shown in Table 6.

The absence of formaldehyde test shall be carried out in the absence of formaldehyde. If a colour change occurs without the apparent presence of formaldehyde, complete absence of formaldehyde might need to be verified.

Table 6 — Test conditions and performance requirements for Type 1 process indicators for FORM

Test condition	Test time	Test temperature	Gas concentration	No change or a change that is markedly different from the visible change as specified by the manufacturer	Visible change as specified by the manufacturer
Absence of formaldehyde	90 min ± 1 min	80 °C ± 2 °C	none	Acceptable result	Unacceptable result
Formaldehyde	20 s ± 5 s	60 °C ± 0,5 °C	1,0 mol/l ± 0,01 mol/l	Acceptable result	Unacceptable result
Formaldehyde	15 min ± 15 s	70 °C ± 2 °C	1,0 mol/l ± 0,01 mol/l	Unacceptable result	Acceptable result

8.6.2 For indicators produced for low temperature steam and formaldehyde sterilization cycles operating at temperatures below 55 °C or above 65 °C, the tests described in Table 6 shall be carried out at the maximum temperature and formaldehyde concentration specified by the manufacturer of the indicator.

NOTE The manufacturer might need to perform additional functional tests on the indicator using a low temperature steam and formaldehyde process in order to demonstrate suitability for that particular process (see 5.7, 5.8 and Annex D).

8.7 Process indicators for vaporized hydrogen peroxide sterilization processes

Following exposure to the specified test conditions, the process indicators shall perform as shown in Table 7.

The absence of hydrogen peroxide test shall be carried out in the absence of hydrogen peroxide. If a colour change occurs without the apparent presence of hydrogen peroxide, complete absence of hydrogen peroxide might need to be verified.

Table 7 — Test conditions and performance requirements for Type 1 process indicators for VH202

Test condition	Test time	Test temperature	Gas concentration	No change or a change that is markedly different from the visible change as specified by the manufacturer	Visible change as specified by the manufacturer
Absence of hydrogen peroxide	45 min ± 5 min	50 °C ± 0,5 °C	None	Acceptable result	Unacceptable result
Hydrogen peroxide	7 s ± 1 s	50 °C ± 0,5 °C	2,3 mg/l ± 0,4 mg/l	Acceptable result	Unacceptable result
Hydrogen peroxide	6 min ± 1 s	50 °C ± 0,5 °C	2,3 mg/l ± 0,4 mg/l	Unacceptable result	Acceptable result

9 Additional requirements for single critical process variable (Type 3) indicators

9.1 Single critical process variable indicators shall be designed for one of the critical process variables to be monitored, as listed in 5.2.

9.2 Single critical process variable indicators tested at the SV (test point 1) shall reach the end point (see Table 8).

9.3 Single critical process variable indicators tested at the SV minus the tolerance (test point 2) shall not reach the end point (see Table 8).

10 Additional requirements for multicritical process variable (Type 4) indicators

10.1 Multicritical process variable indicators shall be designed for two or more of the critical process variables to be monitored, as listed in 5.2.

10.2 Multicritical process variable indicators tested at the SV (test point 1) shall reach the end point (pass condition) (see Table 8).

10.3 Multicritical process variable indicators tested at the SV minus the combined tolerances (test point 2) shall not reach the end point (fail condition) (see Table 8).

10.4 Multicritical process variable indicators for low temperature steam and formaldehyde, tested at the time and temperature SVs in dry heat, i.e. absence of moisture, but with all other parameters at the SVs, shall not reach the end point (see Table 8).

NOTE The dry heat test is designed to ensure that multicritical process variable indicators for low temperature steam and formaldehyde require the presence of steam in order to respond.

Table 8 — Test and performance requirements for Type 3 and Type 4 indicators

Sterilization process	Test point[a]	Test time	Test temperature	Sterilizing agent concentration mg/l	RH %
Steam	1	SV	SV		
	2	−25 %	−2 °C		
Dry heat	1	SV	SV		
	2	−25 %	−5 °C		
Ethylene oxide	1	SV	SV	SV	>30
	2	−25 %	−5 °C	−25 %	>30
Low temperature steam and formaldehyde	1	SV	SV	SV	
	2	−25 %	−3 °C	−20 %	
Vaporized hydrogen peroxide	1	SV	SV	SV	
	2	−25 %	−3 °C	−20 %	

NOTE For examples of testing multicritical process variable (Type 4) indicators, see Annex B.

[a] Test point 1: The indicator, when tested at the SV, shall reach its end point. Test point 2: The indicator, when tested at all SVs minus the combined tolerances, shall not reach its end point.

11 Additional requirements for steam integrating (Type 5) indicators

11.1 Integrating indicators for steam shall be designed for all critical process variables and reach an end point indicating exposure to sterilization conditions at defined critical process variables within the relevant tolerances given in Table 9.

11.2 Integrating indicators for steam shall have SVs at 121 °C and 135 °C and at one or more equally spaced temperature points in the range of 121 °C to 135 °C.

11.3 Integrating indicators for steam tested at all of the SVs (Test point 1) shall reach the end point (pass condition). (See 11.2).

11.4 Integrating indicators for steam tested at all of the SVs minus the time and temperature tolerances (test point 2) shall not reach the end point (fail condition) (see Table 9).

11.5 The SVs for time at 121 °C and 135 °C shall be specified and shall not be less than 16,5 min at 121 °C and 1,2 min at 135 °C.

11.6 The integrating indicator temperature coefficient shall be determined from the slope of the curve created by plotting log SV and/or SV (determined) versus temperature. The integrating indicator temperature coefficient shall be not less than 10 °C and not more than 27 °C, and the correlation coefficient of the curve established by least squares linear regression analysis of the data shall be not less than 0,9.

NOTE See Figure C.1. The manufacturer's SVs can be used to determine the integrating indicator temperature coefficient.

11.7 Integrating indicators for steam exposed to dry heat at 140 °C ± 2 °C for 30 min ± 1 min shall not reach the end point.

NOTE The dry heat test is designed to ensure that integrating indicators for steam require the presence of steam in order to respond.

11.8 The manufacturer shall state clearly any factors of which he is aware that can adversely affect the efficacy of the sterilization process but which are not detectable by the indicator, or not detectable in a manner that will give assurance of satisfactory attainment of that critical process variable [see 5.8 g)].

12 Additional requirements for ethylene oxide integrating (Type 5) indicators

12.1 Integrating indicators for ethylene oxide shall be designed for all critical process variables and reach an end point indicating exposure to sterilization conditions at defined critical process variables within the relevant tolerances given in Table 9.

12.2 Integrating indicators for ethylene oxide shall have SVs for time at 37 °C and 54 °C that shall be specified and that shall not be less than 75 min at 37 °C and 30 min at 54 °C.

12.3 Upon exposure to the SVs for time, temperature, and 60 % relative humidity (RH) ± 10 % RH, but in the absence of ethylene oxide, the integrating indicator shall not reach the end point (fail condition).

12.4 The manufacturer shall state clearly any factors of which he is aware that can adversely affect the efficacy of the sterilization process but which are not detectable by the indicator, or not detectable in a manner that will give assurance of satisfactory attainment of that critical process variable [see 5.8 g)].

NOTE Some regulatory authorities require that demonstration of the performance of an integrating indicator be conducted in parallel with an appropriate BI.

Table 9 — Test and performance requirements for Type 5 indicators

Sterilization process	Test point[a]	Test time	Test temperature[b]	Gas concentration mg EO/l	RH %
Steam	1	SV	SV		
	2	−15 %	1° C		
Ethylene oxide	1	SV	SV	600 ± 30	60 ± 10
	2	−20 %	SV	600 ± 30	60 ± 10

NOTE For an example of testing integrating (Type 5) indicators, see Annex B.

[a] Test point 1: The indicator, when tested at all the SVs, shall reach its end point (pass condition). Test point 2: The indicator, when tested at all SVs minus the time and temperature, shall not reach its end point (fail condition).

[b] There are at least 3 SVs for temperature required for steam Type 5 indicators; at 121 °C, 135 °C, and at one or more equally spaced temperatures in between the first two. There are 2 SVs for temperature for ethylene oxide Type 5 indicators, at 37 °C and 54 °C.

13 Additional requirements for emulating (Type 6) indicators

13.1 Emulating indicators shall be designed for all critical process variables for the process listed in 5.2 and reach an end point indicating exposure to a sterilization process at defined critical process variables within the relevant tolerances given in Table 10.

13.2 Emulating indicators tested at the SV (test point 1) shall reach the end point (pass condition).

13.3 Emulating indicators tested at the SV minus the combined tolerances (test point 2) shall not reach the end point (fail condition).

13.4 Emulating indicators for steam exposed to dry heat at 140 °C ± 2 °C for 30 min ± 1 min shall not reach the end point (see 6.1.4).

NOTE The dry heat test is designed to ensure that emulating indicators for steam require the presence of steam in order to respond.

13.5 The manufacturer shall state clearly any factors of which he is aware that can adversely affect the efficacy of the sterilization process but which are not detectable by the indicator, or not detectable in a manner that will give assurance of satisfactory attainment of that critical process variable [see 5.8 g)].

Table 10 — Test and performance requirements for Type 6 indicators

Sterilization process	Test point[a]	Test time min	Test temperature	Gas concentration mg/l	RH %
Steam	1	SV	SV		
	2	−6 %	−1 °C		
Dry heat	1	SV	SV		
	2	−20 %	−1 °C		
Ethylene oxide	1	SV	SV	SV	>30
	2	−10 %	−2 °C	−15 %	>30

NOTE For an example of testing emulating (Type 6) indicators, see Annex B.

[a] Test point 1: The indicator, when tested at the SV, shall reach its end point (pass condition). Test point 2: The indicator, when tested at all SVs minus the combined tolerances, shall not reach its end point (fail condition).

Annex A
(normative)

Method for demonstrating shelf-life of the product

A.1 Product testing to determine shelf-life shall be performed in accordance with a written protocol. The protocol shall be established before the commencement of the study. The protocol shall specify requirements for sample size, sampling method and data evaluation.

NOTE National or regional regulation can contain additional or different requirements. Compliance with quality management International Standards (in particular ISO 9001 and ISO 13485) could require additional or different provisions.

A.2 Product samples shall be stored in their normal packaging at, or above, the maximum temperature and RH recommended for storage. These conditions shall be controlled and monitored.

A.3 All performance attributes shall maintain the original specifications throughout the shelf-life.

A.4 All results of the storage trial shall be retained for a period of the shelf-life plus one year from completion of the trial. After this period, a summary report shall be retained for as long as the product is commercially available.

Annex B
(informative)

Examples of testing indicators

B.1 Example of testing single critical process variable (Type 3) indicator for dry heat processes

An indicator with a stated value at 160 °C.

The manufacturer will denote this indicator's performance by identifying the SV of 160 °C (see 5.8). When tested at 160 °C (SV, test point 1), using the test methods specified in this part of ISO 11140, the indicator should reach its end point. When tested at 155 °C (SV minus tolerance, test point 2) (see NOTE) the indicator should not reach its end point. There is no requirement to test the indicator between test point 1 and test point 2; however, if the indicator were to be tested between test point 1 and test point 2, it could produce an ambiguous result (i.e. the indicator could reach its end point or it could not reach its end point).

NOTE With reference to Table 8, the tolerance on the test temperature for a Type 3 single-critical process variable indicator is −5 °C. Therefore, 160 °C − 5 °C = 155 °C, which becomes test point 2.

B.2 Example of testing multicritical process variable indicator for steam processes

An indicator with a stated value at 121 °C and 15 min.

The manufacturer will denote this indicator's performance by identifying the SVs of 121 °C and 15 min (see 5.7 and 5.8). The manufacturer can also give additional SVs for the product at different temperatures and times. When tested at 121 °C for 15 min (SV, test point 1), using the test methods specified in this part of ISO 11140, the indicator should reach its end point. When tested at 119 °C for 11 min 15 s (SV minus both temperature and time tolerances or test point 2) the indicator should not reach its end point (see NOTE). There is no requirement to test the indicator between test point 1 and test point 2; however, if the indicator were to be tested between test point 1 and test point 2, it could produce an ambiguous result (i.e. the indicator could reach its end point or not reach its end point).

NOTE With reference to Table 8, the tolerance on the test temperature for a Type 4 multicritical process variable indicator is −2 °C, and the tolerance on the test time is −25 % (25 % of 15 min is 3 min 45 s). Therefore, 121 °C − 2 °C = 119 °C and 15 min minus 3 min 45 s equals 11 min 15 s, which becomes test point 2.

B.3 Example of testing emulating (Type 6) indicators for steam processes

An indicator with a SV at 134 °C and 3,5 min.

The manufacturer will denote this by clearly identifying the SVs of 134 °C and 3,5 min (see 5.7 and 5.8). The manufacturer can also give additional SVs for the product at different temperatures and times. When tested at 134 °C for 3,5 min (SV, test point 1), using the test methods specified in this part of ISO 11140, the indicator should reach its end point. When tested at 133 °C for 3 min 17 s (SV minus both tolerances or test point 2) the indicator should not reach its end point (see NOTE). There is no requirement to test the indicator between test point 1 and test point 2; however, if the indicator were to be tested between

test point 1 and test point 2, it could produce an ambiguous result (i.e. the indicator could reach its end point or not reach its end point).

NOTE With reference to Table 10, the tolerance on the test temperature for a Type 6 emulating indicator is –1 °C, and the tolerance on the test time is –6 % (6 % of 3 min and 30 s is 12,6 s [rounded up to 13 s]). Therefore, 134 °C – 1 °C = 133 °C and 3 min 30 s minus 13 s equals 3 min 17 s, which becomes test point 2.

Annex C
(informative)

Rationale for the requirements for integrating indicators and the link to the requirements for biological indicators specified in ISO 11138 (all parts) and microbial inactivation

C.1 Steam

C.1.1 General

Integrating indicators are designed to respond in a similar manner to that of a biological indicator (BI) when exposed to the critical process variables of a sterilization process. For the purposes of this part of ISO 11140, the performance of integrating indicators is linked to the minimum requirements for a BI for moist heat sterilization as defined in ISO 11138-3. The following provides background information and a detailed rationale for the requirements for Type 5 integrating indicators specified in Clause 11.

C.1.2 Background information

ISO 11138-3 specifies that a BI for moist heat sterilization processes should have a D_{121} of not less than 1,5 min, a minimum population of 10^5 and a *z*-value greater than 6. The *z*-value for many species of *Geobacillus stearothermophilus* is often nearer to 10 and the *z*-value for self-contained BIs can often be even greater than this (often 20 or more). Theoretical calculations relating to validation of moist heat processes, e.g. F_0, normally use a z of 10 in the equation.[6]

The performance of a BI can also be defined by the survivor kill window (SKW) which, at 121 °C and based on the minimum values specified above, would typically be: survives 4,5 min and is killed in 13,5 min.

The SKW is calculated from:

$$\text{Survival time} = (\log \text{P} - 2) \times D_{121} \quad \text{(C.1)}$$

$$\text{Kill time} = (\log \text{P} + 4) \times D_{121} \quad \text{(C.2)}$$

where

log is the log to the base 10 of the number;

P is the nominal population;

D_{121} is the decimal reduction time at 121 °C, in min.

The kill time calculated from the equation represents a 9 log reduction in the initial population, i.e.:

$$\log \text{P} + 4 = \log\left(1 \times 10^5\right) + 4 = 9 \quad \text{(C.3)}$$

It should also be borne in mind that:

— ISO 14161[6] shows that an 8 log reduction would yield 1 % positives and a 9 log reduction zero positives when determined by most probable number enumeration techniques;

— terminal sterilization processes will normally aim to deliver an inactivation level of at least 10^{-6} in populations of microorganisms.[13]

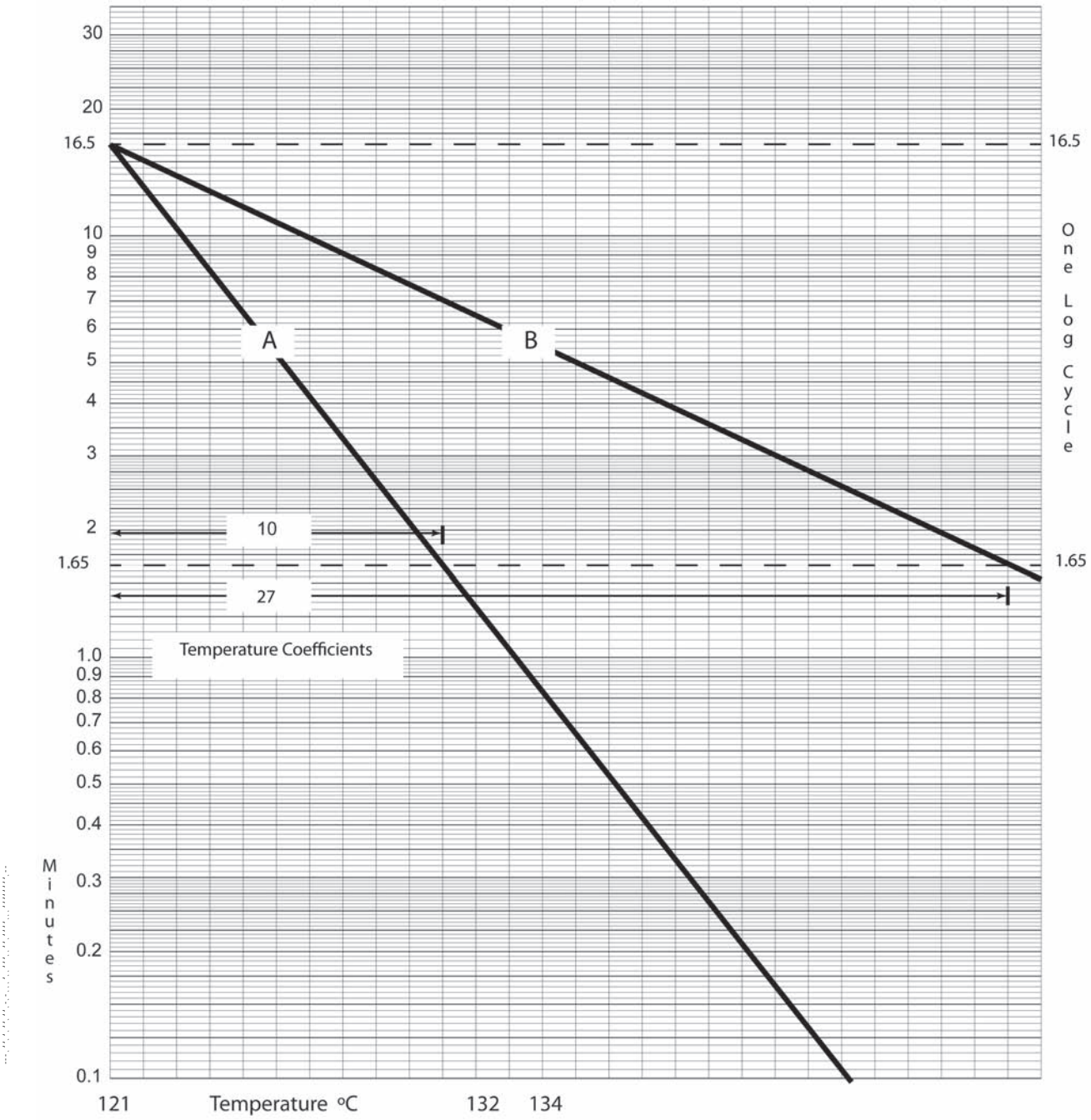


Figure C.1 — Type 5 plot

After plotting SVs at three temperature points, the slope of the line connecting the points should be between lines A and B. This is an example where the 121 °C SV is 16,5 min; if the 121 °C SV is greater than 16,5 min, lines A and B would move upwards and the intersection would be at the 121 °C SV actually utilized.

C.2 The link between the integrating indicator SV and BI inactivation

C.2.1 Accept or pass (end point attained) criteria

Based on the above information it would be necessary to expose a BI with a D_{121} = 1,5 min and a population of 10^5 to a temperature of 121 °C for 16,5 min in order to achieve an inactivation level of 10^{-6}.

Thus

$$\left(\log 10^{5} - \log 10^{-6}\right) \times 1{,}5 = 16{,}5\,\text{min}. \tag{C.4}$$

Thus, for a Type 5 integrating indicator the minimum SV, i.e. the time at which the end point is reached at 121 °C, is required to be not less than 16,5 min (see 11.5). By requiring a minimum SV of 16,5 min a direct relationship is established between the integrating indicator end point and a satisfactory inactivation level in an equivalent BI and therefore the objective of a terminal sterilization process.

In the case where the manufacturer specifies a SV at 121 °C greater than 16,5 min, a greater inactivation level will have been achieved (and therefore a greater safety factor) by the time the indicator reaches its end point. Nevertheless, when tested, the integrating indicator should reach or exceed its end point when exposed for a time equal to the SV (see 11.5).

The above-mentioned represents the pass or accept condition for the integrating indicator.

C.2.2 Reject or fail (end point not attained) criteria

Regarding the fail condition, theoretically a single BI will show no growth when the exposure time is sufficient to reduce the population to less than one surviving organism. However, when actual BIs are in use the exposure time has to be greater than that specified above because of the natural variation associated with biological systems. Typically, if 50 or more BIs are tested then growth will be observed in 1 % of the sample when an exposure time which reduces the population to a theoretical level of 10^{-2} is employed.[6] In practice, exposure times which reduce the population to a theoretical survival level of 10^{-3} to 10^{-4} is required to eliminate all positives for growth.[6] This latter value is reflected in the equation used to calculate the SKW. Thus an exposure period equal to (log P + 4) × D_{121} is used to calculate the kill time, i.e. a further 4 log reduction past the point of one surviving organism per unit, i.e. 10^{-4}. Thus it can be anticipated that some BIs would show positive for growth at a 10^{-2} exposure level but none at a 10^{-4} exposure level. Adopting these criteria for the definition of the "fail" response in an integrating indicator:

At 121 °C with a population of 10^5 and a D-value of 1,5, a 7 log reduction is required to reach the 10^{-2} level. The exposure time required for this is:

$$\left(\log \mathrm{P} + 2\right) \times D = 10{,}5\,\text{min}. \tag{C.5}$$

However, the requirements for an integrating indicator is that it should not reach its end point, i.e. show a Reject or fail response, when exposed to saturated steam at a temperature of 120 °C for a time equal to 85 % of its SV (see 11.4). For an integrating indicator with the minimum SV at 121 °C of 16,5 min this equates to an exposure time of 14 min at 120 °C. This relates to a BI response as follows:

If the BI has a D_{121} of 1,5 and a z of 10 °C, then the *D*-value at 120 °C will be:

$$D_{120} = D_{121} \times 10^{-(T1-Tref/10)} \quad (C.6)$$

where

D_{120} is the *D*-value at 120 °C;

D_{121} is the *D*-value at 121 °C;

T_1 is the working temperature (in this case 120 °C);

T_{ref} is the reference temperature (in this case 121 °C).

$$D_{120} = 1,5 \times 10^{-(120-121/10)} \quad (C.7)$$

$$= 1,88 \text{ min}$$

Assuming the BI has a population of 10^5, then the log reduction achieved by exposing the BI at 120 °C for 14,025 min would be:

$$14,025 / 1,88 = 7,427 \quad (C.8)$$

i.e. a 7,4 log reduction.

Thus the log survivor level in the BI would be:

$$5 - 7,427 = -2,427 \quad (C.9)$$

therefore the surviving population will be

$$1 \times 10^{-2.427} \quad (C.10)$$

$$= 3,7 \times 10^{-3}$$

A value where the detection of survivors would be expected to be periodically but infrequently observed.

Thus, the integrating indicator should not reach its end point when exposed to dry saturated steam at 120 °C for 14 min and this relates to a BI survival level of approximately 4×10^{-3}.

In comparison to a BI, the SVs of the integrating indicator providing a pass or accept result (end point attained) is related to the time required to achieve an 11 log reduction in population (SV_T = 121 °C, SV_t = 16,5 min). In contrast the integrating indicator should show a fail or reject result (end point not attained) when exposed to conditions required to achieve an approximately 7 log reduction in population.

C.3 The response of the integrating indicator at different temperatures and the relationship to the biological indicator's *z*-value

The above discussion considers the relationship between a BI and an integrating indicator's response at one base temperature, 121 °C. However, it is also necessary to consider other temperatures at which sterilization is carried out. In general, sterilization in a health care setting will be carried out at temperatures between 121 °C and 134 °C, usually with a tolerance (see ISO 17665 (all parts) and Reference [10]). In consideration of this there is a requirement that the integrating indicators SV at 135 °C should be not less than 1,2 min (see 11.5) and that SVs are specified at least one other temperature within the range 121 °C to 135 °C (see 11.2).

The *z*-value of a microbial population is the change in temperature required to bring about a tenfold change in *D* value. ISO 11138-3 specifies that the *z*-value for a moist heat BI should be not less than 6. However, as explained above, this is in many cases more likely to be nearer to 10 and in some self-contained BIs can be higher. The requirements for integrating indicators specify that the temperature coefficient should be not less than 10 °C and not more than 27 °C (see 11.6).

Since the SV for an integrating indicator is related to the *D*-value of a BI as shown above, the theoretical *z*-value of the integrating indicator, z_i, will be similarly related to the *z*-value of a BI. Compliance with this requirement should be demonstrated by determining the end point (and hence confirming any specified SVs) of the integrating indicator at a number of fixed temperatures covering the range 121 °C to 135 °C (see 11.2). The z_i for the integrating indicator can then be determined by plotting log end point versus exposure temperature. The slope of the curve is the z_i value. In order to assess the linearity of the curve it would be necessary to conduct end point determinations at at least three, but preferably more than three, fixed points, and for this reason the clause requires end point determinations at 121 °C, 135 °C and at one or more further temperatures selected from within this range. The final requirement is that the z_i curve should be linear and so a correlation coefficient of 0,9 is specified (see 11.6).

Having established that the z_i-value falls within the range 10 °C to 27 °C and therefore that any SVs have a direct link to the performance of a BI, it is necessary to ensure that the integrating indicator reaches its end point when exposed to the maximum temperature in the specified range, i.e. 135 °C after 1,2 min exposure.

The integrating indicator is then shown to yield a reject or fail response (end point not achieved) when exposed to the SVs for temperature, *T*, less 1 °C (134 °C) and time, *t*, less 15 % (1 min) (see 11.4).

Tests to ensure the correct response for pass and fail conditions are then carried out at a selection of temperatures falling within the range 121 °C to 135 °C (see 11.2).

In terms of a BI response at a temperature of 135 °C:

The integrating indicator has an SV at 135 °C of not less than 1,2 min and a z_i = 12 (see Figure C.1).

For a BI with a D_{121} of 1,5, a population of 10^5 and a z = 10 °C:

$$D_{135} = 1{,}5 \times 10^{-(135-121/10)} \tag{C.11}$$

= 0,06 min.

For the pass or accept condition an 11 log reduction is achieved in:

11 × 0,06 min = 0,66 min. Since the integrating indicator is required to have an end point of not less than 1,2 min at 135 º C the log reduction in a BI would be:

$$\text{LR} = t/D_{135} \tag{C.12}$$

LR = 1,2/0,06;

LR = 20.

Thus the BI survival level would be:

$$\log \text{P} - \text{LR} = 5 - 20 = 1 \times 10^{-15} \tag{C.13}$$

For the fail or reject condition not less than a 7 log reduction is required after exposure to 134 °C for 1 min:

$$D_{134} = 1{,}5 \times 10^{-(134-121/10)} \tag{C.14}$$

D_{134} = 0,075;

LR = t/D;

LR = 1,0/0,075;

LR = 13,3.

Thus the BI survival level would be;

$$\log P - LR = 5 - 13,3 = -8,3 \quad \text{(C.15)}$$

$= 4,6 \times 10^{-9}$.

C.4 Ethylene oxide

ISO 11138-2 specifies that an ethylene oxide (EO) BI should have a D-value of not less than 2,5 min at 54 °C, 60 % relative humidity (RH), and 600 mg EO/litre with a maximum population of 10^6. The performance of the BI can be defined by the survivor/kill window which would typically be: survives at least 10 min, killed in no more than 25 min at 54 °C based on the minimum values specified above. The survival/kill window can be calculated from:

$$\text{Survival time} = (\log P - 2) \times D \quad \text{(C.16)}$$

$$\text{Kill time} = (\log P + 4) \times D \quad \text{(C.17)}$$

It is common to aim for a final probability of survival in a population of microorganisms of 10^{-6} before a product can be labelled sterile.

Based on the above information it would be necessary to expose a BI with a D = 2,5 and a population of 10^6 to a temperature of 54 °C, 600 mg EO/litre, and 60 % RH for 30 min in order to achieve an inactivation level of 10^{-6}.

Thus:

$$\left(1 \times 10^6 - 1 \times 10^{-6}\right) \times 2,5 = 30,0 \text{ min}. \quad \text{(C.18)}$$

Thus for a Type 5 integrating indicator the minimum SV, i.e. the time at which the end point is reached, should not be less than 30,0 min in order to ensure an adequate inactivation factor has been achieved in an equivalent BI.

In the case when the SV at 54 °C, 600 mg EO/litre, and 60 % RH is greater than 30,0 min, then clearly a greater inactivation level will have been achieved by the time the indicator reaches its end point. Nevertheless, the integrating indicator should reach or exceed its end point when exposed for a time equal to the SV.

The above-mentioned represents the pass condition. Now to consider the fail condition.

Theoretically, a single BI will show no growth when the exposure time is sufficient to reduce the population to less than one surviving organism. However, when actual BIs are in use, the exposure time has to be greater than that specified above because of the natural variation associated with biological systems. Typically if 50 or more BIs were tested, then an exposure time which reduces the population to a theoretical level of less than 10^0 would be required to eliminate most positives for growth. This is reflected in the determination of the survival/kill characteristics where an exposure period of (log P + 4) × D is used to define the kill time, i.e. a further 4 log reduction past the point of one surviving organism per unit, i.e. 10^{-4}. Thus we might expect to see some BIs showing positive for growth at a 10° exposure level but none at a 10^{-4} exposure level. Adopting these criteria for the definition of the fail response in an integrating indicator:

At 54 °C, 600 mg EO/litre, and 60 % RH with a population of 10^6 and a *D*-value of 2,5, an 8 log reduction is required to reach the 10^{-2} level. The exposure time required for this is:

$$(\log \mathrm{P} + 2) \times D = 20\,\mathrm{min}. \tag{C.19}$$

Thus the ethylene oxide integrating indicator in biological terms should not reach its end point when exposed to 600 mg EO/litre and 60 % RH at 54 °C for 20 min or less. However, according to this part of ISO 11140, the fail condition is to be reached at 80 % of the SV time to provide an additional safety margin. The manufacturer's SV for time at 54 °C may be greater than 30,0 min; therefore, the fail condition should be reached at the time of (SV × 0,8). The fail condition at 37 °C should also be reached at the time of (SV × 0,8).

Annex D
(informative)

Rationale for the liquid-phase test method for low temperature steam and formaldehyde indicators

D.1 General

In order to test indicators in a reproducible manner, specific test equipment (resistometers) and methods are used. For the low temperature steam and formaldehyde process, it is extremely difficult to create a stable formaldehyde gas concentration in a resistometer, since defined amounts of formaldehyde injected into a vessel will dissolve in the small amounts of water (condensate) droplets present. The concentration of formaldehyde in this water is 1 000 times to 10 000 times higher in concentration than in the gas phase, depending on the temperature.[17]

It is for this reason that ISO 11138-5 utilizes a liquid-phase test method where the formaldehyde concentration is clearly defined and allows reproducible conditions.

D.2 The low temperature steam and formaldehyde process

Even with constant steam conditions and stable formaldehyde gas concentrations, the sterilization process depends heavily upon the design of the sterilizer chamber and the nature of the load. The low temperature steam and formaldehyde sterilization process can be considered simplistically in two steps:

— as in steam sterilization processes, an aqueous condensate film is created on the surface of the load; this condensation will occur very rapidly;

— since the concentration of formaldehyde at equilibrium between the gas and liquid phases is extremely different (1:1 000 to 1:10,000), the time taken for this equilibrium to occur will be relatively long. In practical situations, this can require a time frame from 10 min up to 2 h.

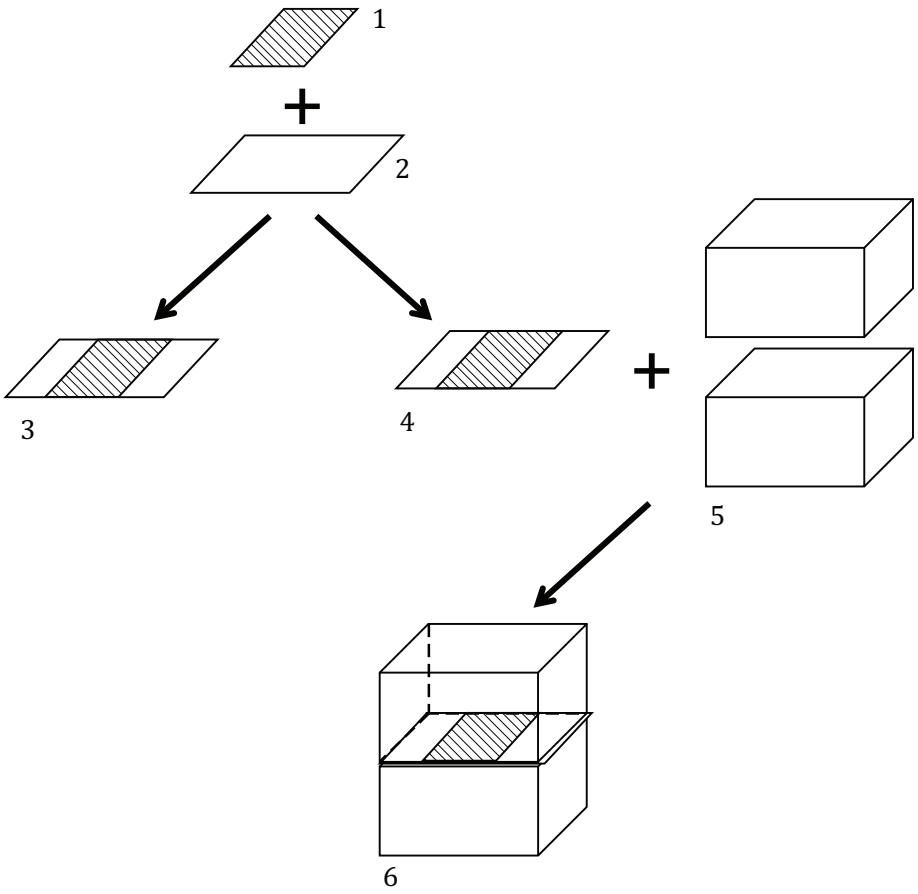
The lethality of the sterilization process thus depends heavily on the formaldehyde concentration in the liquid phase, i.e. surface condensate. It can be very difficult to determine in absolute terms the time taken for these equilibrium conditions to be achieved.

D.3 Chemical indicators

For chemical indicators that do not have water soluble components, it is desirable to utilize a similar liquid-phase test method, for the reasons given above. However, for chemical indicators which do have water-soluble components, the indicators could have to be tested and calibrated in the gas phase, using BIs in conformance to ISO 11138-5, as a process reference, and in a low temperature steam and formaldehyde sterilizer. This will also apply to indicators other than Type 1 indicators.

Annex E
(informative)

Relationship of indicator and indicator system components



Key

1 indicator agent
2 substrate
3 indicator (to be used as such)
4 indicator (to be used with a specific test load)
5 specific test load
6 indicator system

Figure E.1 — Relationship of indicator and indicator system components

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1) Withdrawn and replaced by ISO 11135.

2) Withdrawn and replaced by ISO 11137.

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