INTERNATIONAL STANDARD

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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

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Foreword

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

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

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 11140-1 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

This second edition cancels and replaces the first edition (ISO 11140-1:1995 and ISO 11140-1:1995/Amd.1:1998), 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 2: Test equipment and methods*

— *Part 3: Class 2 indicators for steam penetration test sheets*

— *Part 4: Class 2 indicators for steam penetration test packs*

— *Part 5: Class 2 indicators for air removal test sheets and packs*

NOTE ISO 11140-2 is to be replaced by ISO 18472, *Sterilization of health care products — Biological and chemical indicators — Test equipment*.

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, steam-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) 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. Subsequent parts of this International Standard specify the particular requirements for chemical indicators for particular applications and for defined tests of particular sterilization processes used in health care, including industry. The use of the indicators specified in this part of ISO 11140 are described in ISO 15882, EN 285, ISO 11135 and ISO 17665.

Resistometers (see ISO 18472) are used to characterize the performance of the chemical indicators described in this part of ISO 11140. Resistometers allow for precise variation of the specific test conditions and cycle sequences in order to produce controlled physical studies. Resistometers differ from conventional sterilizers; therefore, if conventional sterilizers are used to attempt to duplicate resistometer conditions, erroneous and/or misleading results may occur.

Sterilization of health care products — Chemical indicators —

Part 1:
General requirements

WARNING — The use of this part of ISO 11140 may involve hazardous materials, operations and equipment. This part of ISO 11140 does not purport to address to all the safety problems associated with its use. It is the responsibility of the user of this part of ISO 11140 to establish appropriate safety and health practise and determine the applicability of regulatory limitations prior to use.

1 Scope

1.1 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 variables required for a sterilization process. They are not dependent for their action on the presence or absence of a living organism.

NOTE Biological test systems are regarded as those tests 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).

1.2 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 Additional requirements for specific test indicators (Class 2 indicators) are given in ISO 11140-3, ISO 11140-4 and ISO 11140-5.

2 Normative references

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

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

ISO 11138 (all parts), *Sterilization of health care products — Biological indicator systems*

ISO 11607, *Packaging for terminally sterilized medical devices*

ISO 18472[1)], *Sterilization of health care products — Biological and chemical indicators — Test equipment*

1) To be published.

3 Terms and definitions

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

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

3.2
critical variable
parameters identified as being essential to the sterilization process (and requiring monitoring)

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

3.4
graduated response
progressive observable change occurring on exposure to one or more process variables allowing assessment of the level achieved

3.5
indicator
combination of the indicator agent and its substrate in the final form in which it is intended to be used (see Annex E)

NOTE An indicator system in combination with a specific test load is also termed an indicator.

3.6
indicator agent/indicator reagent
active substance(s) or combination of substances (see Annex E)

3.7
indicator system
combination of the indicator agent and its substrate subsequently intended to be used in combination with a specific test load

3.8
off-set
transfer of indicator agent to a material in intimate contact with the surface of the indicator

3.9
parameter
specified value for a process variable

3.10
penetration
migration of the indicator agent through the substrate to the surface opposite the one to which the indicator agent was applied

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 variable at which the indicator is designed to reach its endpoint as defined by the manufacturer

3.13
substrate
carrier or support material onto which the indicator is applied (see Annex E)

3.14
variable
condition within a sterilization process, changes that alter microbicidal effectiveness

3.15
visible change
change defined by the manufacturer, which can be seen in the indicator after exposure to one or more critical variables of the process

NOTE Visible change is used to describe the response of Class 1 process indicators.

4 Classification

4.1 General

In subsequent parts of ISO 11140, indicators are classified by their intended use. The chemical indicators described in this part of ISO 11140 are classified into six groups. The chemical indicators within each of these classifications are further subdivided by the sterilization process for which they are designed to be used. The classification structure used is solely to denote the characteristics and intended use of each type of indicator when used as defined by the manufacturer. This classification has no hierarchical significance.

4.2 Class 1: process indicators

Process indicators are intended for use with individual units (e.g. packs, containers) to indicate that the unit has been directly exposed to the sterilization process and to distinguish between processed and unprocessed units. They shall be designed to react to one or more of the critical process variables (see Tables 1 to 6).

4.3 Class 2: indicators for use in specific tests

Class 2 indicators are intended for use in specific test procedures as defined in relevant sterilizer/sterilization standards.

NOTE The requirements for specific test indicators (Class 2 indicators) are provided in other parts of ISO 11140.

4.4 Class 3: single variable indicators

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

4.5 Class 4: multi-variable indicators

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

4.6 Class 5: integrating indicators

Integrating indicators shall be designed to react to all critical variables. The SVs are generated to be equivalent to, or exceed the performance requirements given in the ISO 11138 series for BIs (see Clauses 11, 12 and 13).

4.7 Class 6: emulating indicators

Emulating indicators are cycle verification indicators which shall be designed to react to all critical variables for specified sterilization cycles. The SVs are generated from the critical variables of the specified sterilization process.

5 General requirements

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

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

- STEAM — Time, temperature and water (as delivered by saturated steam)
- DRY HEAT — Time and temperature
- ETHYLENE OXIDE — Time, temperature, relative humidity and ethylene oxide (EO) concentration
- IRRADIATION — Total absorbed dose
- STEAM-FORMALDEHYDE — Time, temperature, water (as delivered by saturated steam) and formaldehyde concentration
- VAPOURIZED HYDROGEN PEROXIDE — Time, temperature, hydrogen peroxide concentration, and, if applicable, plasma .

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), with the the class of indicator (see Clause 4), and for Class 3, 4, 5 and 6 indicators, with the the SVs.

Where the size or format of the indicator does not permit this information to be stated in a font of 6 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 steam/formaldehyde sterilization processes

VH2O2

— all vaporized hydrogen peroxide sterilization processes

These descriptions are symbols and should not be translated.

5.7 If the indicator is designed for use in specific sterilization cycles, this information shall be stated or coded on the indicator, e.g.

STEAM

121 °C 15 min

(See 3.12 and 5.6.)

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

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

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

c) the class (see Clause 4), process (see 5.6) and intended use (see 5.7) for which the indicator is designed;

d) the storage conditions, before and after use;

e) 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);

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

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

h) 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;

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

j) the manufacturer's or supplier's name and address;

k) the nature of any change that can occur when completely/incompletely changed indicators are stored according to the manufacturer’s instructions.

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.

6 Performance requirements

6.1 General

6.1.1 The condition of the indicator after exposure to a sterilization process, during which all the variables met or exceeded the specified level to produce a visible change, graduated response or endpoint, 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.

6.1.2 Incompletely changed indicators can deteriorate on storage, either returning to the unchanged condition or slowly completing the change reaction. If such deterioration can occur, this information should be stated in the technical information supplied by the manufacturer [see 5.8 k)].

6.2 Class 1 Indicators

6.2.1 The visible change that occurs after exposure of the indicator shall be clearly observable and shall be either from light to dark, dark to light, or shall be from one colour to a distinctly different colour (see Clause 8).

6.2.2 When printed on to single-use packaging material complying with ISO 11607, the indicator agent shall not bleed or offset to such an extent that it compromises the utility of the indicator or presents a hazard for the use of the packaging material. Penetration 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 Class 2 indicators

Specific requirements for Class 2 indicators are given in Parts 3, 4 and 5 of ISO 11140.

6.4 Classes 3, 4, 5 and 6 indicators

6.4.1 The endpoint which occurs after exposure of the indicator to the SVs of critical variables shall be clearly observable and shall be either from light to dark, dark to light or shall be from one colour to a distinctly different colour.

6.4.2 The indicator agent shall not off-set or penetrate the substrate to which it is applied, or materials with which it is in contact 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).

7 Test methods

7.1 General

Tests for compliance with the requirements given in Clauses 6 and 7 to 14 of this part of ISO 11140 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 irradiation indicators are not given. Performance requirements are given in 8.5.

NOTE Test equipment and methods for Class 2 indicators are contained in Parts 3, 4 and 5 of ISO 11140.

7.2 Off-set (transference)

Place a second layer of a similar substrate to that of the indicator in intimate contact with the indicator reagent. 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.

The sample holder should allow the indicator to be exposed to the test conditions in the manner specified by the indicator manufacturer. Different indicators may require different sample holder designs. 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 2 min [manufacturers of chemical indicators may choose to specify the use of a different depth of vacuum; if they do so, this information shall be included in each package of indicators, or in the technical information leaflet supplied with each package (see 5.8)].

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 1 min, then admit air to ambient pressure.

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

The indicator should be removed from the resistometer as quickly as possible to avoid prolonged exposure to process-critical variables during testing.

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.

The sample holder should allow the indicator to be exposed to the test conditions in the manner specified by the indicator manufacturer. Different indicators may require different sample holder designs. 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 required 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 samples from the resistometer and cool to 100 °C or less in a period not exceeding 1 min.

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

The indicator should be removed from the resistometer as quickly as possible to avoid prolonged exposure to process-critical variables during testing.

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.

The sample holder should allow the indicator to be exposed to the test conditions in the manner specified by the indicator manufacturer. Different indicators may require different sample holder designs. 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 [manufacturers of chemical indicators may choose to specify the use of a different depth of vacuum; if they do so, this information shall be included in each package of indicators, or in the technical information leaflet supplied with each package (see 5.8)].

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 should be admitted. If applicable, the diluent gas should be admitted to the working pressure. This test should not be carried out in a vessel where traces of ethylene oxide may be present.)

d) Maintain these conditions for the required exposure time.

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

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

The indicator should be removed from the resistometer as quickly as possible to avoid prolonged exposure to process-critical variables during testing.

7.6 Procedure — Steam-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 Pre-heat 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.

The sample holder should allow the indicator to be exposed to the test conditions in the manner specified by the indicator manufacturer. Different indicators may require different sample holder designs. 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. Visually examine the indicator for compliance. Record the result.

The indicator should be removed from the formaldehyde solution as quickly as possible.

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.

The sample holder should allow the indicator to be exposed to the test conditions in the manner specified by the indicator manufacturer. Different indicators may require different sample holder designs. 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 should 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 and visually examine for compliance. Record the result.

The indicator should be removed from the resistometer as quickly as possible to avoid prolonged exposure to process-critical variables during testing.

8 Additional requirements for process (Class 1) indicators

8.1 Process indicators printed or applied onto 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 1.

Table 1 — Test and performance requirements for Class 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	3,0 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	0,5 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.				

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 2.

Table 2 — Test and performance requirements for Class 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 3.

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

Table 3 — Test and performance requirements for Class 1 process indicators for EO

Test environment	Test time	Test temperature	RH %	Gas concentration mg/l	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:	5 min ± 15 s	30 °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:	30 min ± 15 s	30 °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 h)].

8.5 Process indicators for irradiation sterilization processes

Following exposure to the specified test conditions, the process indicator shall perform as shown in Table 4.

Table 4 — Test and performance requirements for Class 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/m²	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 steam-formaldehyde sterilization processes

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

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

Table 5 — Test conditions and performance requirements for Class 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 steam-formaldehyde sterilization cycles operating at temperatures below 55 °C or above 65 °C, the tests described in Table 5 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 formaldehyde process in order to demonstrate suitability for that particular process (see 5.7, 5.8 and Annex D).

8.7 Process indicators for vaporised hydrogen peroxide sterilization processes

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

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

Table 6 — Test conditions and performance requirements for Class 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 Test at:	45 min ± 5 min	50 °C ± 0,5 °C	None	Acceptable result	Unacceptable result
	45 min ± 5 min	27 °C ± 0,5 °C	None		
Hydrogen peroxide Test at:	7 s ± 1 s	50 °C ± 0,5 °C	2,3 mg/l ± 0,4 mg/l	Acceptable result	Unacceptable result
	10 s ± 1 s	27 °C ± 0,5 °C	2,3 mg/l ± 0,4 mg/l		
Hydrogen peroxide Test at:	6 min ± 1 s	50 °C ± 0,5 °C	2,3 mg/l ± 0,4 mg/l	Unacceptable result	Acceptable result
	10 min ± 1 s	27 °C ± 0,5 °C	2,3 mg/l ± 0,4 mg/l		

9 Additional requirements for single variable (Class 3) indicators

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

9.2 Single variable indicators tested at the SV (Test Point 1) shall reach the endpoint (see Table 7).

9.3 Single variable indicators tested at the SV minus the tolerance (Test Point 2) shall not reach the endpoint (see Table 7).

10 Additional requirements for multi-variable (Class 4) indicators

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

10.2 Multi-variable indicators tested at the SV (Test Point 1) shall reach the endpoint (see Table 7).

10.3 Multi-variable indicators tested at the SV minus the combined tolerances (Test Point 2) shall not reach the endpoint (see Table 7).

10.4 Multi-variable indicators for steam and steam-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 endpoint (see Table 7).

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

Table 7 — Test and performance requirements for Class 3 and Class 4 indicators

Sterilization process	Test point [a]	Test time	Test temperature	Sterilizing agent concentration mg/l	RH %
Steam	1	SV 0 %	SV 0 °C		
	2	SV – 25 %	SV – 2 °C		
Dry heat	1	SV 0 %	SV 0 °C		
	2	SV – 25 %	SV – 5 °C		
Ethylene oxide	1	SV 0 %	SV 0 °C	SV 0 %	> 30
	2	SV – 25 %	SV – 5 °C	SV – 25 %	> 30
Steam formaldehyde	1	SV 0 %	SV 0 °C	SV 0 %	
	2	SV – 25 %	SV – 3 °C	SV – 20 %	

NOTE For examples of testing multi-variable (Class 4) indicators, see Annex B.

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

11 Additional requirements for steam integrating (Class 5) indicators

NOTE See Annex C.

11.1 Integrating indicators for steam processes shall undergo an endpoint indicating exposure to a steam sterilization cycle at defined variables within the relevant tolerances given in 11.2 to 11.10.

11.2 The SV at 121 °C shall be specified and shall be not less than 16,5 min.

11.3 Upon exposure to saturated steam at 121 °C ± 0,5 °C to a time equal to the SV at 121 °C, the integrator shall have reached or exceeded its endpoint (pass condition).

11.4 Upon exposure to saturated steam at 121 °C ± 0,5 °C to a time equal to 63,6 % of the SV at 121 °C, the integrator shall not have reached its endpoint (fail condition).

11.5 The endpoint shall be determined in dry saturated steam at 135 °C ± 0,5 °C and at one or more equally spaced temperature test points in the range of 121 °C to 135 °C. The time to reach the endpoint at these temperatures shall be the SVs determined and given by the manufacturer (see 5.8).

11.6 The integrator temperature coefficient shall be determined from the slope of the curve created by plotting log SV and/or SV (determined) versus temperature.

NOTE The manufacturer's SVs at these additional temperatures can be used to determine the integrator temperature coefficient.

11.7 The integrator temperature coefficient shall be not less than 6 °C and not more than 14 °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.

11.8 Upon exposure to saturated steam at 135 °C ± 0,5 °C to a time equal to 63,6 % of the SV at 135 °C (determined) the integrator shall not reach its endpoint (fail condition).

11.9 Upon exposure to saturated steam at the temperatures as used in 11.5 at a time equal to 63,6 % of the SV (determined) the integrator shall not reach its endpoint (fail condition).

11.10 Upon exposure to dry heat at 137 °C $^{+1}_{0}$ °C for 30 min $^{+1}_{0}$ min the endpoint shall not be reached.

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

NOTE Some regulatory authorities require that demonstration of the performance of a steam integrator be conducted in parallel with an appropriate BI.

12 Additional requirements for dry heat integrating (Class 5) indicators

12.1 Integrating indicators for dry heat processes shall undergo a clearly detectable change indicating exposure to a dry heat sterilization cycle at defined variables within the relevant tolerances given in 12.2 to 12.9.

12.2 The SV at 160 °C shall be specified and shall be greater than 30 min.

12.3 Upon exposure to dry heat at 160 °C ± 1,5 °C to a time equal to the SV at 160 °C, the integrator shall have reached or exceeded its endpoint (pass condition).

12.4 Upon exposure to dry heat at 160 °C ± 1,5 °C to a time equal to 63,6 % of the SV at 160 °C, the integrator shall not have reached its endpoint (fail condition).

12.5 The endpoint shall be determined in dry heat at 180 °C ± 1,5 °C and at one or more of the following temperatures: 140 °C ± 1,5 °C; 170 °C ± 1,5 °C. The time to reach the endpoint at these temperatures shall be the SVs (determined) given by the manufacturer.

12.6 The integrator temperature coefficient shall be determined from the slope of the curve created by plotting log SV and/or SV (determined) versus temperature.

NOTE The manufacturer's SVs at these additional temperatures can be used to determine the integrator temperature coefficient.

12.7 The integrator temperature coefficient shall be not less than 20 °C and not more than 40 °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.

12.8 Upon exposure to dry heat at 180 °C ± 1,5 °C to a time equal to 63,6 % of the SV at 180 °C (determined), the integrator shall not reach its endpoint (fail condition).

12.9 Upon exposure to dry heat at the temperatures of 140 °C ± 1,5 °C or 170 °C ± 1,5 °C as used in 12.5 at a time equal to 63,6 % of the SV (determined), the integrator shall not reach its endpoint (fail condition).

12.10 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 detected by the indicator, or not detected in a manner that will give assurance of satisfactory attainment of that critical variable [see 5.8 h)].

NOTE Some regulatory authorities require that demonstration of the performance of a dry heat integrator be conducted in parallel with an appropriate BI.

13 Additional requirements for ethylene oxide integrating (Class 5) indicators

NOTE See Annex C.

13.1 Integrating indicators for ethylene oxide processes shall undergo a clearly detectable change indicating exposure to an ethylene oxide sterilization cycle at defined variables within the relevant tolerances given in 13.2 to 13.5.

13.2 The SV at 54 °C ± 0,5 °C, 600 mg EO/l ± 30 mg EO/l and 60 % relative humidity (RH) ± 10 % RH shall be at least 30 min, and/or the SV at 37 °C ± 0,5 °C, 600 mg EO/l ± 30 mg EO/l and 60 % relative humidity ± 10 % RH shall be at least 90 min (see 5.7 and 5.8).

13.3 Upon exposure to an ethylene oxide process of 54 °C ± 0,5 °C, 600 mg EO/l ± 30 mg EO/l and 60 % RH ± 10 % RH to a time equal to the SV, and upon exposure to 37 °C ± 0,5 °C, 600 mg EO/l ± 30 mg EO/l and 60 % RH ± 10 % RH to a time equal to the SV, the integrator shall reach its endpoint (pass condition).

13.4 Upon exposure to an ethylene oxide process of 54 °C ± 0,5 °C, 600 mg EO/l ± 30 mg EO/l and 60 % RH ± 10 % RH to a time equal to 66,7% of the SV, and upon exposure to an ethylene oxide process of 37 °C ± 0,5 °C, 600 mg EO/l ± 30 mg EO/l and 60 % RH ± 10 % RH to a time equal to 66,7 % of the SV, the integrator shall not reach its endpoint (fail condition).

13.5 Upon exposure to 54 °C ± 0,5 °C and 60 % RH ± 10 % RH to a time equal to the SV in the absence of ethylene oxide, and upon exposure to 37 °C ± 0,5 °C and 60 % RH ± 10 % RH to a time equal to the SV in the absence of ethylene oxide, the integrator shall not reach its endpoint (fail condition).

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

13.6 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 detected by the indicator, or not detected in a manner that will give assurance of satisfactory attainment of that critical variable [see 5.8 h)].

14 Additional requirements for emulating (Class 6) indicators

14.1 Emulating indicators shall be designed for all critical variables for the process listed in 5.2 and undergo an endpoint indicating exposure to a sterilization cycle at defined variables within the relevant tolerances given in Table 8.

14.2 Emulating indicators tested at the SV (Test Point 1) shall reach the endpoint (pass condition).

14.3 Emulating indicators tested at the SV minus the combined tolerances (Test Point 2) shall not reach the endpoint (fail condition).

14.4 Emulating indicators for steam exposed to dry heat at 137 °C $^{+1}_{0}$ °C for 30 minutes $^{+1}_{0}$ minutes shall not reach the endpoint.

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

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

Table 8 — Test and performance requirements for Class 6 indicators

Sterilization process	Test point [a]	Test time min	Test temperature	Gas concentration mg/l	RH %
Steam	1	SV 0 %	SV 0 °C		
	2	SV – 6 %	SV – 1 °C		
Dry heat	1	SV 0 %	SV 0 °C		
	2	SV – 20 %	SV – 1 °C		
Ethylene oxide	1	SV 0 %	SV 0 °C	SV 0 %	> 30
	2	SV – 10 %	SV – 2 °C	SV – 15 %	> 30

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

[a] Test point 1: The indicator, when tested at the SV, shall reach its endpoint (pass condition).

Test point 2: The indicator, when tested at all SVs minus the combined tolerances, shall not reach its endpoint (fail condition).

Annex A
(informative)

Method for demonstrating shelf life of the product

A.1 Product testing to determine shelf life should be performed in accordance with a written protocol. The protocol should be established before the commencement of the study. The protocol should 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 standards (in particular ISO 9001 and ISO 13485) could require additional or different provisions.

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

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

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

Annex B
(informative)

Examples of testing indicators

B.1 Example of testing single variable (Class 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 Stated Value (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 shall reach its endpoint. When tested at 155 °C (SV minus tolerance, Test Point 2) (see NOTE) the indicator shall not reach its endpoint. 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 may produce an ambiguous result (i.e., the indicator may reach its endpoint or it may not reach its endpoint).

NOTE With reference to Table 7, the tolerance on the test temperature for a Class 3 single-variable indicator is – 5 °C. Therefore, 160 °C – 5 °C = 155 °C, which becomes Test Point 2.

B.2 Example of testing multi-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 may 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 shall reach its endpoint. When tested at 119 °C for 11 min 15 s (SV minus both temperature and time tolerances or Test Point 2) the indicator shall not reach its endpoint (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 may produce an ambiguous result (i.e., the indicator could reach its endpoint or not reach its endpoint).

NOTE With reference to Table 7, the tolerance on the test temperature for a Class 4 multi-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 integrating (Class 5) indicators

Test procedures and background for integrating indicators are found in Annex C.

B.4 Example of testing emulating (Class 6) indicators for steam processes

An indicator with a stated value 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 may 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 shall reach its endpoint. When tested at 133 °C for 3 min 17 s (SV minus both tolerances or Test Point 2) the indicator shall not reach its endpoint (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 may produce an ambiguous result (i.e., the indicator could reach its endpoint or not reach its endpoint).

NOTE With reference to Table 8, the tolerance on the test temperature for a Class 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 (BIs) specified in ISO 11138 and microbial inactivation

C.1 Steam

C.1.1 Introduction

Integrating indicators are designed to respond in a similar manner to that of a BI when exposed to the critical variables of a sterilization process. For the purposes of this document, 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 the Class 5 integrating indicators specified in Clause 11.

C.1.2 Background information

ISO 11138-3 specifies that a BI for moist heat sterilization processes shall have a D_{121} of not less than 1,5 minutes, a minimum population of 1×10^5 and a z value greater than 6. The z value for many species of *Geobacillus stearothermophilus* is often nearer to 10 (ISO 14161). Theoretical calculations relating to validation of moist heat processes, e.g. F_o, normally use a z of 10 (Pflug 1999[18]).

The performance of a BI may 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 mins and is killed in 13,5 min. The SKW is calculated from:

Survival time = $(\log P - 2) \times D_{121}$;

Kill time = $(\log P + 4) \times D_{121}$;

where

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

P is the nominal population;

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

C.2 The link between the integrator stated value (SV) and BI inactivation

In order to achieve an inactivation of at least 1×10^{-6} in populations of micro-organisms, it would be necessary to expose a BI with a D_{121} = 1,5 min and a population of 1×10^5 to a temperature of 121 °C for 16,5 min.

Thus

$(\log 10^5 - \log 10^{-6}) \times 1{,}5 = 16{,}5$ min

Thus for a Class 5 integrator the minimum SV, i.e., the time at which the endpoint is reached at 121 °C, is required to be not less than 16,5 min. By requiring a minimum SV of 16,5 min, a direct relationship is established between the integrator endpoint 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 an 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 endpoint. Nevertheless, when tested, the integrator should reach or exceed its endpoint when exposed for a time equal to the SV.

The above represents the pass or accept condition for the integrator.

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 an exposure time that reduces the population to a theoretical level of less than 10^{-2} would be required to eliminate any positives for growth (ISO 14161). The determination of the SKW provides an indication of how much more exposure time is required. Thus an exposure period of $(\log P + 4) \times D$ 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., 1×10^{-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:

$$(\log P + 2) \times D = 10{,}5 \text{ min}$$

Thus, the integrating indicator should not reach its endpoint when exposed to dry saturated steam at 121 °C for 10,5 min. However, the manufacturer's SV at 121 °C may be greater than 16,5 min, in which case the exposure conditions required to create a failure or reject response in the integrator must be linked to the manufacturer's SV and be at least 10,5 min. Using 10,5 min as a baseline for failure and 16,5 min as a baseline for a pass,

$$\frac{10{,}5}{16{,}5} = 0{,}636$$

Thus, for an indicator with an SV greater than 16,5 min, the fail condition at which it is tested should be an exposure time which is 63,6 % of its SV. Thus, the indicator must show a fail or reject response when exposed to dry saturated steam at 121 °C for 63,6 % of the SV.

In comparison to a BI, the SV of the integrator is related to the time required to achieve an 11 log reduction in population. 63,6 % of the SV is related to the time required to achieve a 7 log reduction in population. Thus the D value of a BI complying with ISO 11138-3 is related to the SV of the integrator through the following:

$$(\log P + 6) \times D = \text{SV}$$

$$(5 + 6) \times 1{,}5 = 16{,}5$$

i.e., an 11 log reduction in population to an inactivation level of 1×10^{-6}.

Therefore

$$D = \frac{\text{SV}}{(\log P + 6)} = \frac{\text{SV}}{11}$$

In a BI, survivors will be observed when the exposure time (survivor time, ST) is

$$(\log P + 2) \times D = \text{ST}$$

substituting for D:

$$(\log P + 2) \times \frac{\text{SV}}{11} = \text{ST}$$

Now

$$\log P + 2 = 7$$

Therefore

$$7 \times \frac{\text{SV}}{11} = \text{ST}$$

Therefore

$$\text{SV} \times \frac{7}{11} = \text{SV} \times 0{,}636 = \text{ST}$$

Thus, for the integrator the survivor time, i.e., the fail response in the integrator, and hence the time when the endpoint must not be reached, is 63,6 % of the SV.

C.3 Comparison with the requirements for integrating indicators in ISO 11140-1:1995

ISO 11140-1:1995 requires that an integrator shall show a fail response when exposed to its SV minus 1 °C on temperature and minus 15 % on time. For an integrator with a SV of 16,5 min at 121 °C, a fail condition should be observed when the indicator is exposed to 120 °C for 14,025 min. Relating this to a BI response, if the BI has a D_{121} of 1,5 and a z of 10 °C, then the D at 120 °C will be:

$$D_{120} = D_{121} \times 10^{-[(T_1 - T_{\text{ref}})/10]}$$

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);

$$D_{120} = 1{,}5 \times 10^{-[(120 - 121)/10]} = 1{,}88 \text{ min.}$$

Assuming the BI has a population of 1×10^5, then the log reduction achieved by exposing the BI at 120 °C for 14,025 min would be:

$$\frac{14{,}025}{1{,}88} = 7{,}427$$

i.e., a 7,4 log reduction.

