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Sterilization of health care products — Chemical indicators —

Part 4: Class 2 indicators for steam penetration test packs

Stérilisation des produits de santé — Indicateurs chimiques —

Partie 4: Indicateurs de classe 2 pour paquets prépliés servant à l'essai de pénétration de la vapeur

Reference number
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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 3.

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 part of ISO 11140 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 11140-4 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

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*

Annexes A, B, C, D, E, F, G, H, I, J, K and L form a normative part of this part of ISO 11140.

Introduction

The Bowie and Dick test was conceived as a test for successful air removal from high vacuum porous load sterilizers [1]. A successful Bowie and Dick test indicates rapid and even penetration of steam into the test pack. The presence of air within the pack, due either to an inefficient air removal stage, an air leak during this stage or non-condensable gases in the steam supply, are circumstances which can lead to failure of the test. The result of the test may also be affected by other factors which inhibit steam penetration. The test does not necessarily demonstrate either achievement of the required temperature or maintenance of that temperature for the required time to achieve sterilization.

Failure of the Bowie and Dick test is not conclusive proof that the fault in the sterilizer is due to air retention, air leakage or non-condensable gases, and it may be necessary to investigate other causes of failure.

The Bowie and Dick test is a performance test for steam sterilizers for wrapped goods and porous loads. As such it is performed during the demonstration of conformance of steam sterilizers to EN 285 and as a routine test of performance in ISO 11134. The test procedure is described in EN 285.

A test pack for the Bowie and Dick test consists of two components:

a) a small standardized test load and

b) a chemical indicator system to detect the presence of steam (see ISO 11140-3 and ISO 11140-4).

The Bowie and Dick test as originally described [1] utilized huckaback towels as the material for the test load. The test described in EN 285 uses cotton sheets for this purpose.

Indicators intended as an alternative to the Bowie and Dick Test use different materials for the test load and employ indicator systems specifically formulated for use with the defined test load. This part of ISO 11140 specifies the performance of the indicator system in combination with the test load with which it is intended to be used. The test load may be presented with the indicator system already incorporated and intended for single-use, or it may be intended for multiple use with a new indicator system to be inserted prior to each use.

The indicator for which the performance is specified in this part of ISO 11140 is intended to indicate when steam penetration has been inadequate. The performance of the indicator specified in this part of ISO 11140 should be equivalent, but not necessarily identical, to the performance obtained in a Bowie and Dick test as described in EN 285. Equivalence should be regarded as providing a similar response to steam penetration with any differences being predictable and such that the necessary level of assurance of satisfactory steam penetration is provided. An indicator meeting this specification is not intended to identify which of the potential causes of poor steam penetration was responsible for the failure indicated by the test.

Sterilization of health care products — Chemical indicators —

Part 4:
Class 2 indicators for steam penetration test packs

1 Scope

This part of ISO 11140 specifies the performance requirements for a Class 2 indicator to be used as an alternative to the Bowie and Dick test for steam sterilizers for wrapped health care goods (instruments, etc. and porous loads).

An indicator complying with this part of ISO 11140 incorporates a specified material which is used as a test load. This test load may, or may not, be re-usable. This part of ISO 11140 does not specify requirements for the test load, but specifies the performance of the indicator system in combination with the test load with which it is intended to be used. The indicator specified in this part of ISO 11140 is intended to identify poor steam penetration but does not indicate necessarily the cause of this poor steam penetration.

This part of ISO 11140 does not include test methods to establish the suitability of these indicators for use in sterilizers in which the air removal stage does not include evacuation below atmospheric pressure.

NOTE The Bowie and Dick Test is performed to demonstrate conformance of a steam sterilizer for wrapped health care goods to EN 285 and may be used as a routine test of performance of such a sterilizer (see ISO 11134). The test procedure is described in EN 285.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 11140. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 11140 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 5-1, *Photography — Density measurements — Part 1: Terms, symbols and notations*.

ISO 5-3, *Photography — Density measurements — Part 3: Spectral conditions*.

ISO 5-4:1995, *Photography — Density measurements — Part 4: Geometric conditions for reflection density*.

ISO 187, *Paper, board and pulps — Standard atmosphere for conditioning and testing and procedure for monitoring the atmosphere and conditioning of samples*.

ISO 2248, *Packaging — Complete, filled transport packages — Vertical impact test by dropping*.

ISO 9001:1994, *Quality systems — Model for quality assurance in design, development, production, installation and servicing*.

ISO 10012-1, *Quality assurance requirements for measuring equipment — Part 1: Metrological confirmation system for measuring equipment*.

ISO 11140-1:1995, *Sterilization of health care products — Chemical indicators — Part 1: General requirements*.

IEC 60584-2:1982 + A1:1989, *Thermocouples — Part 2: Tolerances*.

IEC 60751:1983 + A1:1986, *Industrial platinum resistance thermometer sensors*.

EN 285:1996, *Sterilization — Steam sterilizers — Large sterilizers*.

3 Terms and definitions

For the purposes of this part of ISO 11140, the following terms and definitions apply.

3.1
air pocket
concentration of residual, induced or injected air or non-condensable gases in the standard test pack

3.2
chamber reference temperature
temperature measured at a defined reference point within the steam exposure apparatus

NOTE The defined reference point is usually located in the chamber drain or active chamber discharge.

3.3
defined end-point
visible change occurring after exposure to the specified variable(s) at a level equal to or greater than that specified for the indicator

3.4
dry saturated steam
steam with a dryness value between 0,9 and 1,0 and a non-condensable gas content of not more than 3,5 % (volume fraction) when determined by the methods given in EN 285

3.5
exposure time
period for which the chamber reference temperature lies within the sterilization temperature band

3.6
graduated response
progressive visible change, occurring on exposure to one or more process variables, which allows assessment of the level achieved

3.7
indicator
indicator system in the form in which it is intended to be used

3.8
indicator reagent
active ingredient or combination of ingredients before conversion into the indicator

[ISO 11140-1:1995, 3.4]

3.9
indicator system
combination of the indicator reagent and its substrate

3.10
pre-assembled pack
indicator in which the indicator system is incorporated into the test load during the manufacturing process and which is supplied ready for use

3.11
reference fault period
period of 30 s commencing when the chamber reference temperature attains the set operating temperature

3.12
sterilization temperature
minimum temperature of the sterilization temperature band

NOTE The use of the word “sterilization” within this and subsequent definitions is not intended to imply that sterilizing conditions will take place under the test cycle conditions.

3.13
sterilization temperature band
range of temperatures from the sterilization temperature to the maximum allowable temperature which may prevail throughout the load during the holding time

NOTE These temperatures are usually stated in whole degrees Celsius.

3.14
temperature depression
thermodynamic temperature difference in kelvin given by (chamber reference temperature, in degrees Celsius) minus (temperature in the standard test pack, in degrees Celsius)

3.15
test equilibration time
time elapsed after the chamber reference temperature attains the set operating temperature until the temperature within the standard test pack is the same as the chamber reference temperature, within the limits of accuracy of the temperature-measuring equipment

3.16
user-assembled pack
indicator in which the user combines the indicator system with the test load prior to use

4 General requirements

4.1 The requirements of ISO 11140-1 apply.

4.2 Test samples shall be conditioned in accordance with ISO 187 prior to testing for performance.

4.3 Compliance with the requirements of this part of ISO 11140 shall be determined by establishing conformity with the requirements of clause 6.

4.4 The indicator shall have sufficient strength to withstand steam sterilization and subsequent handling.

Compliance shall be tested in accordance with annex A.

4.5 Test cycles for demonstrating compliance with the requirements of this part of ISO 11140 shall employ sub-atmospheric, trans-atmospheric and super-atmospheric air removal stages (see Table 1 and clauses B.1, B.2 and B.3 respectively in annex B) except when the indicator, or indicator system, is intended solely for use with one type of air removal system, in which case only the specified air removal system needs to be used during compliance testing.

4.6 A thermometric recording instrument(s) shall be used in conjunction with temperature sensors to record the temperatures measured in the locations specified in the tests described in this part of ISO 11140. The temperature-measuring equipment used in all test methods for demonstrating compliance with this part of ISO 11140 shall meet the following requirements.

a) Temperature sensors shall be either platinum resistance and comply with Class A of IEC 60751:1983 + A1:1986 or thermocouple and comply with one of the tables of tolerance class 1 of IEC 60584-2:1982 and A1:1989.

b) The performance characteristic of the temperature sensor shall not be affected by the environment in which it is used, e.g. pressure, steam or vacuum.

c) The temperature sensors shall have a response time in water of $\pi_{90} \leqslant 0{,}5$ s.

d) The temperature measured by all temperature sensors when immersed in a temperature source at a temperature known to within ± 0,1 K, and within the sterilization temperature band, shall not differ by more than 0,5 K.

e) The recording instrument shall record the temperature from a minimum of 12 sensors. The sampling duration shall not exceed 2,5 s. All data sampled shall be used for the interpretation of results.

f) The scale range shall include 0 °C to 150 °C. For analog instruments, the minor mark interval shall not exceed 1 K, the resolution shall be not less than 0,5 K and the chart speed shall be not less than 15 mm/min. Digital instruments shall register and record in increments of not more than 0,1 K.

g) The limit of error of the recording instrument between 0 °C and 150 °C (excluding temperature sensors) shall not exceed 0,25 % when tested in an ambient temperature of (20 ± 3) °C. The additional error due to change in the environmental temperature shall not exceed 0,04 K/K.

h) Calibration shall be carried out using a working or reference standard which is traceable to a national standard or a primary standard. The instrument shall have a valid test certificate.

5 Indicator system format

5.1 When the indicator system is one in which the indicator reagent is distributed on a substrate, it shall meet the following requirements.

a) The indicator reagent shall be distributed to cover not less than 30 % of the surface area of the substrate. The distance between adjacent areas of indicator reagent shall not exceed 20 mm.

The pattern of indicator reagent distribution should permit clear interpretation of the colour change.

b) The substrate shall have a colour which is uniform to visual observation.

c) The indicator system shall have a difference in relative reflectance density of not less than 0,3 between the colour of the substrate and either the changed indicator or unchanged indicator as specified by the manufacturer.

Compliance shall be tested in accordance with annex C.

5.2 When the indicator system depends on migration of the indicator reagent to demonstrate change, the pattern of indicator reagent distribution before and after use shall permit clear interpretation of the result.

5.3 When the indicator system is intended for use with a user-assembled pack, the indicator system shall permit writing in permanent ink to be made legibly on both processed and unprocessed materials. Those markings made before processing shall remain legible after processing.

5.4 When the indicator system is provided by the manufacturer already incorporated into the test load, the material of either the indicator or the indicator system, as appropriate, shall permit writing to be made after processing.

6 Performance requirements

6.1 The indicator, when tested in combination with the test load specified by the manufacturer, shall show a uniform colour change complying with 5.1 c) after exposure to dry saturated steam at 134 °C for 3,5 min, or at 121 °C for 15 min or at any other time/temperature combination specified by the manufacturer when the temperature tolerance shall be $\binom{+1,5}{0}$ °C and the time tolerance shall be ± 5 s.

Compliance shall be tested in accordance with annex D using the steam exposure apparatus. The steam exposure apparatus shall be operated with the standard test cycles described in annex B as shown in Table 1.

Indicators intended for use only with specific air removal cycles shall be tested with those specific cycles only (see ISO 11140-1).

NOTE Indicators intended to be used over a wide range of sterilization temperatures, e.g. both for cycles operating at 121 °C and for those operating at 134 °C, may not give the same depth or intensity of colour change at both temperatures. This should be regarded as in compliance if:

a) all other performance characteristics required by this part of ISO 11140 are met and

b) the nature of the colour change is unambiguously defined in the instructions for use (see ISO 11140-1).

6.2 The indicator shall show no colour change, an incomplete, or an uneven colour change when exposed to a test cycle previously demonstrated to produce a reference fault condition, whether the system used to produce the fault depends on air retention, air leak or air injection. The test cycles used to generate the reference fault conditions shall be as shown in Table 1. The chamber reference temperatures and holding times shall include 134 °C for 3,5 min, or 121 °C for 15 min or other time/temperature combination specified by the manufacturer (see 6.1) when the temperature tolerance shall be $\binom{+1,5}{0}$ °C and the time tolerance shall be ± 5 s.

Compliance shall be tested in accordance with annex E.

Compliance of the fault condition reproducibility shall be demonstrated in accordance with annex F.

Table 1 — Schedule of test cycles to be used

Test condition	Standard test cycle of annex B		
	B.1	B.2	B.3
"Pass" cycle (see 6.1)	✓	✓	✓
"Fail" cycle — modified air removal stage (see 6.2)	✓	✓	×
"Fail" cycle — induced leak (see 6.2)	✓	×	×
"Fail" cycle — air injection (see 6.2)	✓	×	✓
✓ = test required; × = test not required.			

6.3 The indicator system shall show no discernible colour change after exposure to dry heat at (140 ± 2) °C for not less than 30 min.

With some indicators the indicator system may show a slight colour change after exposure to dry heat; this shall be acceptable if the change that occurs is slight or markedly different from that brought about by exposure to steam in accordance with 6.1 and within the limits specified by the manufacturer.

Compliance shall be tested in accordance with annex G.

6.4 Indicators intended for use only with a sterilization temperature 121 °C shall be tested by exposure to dry heat at (130 ± 2) °C for not less than 45 min if the indicator will not withstand heating to 140 °C.

Compliance shall be tested in accordance with annex G.

6.5 Indicator systems intended for use with re-usable user-assembled packs shall not visibly transfer indicator reagent to the material of the test load during processing. Pre-assembled packs and indicator systems intended for use with single-use user-assembled packs shall not transfer indicator reagent to the material of the test load during processing to an extent which impairs the utility of the product.

Compliance shall be demonstrated by visual examination after testing in accordance with the requirements of 6.1 and annex D.

6.6 The indicator shall comply with the requirements of this part of ISO 11140 for the duration of the shelf life specified by the manufacturer.

If any change in the indicator occurs during ageing, it shall be different to the change on exposure to dry saturated steam (as described in 6.1) and have either inactivated the indicator system so that no further change can take place or not affected the performance of the indicator system with respect to the requirements of 6.1 and 6.2.

Compliance shall be tested in accordance with annex H or by performance testing after accelerated ageing in accordance with annex I.

7 Packaging and labelling

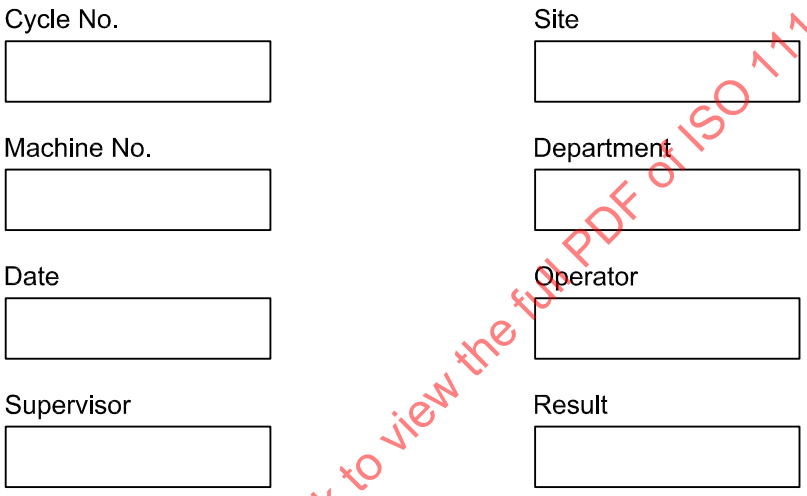
7.1 Each indicator, or indicator system, shall be marked with

a) the sterilization temperature(s) at which the product is designed to be used,

b) a unique code from which the manufacturing history can be traced,

c) the expiry date under the specified storage conditions,

d) at least the information summarized in Figure 1. Adjacent to each heading there shall be a clear space not less than 5 mm × 20 mm for the user to enter the required information at the time of use, or, if the size of the indicator system does not permit this, each indicator or indicator system shall be supplied with means of retaining the indicator or indicator system as a permanent record which shall be printed with the information given in Figure 1. The means of retention shall permit writing in permanent ink to be made in association with the indicator.



NOTE This is an example of a suitable format. Other formats and/or text can be used.

Figure 1 — Provision for recording information to be provided on or with each indicator

7.2 When the indicator is supplied assembled, i.e. with the indicator system within the test load, the exterior of the test load shall be marked with the sterilization temperature(s) at which the product is suitable for use, the manufacturer's name, batch number and date of manufacture. In addition, either a means of uniquely identifying the individual indicator or an area on the outside of the test load onto which the operator can write the number of the machine tested and the date shall be provided.

When a manufacturer provides similar products which are intended only for specific sterilization cycles, the product shall include identification sufficient to enable the user to determine, from the instructions for use, any restrictions on the use of the product. The identification shall be on the indicator or indicator system and, if not visible to the user before use, shall also be on the outside of the test load.

7.3 The transport package shall be such that the product can be removed easily. The package shall protect the product to the extent necessary to ensure that the indicator retains its performance throughout the stated shelf-life when stored and transported in accordance with the manufacturer's instructions.

The manufacturer shall retain documentary evidence demonstrating compliance.

7.4 The outside of each package shall be marked with the sterilization temperature(s) at which the product is suitable for use.

7.5 The information supplied by the manufacturer (see 5.6 of ISO 11140-1:1995) shall include sufficient instructions on the use of the indicator to enable correct interpretation of the test results.

7.6 When requested by the purchaser, the manufacturer shall supply a certificate of conformity to the requirements of this part of ISO 11140 for each batch of product supplied.

8 Quality assurance

8.1 The manufacturer's quality system shall ensure that an acceptable quality level (AQL) of 1,0 or less is maintained for performance requirements given in clause 6 of this part of ISO 11140. Other statistical control systems which provide equivalent or better assurance of consistent product quality also shall be acceptable.

NOTE The AQL is the maximum number of defects per hundred units that, for the purposes of sampling inspection, can be considered satisfactory as a process average.

8.2 Suitable records shall be maintained to ensure that, if necessary, faulty batches can be recalled from use.

8.3 The manufacturing and distribution records shall be retained for a period of five years, or twice the declared shelf-life of the product, whichever is greater. These records shall be maintained in accordance with the requirements of 4.16 of ISO 9001:1994.

Annex A
(normative)

Determination of indicator strength during and after steam sterilization

A.1 Apparatus

A.1.1 **Steam exposure apparatus**, complying with annex J.

A.2 Procedure

A.2.1 Expose the indicator to three successive test cycles at the stated sterilization temperature of the indicator or indicator system. The indicator shall be tested using both the standard test cycles specified in B.1 and B.2 (see 4.5) unless the indicator is intended for use with only one type of air removal stage, in which case the appropriate test cycle shall be used. The rate of pressure change of evacuation during the air removal pulse and during the drying stage shall be not less than 400 kPa · min^{-1}.

The rate of pressure change shall be determined as follows (see Figure A.1):

$$p_3 = 0{,}125\,(7p_1 + p_2) \tag{A.1}$$

$$p_4 = 0{,}5\,(p_1 + p_2) \tag{A.2}$$

$$\frac{\Delta p}{\Delta t} = \frac{(p_3 - p_4)}{(t_4 - t_3)} \tag{A.3}$$

where

p_1 is the maximum absolute pressure attained during the last air removal pulse and the operating stage, in kilopascals;

p_2 is the minimum absolute pressure attained during the last air removal pulse (prior to the admission of steam to the operating pressure required for the chamber reference temperature to attain the sterilization temperature) and the drying stage, in kilopascals;

p_3 is the pressure calculated from (A.1), in kilopascals;

p_4 is the pressure calculated from (A.2), in kilopascals;

t_3 is the time at p_3, in minutes;

t_4 is the time at p_4, in minutes;

$\frac{\Delta p}{\Delta t}$ is the rate of pressure change, in kilopascals per minute.

A.2.2 Remove the pre-assembled or user-assembled indicator from the exposure apparatus and examine the indicator for visible damage, including for example opening or distortion of seals. Record the result.

A.2.3 If the indicator has remained intact, perform a drop test in accordance with ISO 2248 from a height of 1 m onto a firm horizontal surface. Record the result.

NOTE Concrete or terrazzo surfaces are suitable.

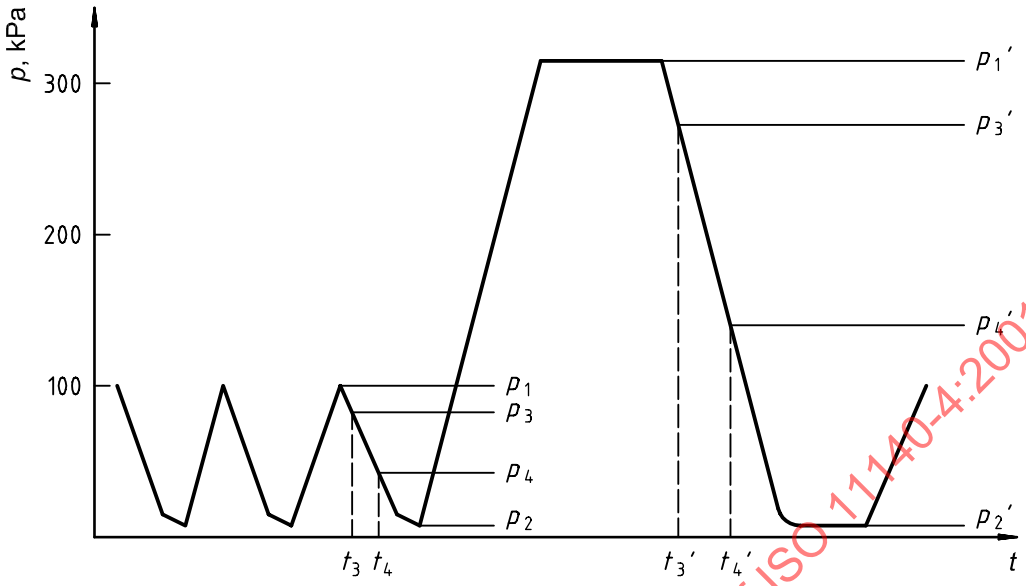


Figure A.1 — Determination of the rate of pressure change

A.2.4 Carry out this test with three samples for each of three separate production batches.

NOTE All nine samples can be processed simultaneously.

A.2.5 Damage occurring during the drop test which can be demonstrated as not impairing the interpretation of the indicator in normal use nor, for re-usable test loads, the subsequent re-use of the test load shall not constitute a failure.

Annex B
(normative)

Standard test cycles

B.1 General

Each of the test cycles described within this annex consists of three principal stages: air removal, exposure time (equivalent to the sterilization stage) and evacuation stage. The temperatures attained during the air removal stage and the duration of the air removal stage can have a significant effect on indicator performance. The cycles described are not intended to imitate any of the many cycles which are commercially available. They are intended to provide an overall range of effects within which most commercially available cycles will occur.

B.2 Cycle 1: Air removal by sub-atmospheric pulsing

The standard test cycle for sub-atmospheric air removal shall consist of the following steps:

a) evacuation of the chamber to 5,0 kPa;

b) steam admission to 97,0 kPa;

c) repetition of steps a) and b) for a further three times;

d) if air injection is being used, it shall take place and be completed during steam admission to the exposure time at a pressure between 75 kPa and 105 kPa (indicated with an arrow on Figure B.1);

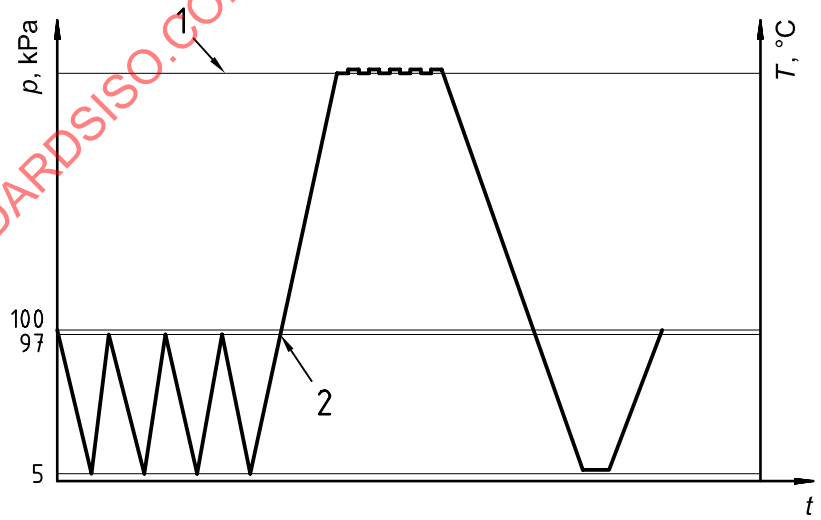
e) steam admission to set operating pressure (see specific requirements for steam admission stage in B.5);

f) exposure time;

g) evacuation to 5,0 kPa;

h) air admission.

NOTE The actual pressures achieved at the set points will be determined by the tolerance permitted for the steam exposure apparatus (see annex J).



Key

1 Set operating pressure

2 Air injection

Figure B.1 — Standard test cycle — Sub-atmospheric air removal

B.3 Cycle 2: Air removal by trans-atmospheric pulsing

The standard test cycle for air removal by trans-atmospheric pulsing shall consist of the following steps:

a) evacuation of the chamber to 5,0 kPa;

b) steam admission to 150 kPa;

c) evacuation of the chamber to 50 kPa;

d) repetition of steps b) and c) for a further three times;

e) steam admission to (set operating pressure minus 10,0 kPa);

f) evacuation of chamber to 110 kPa to 120 kPa;

g) repetition of steps e) and f) for one further time;

h) if air injection is being used, it shall take place during steam admission to the exposure time at a pressure between 120 kPa and 130 kPa (indicated with an arrow in Figure B.2);

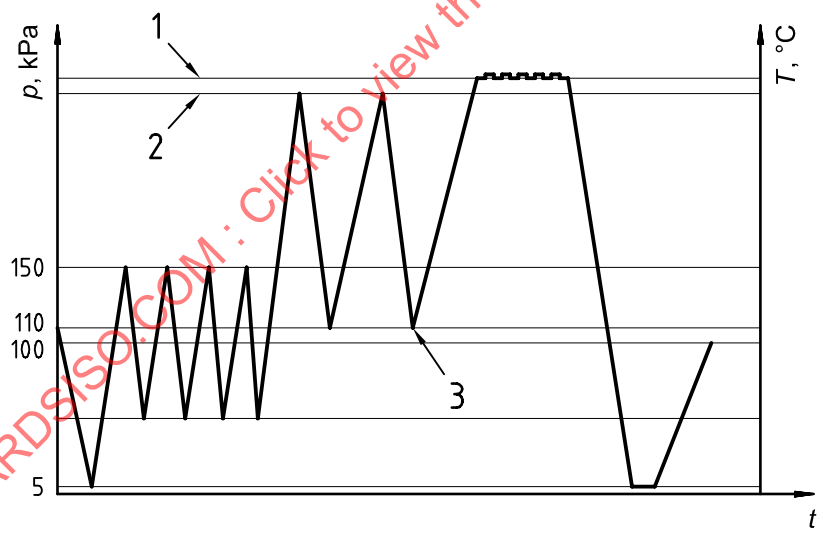
i) steam admission to set operating pressure (see specific requirements for steam admission stage in B.5).

j) exposure time;

k) evacuation to 5,0 kPa;

l) air admission.

NOTE The actual pressures achieved at the set points will be determined by the tolerance permitted for the steam exposure apparatus (see annex J).



Key

1 Set operating pressure

2 Set operating pressure minus 10 kPa

3 Air injection

Figure B.2 — Standard test cycle — Trans-atmospheric air removal

B.4 Cycle 3: Air removal by super-atmospheric pulsing

The standard test cycle for air removal by super-atmospheric pulsing shall consist of the following steps:

a) evacuation of the chamber to 5,0 kPa;

b) steam admission to 95 kPa;

c) evacuation of the chamber to 5,0 kPa;

d) repetition of steps b) and c);

e) steam admission to (set operating pressure minus 20,0 kPa);

f) evacuation of the chamber to 105 kPa to 120 kPa;

g) repetition of steps e) and f) for a further two times;

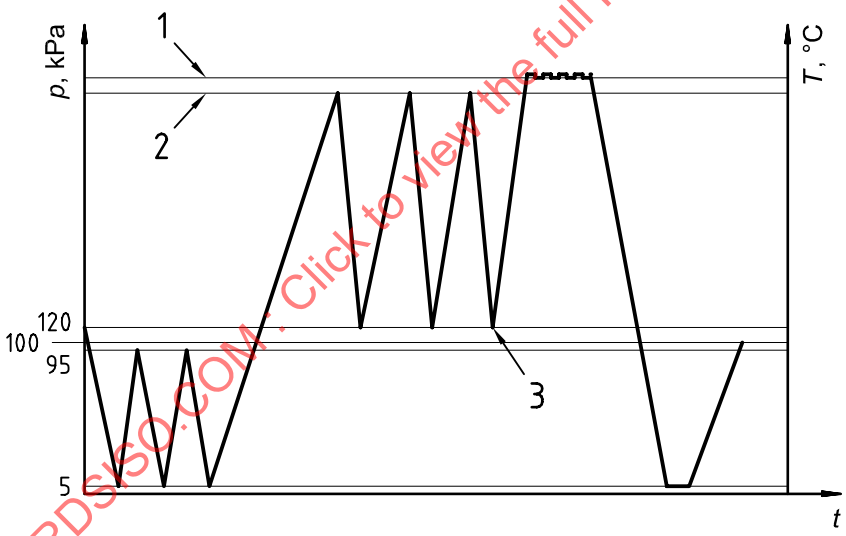
h) if air injection is being used, it shall take place during steam admission to the exposure time at a pressure between 120 kPa and 130 kPa (indicated with an arrow in Figure B.3);

i) steam admission to set operating pressure (see specific requirements for steam admission stage in B.5);

j) exposure time;

k) evacuation to 5,0 kPa;

l) air admission.



Key

1 Set operating pressure
2 Set operating pressure minus 20 kPa
3 Air injection

Figure B.3 — Standard test cycle — Super-atmospheric air removal

B.5 Acceptance limits during steam admission

B.5.1 The rate of pressure rise during steam admission to set operating pressure over the range 100 kPa or lowest pressure at the bottom of the last super-atmospheric pulse to the set operating pressure of the exposure time shall be between 100 kPa · min^{-1} and 250 kPa · min^{-1} as indicated in Figure B.4.

B.5.2 Select the operating temperature of the exposure time such that it corresponds to the temperature as stated for the indicator. Set the operating pressure such that it corresponds to a saturated steam temperature of (selected operating temperature, in degrees Celsius + 0,2 °C).

B.5.3 The integral [Integrated Come-up Exposure (ICE)] between the chamber reference temperature when the chamber reaches 100 kPa or at the bottom of the last super-atmospheric pulse, whichever is the greater, and the set temperature during the steam admission period, bounded by the chamber reference temperature on the graph, shall not exceed

$$T_R \cdot (12 \cdot T_R/6)$$

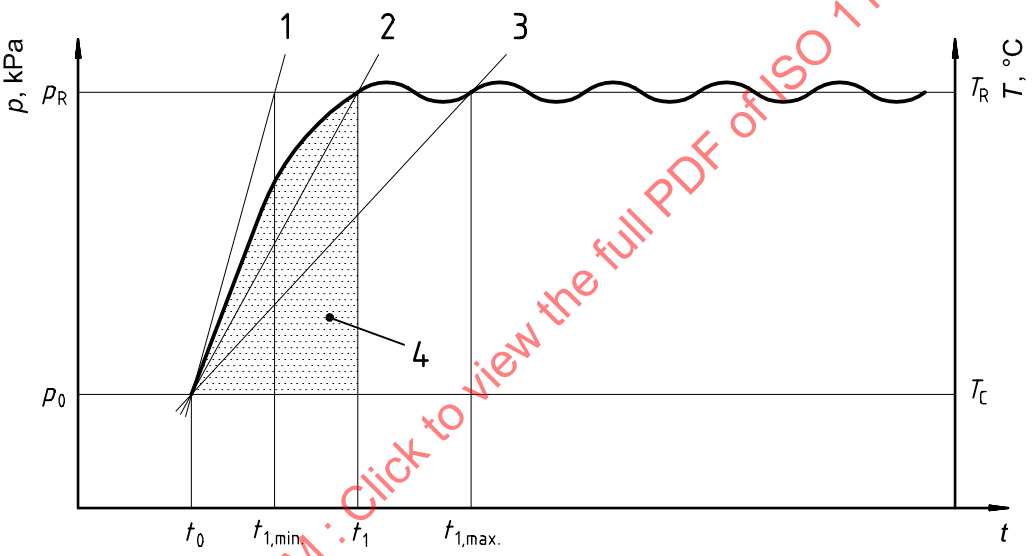
where T_R is the set temperature in degrees Celsius minus 100 °C.

EXAMPLE

at a set temperature 134 °C the integral shall not exceed $[34\,(12 \times 34)\,/6] = 2\,312$ s·K, or

at a set temperature 121 °C the integral shall not exceed $[21\,(12 \times 21)\,/6] = 882$ s·K.

NOTE These limits are intended to ensure that steam admission does not contribute to excessive exposure of the indicator to untypical conditions.



Key

1 Maximum rate of pressure rise during steam admission $\frac{(p_R - p_0)}{t_{1\ min}}$

2 Rate of pressure rise

3 Minimum rate of pressure rise during steam admission $\frac{(p_R - p_0)}{t_{1\ max}}$

4 Integrated Come-up Exposure: Area bounded by T_0 and the curve traced by T_C over the time t_0 to t_1

p_R is the pressure of saturated steam, corresponding to the set operating temperature, in kilopascals;

p_0 is the pressure of saturated steam, corresponding to the temperature T_0, in kilopascals

Figure B.4 — Steam admission

ICE shall be calculated using the equation:

$$\text{ICE} = \sum_{t_0}^{t_1} (T_1 - T_0) \cdot \mathrm{d}t$$

where

T_1 is the chamber reference temperature at time t, in degrees Celsius;

T_0 is 100 °C or the lowest temperature of the last positive pulse, in degrees Celsius;

dt is 1 s;

t_0 is the time at which chamber reference temperature attains T_0, in seconds;

t_1 is the time after t_0 at which the chamber reference temperature (T_C) attains set operating temperature (T_R) e.g. 134 °C, in seconds;

T_C is the chamber reference temperature, in degrees Celsius;

T_R is the set operating temperature of the exposure time, in degrees Celsius.

Annex C
(normative)

Estimation of visual difference between colours of the substrate and of the changed or unchanged indicator system by determination of relative reflectance density

C.1 Principle

The relative reflectance density, as defined in ISO 5-1, of the changed indicator and the substrate shall be determined in accordance with the methods given below which are based on ISO 5-3 and ISO 5-4, to which reference shall also be made.

Relative reflectance density, D_{Rf}, is calculated as follows

$$D_{\mathrm{Rf}} = -\log_{10} R_{\mathrm{f}} \qquad \text{(C.1)}$$

$$R_{\mathrm{f}} = \phi_{\mathrm{c}} / \phi_{\mathrm{ce}} \qquad \text{(C.2)}$$

where

ϕ_{c} is the reflected flux from the indicator;

ϕ_{ce} is the reflected flux from the substrate.

To completely define a type of density spectrally, it is necessary to specify the light source, optics and spectral response of the measuring system.

C.2 Apparatus

C.2.1 Steam exposure apparatus, complying with annex J.

C.2.2 Illuminant.

The relative spectral power distribution of the incident flux shall conform to CIE standard illuminant D_{65}.

NOTE This is regarded as equivalent to “Daylight – cloudy northern sky”.

C.2.3 Photoelectric reflectance photometer, giving within 0,3 % an indicated reading proportional to the intensity of light reflected from the surface under test. The instrument shall have the following characteristics.

a) **Optical geometry**

Optical geometry shall conform to the requirements of ISO 5-4; this includes illumination of the specimen at angles between 40° and 50°, viewed along the normal (0°) with an angle of acceptance (observer angle) of 10°.

The dimensions of the measurement aperture of the instrument shall permit the measurement aperture to be entirely filled with substrate or indicator reagent.

To minimize measurement errors, the optical system should be equipped with a polarizing filter if the surface to be measured is highly reflecting, e. g. a plastics coated surface.

b) **Spectral response**

For the visual reflectance density, the combined spectral sensitivity of the receiver and spectral characteristics of the components on the efflux section of the measuring instrument shall match the spectral luminance efficiency in photopic vision, designated $V_{(\lambda)}$. The product of $V_{(\lambda)}$ and the reflection densitometer illuminance S_{A}, wave-

length by wavelength, defines the spectral products required of the measuring instrument in order to provide comparison of visual densities. The spectral product of the measuring instrument shall be within ± 20 % of the values given in Table C.1.

The logarithms of the products are given in Table C.1.

NOTE These conditions assume that there is no fluorescence in the optical elements of the instrument or the sample.

c) **Calibration**

NOTE Reflectance density is determined using a perfectly-reflecting and perfectly-diffusing material as a reference standard. Such a material does not exist, but the response that would theoretically be obtained from such a material can be compared with a suitable secondary reference standard, e. g. compressed barium sulfate, enamelled metal plaques which can then be used to calibrate the densitometer.

The measuring instrument shall be calibrated against reference samples previously calibrated by a National Reference Laboratory.

The instrument shall indicate values within ± 3 % of the calibrated values of the reference samples.

d) **Background**

While readings of the reflectance density of the substrate and the indicator are being made the sample shall be in contact with a backing material which is spectrally non-selective and diffuse-reflecting and which has an ISO reflection density greater than 1,50 (see annex A of ISO 5-4:1995).

C.3 Sample conditioning

Samples shall be conditioned to, and in equilibrium with, (23 ± 2) °C and (50 ± 5) % relative humidity when tested.

Standardized conditions are recommended because some materials change density with variations in temperature and relative humidity.

C.4 Test procedure

Expose the indicator system within the indicator to a cycle of the steam exposure apparatus at the specified operating temperature for the indicator, to produce a uniform colour change in the indicator reagent in accordance with 6.1.

Determine the relative reflectance density of the indicator reagent on the substrate by using the substrate as the reference reflectance.

Carry out this measurement on three samples for each of three batches of the indicator system and on aged samples (see annex H and annex I) in accordance with 6.6.

C.5 Test report

The test report shall contain at least the following information:

a) name and address of the indicator manufacturer;
b) batch numbers of the individual batches of indicator tested;
c) make, model and serial number of the test instrument;
d) calibration details traceable to a national standards authority;
e) temperature chart records of the steam exposure to which the indicators were exposed;
f) mean and range of the relative reflectance density measurements;
g) date of test;
h) identification of the test operator.

Table C.1 — Values of spectral product required of the reflectance photometer at the given values of wavelength and illuminance

Wavelength nm	Reflection densitometer illuminance S_A	Visual density spectral product πv
340	4	
350	5	
360	6	
370	8	
380	10	
390	12	
400	15	< 1 000
410	18	1 322
420	21	1 914
430	25	2 447
440	29	2 811
450	33	3 090
460	38	3 346
470	43	3 582
480	48	3 818
490	54	4 041
500	60	4 276
510	66	4 513
520	72	4 702
530	79	4 825
540	86	4 905
550	93	4 957
560	100	4 989
570	107	5 000
580	114	4 989
590	122	4 956
600	129	4 902
610	136	4 827
620	144	4 731
630	151	4 593
640	158	4 433
650	165	4 238
660	172	4 013
670	179	3 749
680	185	3 490
690	192	3 188
700	198	2 901
710	204	2 622
720	210	2 334
730	216	2 041
740	222	1 732
750	227	1 431
760	232	1 146
770	237	< 1 000

Annex D
(normative)

Demonstration of uniform colour change on exposure to saturated steam

D.1 Apparatus

D.1.1 **Steam exposure apparatus**, complying with annex J and set to an operating cycle previously demonstrated to produce satisfactory air removal and rapid steam penetration in a standard test pack (see annex K).

D.1.2 **Standard test pack**, complying with annex K.

D.1.3 **Temperature sensors and thermometric recording instruments**, complying with the requirements for test instrumentation given in 4.6.

D.2 Test procedure

D.2.1 Expose the indicator (or the indicator system combined with its test load for user-assembled packs) to a cycle of the steam exposure apparatus with the holding time and temperature preset to those specified in 6.1.

D.2.2 At the end of the operating cycle, remove the indicator from the steam exposure apparatus and examine for compliance with 6.1.

D.2.3 Carry out the test on three samples for each of three production batches using operating cycles with a sub-atmospheric air removal stage, and on further sets of samples with operating cycles employing a trans-atmospheric and a super-atmospheric air removal stage (see Table 1 and annex B).

NOTE The obtained samples can be used in the tests according to annex C.

D.2.4 Before and after each series of three tests, run an operating cycle containing a standard test pack, monitored with temperature sensors, to verify that the operating cycle is performing within the required limits.

D.2.5 Run the tests required on any one production batch on separate operating cycles.

NOTE The oven door cools down between consecutive tests, thus the highest reproducibility is obtained if the time period for the unloading-loading-start sequence is kept short and constant.

Annex E
(normative)

Determination of equivalence of the alternative indicator to the Bowie and Dick test

E.1 Principle

The method provides three different systems for comparing the sensitivity of the alternative indicator to the Bowie and Dick test performed using a standard test pack (see annex K) and thermometric measurement. The three systems produce similar but not identical results in a Bowie and Dick test pack and rely on the air pocket produced when:

a) air is injected into the steam exposure apparatus;

b) residual air is allowed to remain within the chamber of the steam exposure apparatus;

c) air is allowed to leak into the chamber of the steam exposure apparatus (sub-atmospheric air removal stages only).

E.2 Apparatus

E.2.1 **Steam exposure apparatus**, in accordance with annex J.

The steam exposure apparatus shall be fitted with an air injection system as specified in annex L, and fitted with a valved system, including a non-return valve, to provide a controlled leak of ambient air into the chamber while the latter is at pressures below ambient, and a flow meter to measure the rate of air ingress.

E.2.2 **Standard test pack**, complying with annex K.

E.2.3 **Thermometric recording instrument**, as given in 4.6.

The recording instrument shall record the temperature from a minimum of 12 temperature sensors. The temperature sensors shall be introduced into the chamber through the temperature-sensor entry connection and fitting.

E.3 Test procedure

E.3.1 Perform the tests on the standard test loads at the operating temperature(s) of the indicator.

E.3.2 Remove the wrapping from the standard test pack and place not less than five, and not more than ten, temperature sensors in the test pack, of which one shall be placed at the geometric centre of the test pack. The others shall be arranged in a pattern around the geometric centre of the test pack to detect a temperature depression occurring within a radius of 30 mm of the geometric centre. Place one temperature sensor at the defined reference point within the chamber to measure the chamber reference temperature. Reassemble the test pack as described in annex L.

NOTE 1 As the coolest spot within the standard test pack will not be predictably at the exact geometric centre, the additional temperature sensors in the standard test pack are used to improve the reproducibility of the test results.

NOTE 2 In setting up the standard test pack, the use of a chemical indicator test sheet complying with ISO 11140-3, placed within the pack, may be helpful in visualizing the position of the air pocket and determining the optimum position for the temperature sensors.

E.3.3 Provided at least two of the sensors in the standard test pack show a temperature depression, the lowest shall be used from them to calculate the reference fault condition.

E.3.4 Use the following conditions to produce a reference fault condition and when testing the indicator.

a) Inject the volume of air into the chamber at the time specified in annex B.

The volume and the rate of injection should be determined by prior trial.

b) Determine the air leak into the chamber by the method given in EN 285:1996, clause 20;

The rate of air leak required should be determined by prior trial.

c) Modify the air removal stage.

The reduction in the pressure range, and if necessary in the number of pulses in the air removal stage, should be determined by prior trial.

E.3.5 During the tests, vary the orientation of the product between replicate tests (within any limits given in the instructions for use of the product).

E.3.6 Carry out the test three times for each of three production batches, using operating cycles with a sub-atmospheric air removal stage, and on further sets of samples with operating cycles employing a trans-atmospheric air removal stage and a super-atmospheric air removal stage as specified in Table 1 and annex B.

E.3.7 Before and after each series of three tests, run an operating cycle containing a standard test pack and monitor with thermocouples to verify that the operating cycle is performing within the required limits.

E.3.8 Run the three tests required on any one production batch on separate operating cycles to ensure that the results are consistent, within the variation that inevitably occurs between successive cycles on apparatus of this sort.

E.3.9 After each test, inspect the indicator system visually for compliance with 6.2.

E.3.10 The position of all temperature sensors in the test pack (see E.3.2) shall be described in the test report.

Annex F
(normative)

Determination of reproducibility of fail conditions created in a standard test pack by air injection, air leak and retained air systems

F.1 General

It is not possible simultaneously to determine the effect in a standard test pack and in an alternative indicator, since the two systems are each intended to be used in a sterilizer chamber which contains no other load (i.e. is empty except for the chamber furniture).

It is therefore necessary to create a system in which a standard set of operating conditions can be shown reproducibly to yield the desired results with a standard test pack; the alternative indicator can then be tested under these conditions to determine whether the results obtained correspond with those which would have been expected for a standard test pack.

Although many of the variables of the operating cycle for the steam exposure apparatus can be controlled with great precision, the key variable, i. e. the disposition of air within the chamber and load, is the least amenable to control, the least stable, and cannot be simultaneously independently measured.

No difficulty should be experienced in producing operating cycles which show satisfactory steam penetration in a standard test pack. It is only when air is present that it becomes less predictable.

A statistical basis is required so that, with the minimum number of cycles and a suitable means to measure variability, appropriate acceptance criteria can be determined:

— for cycles intended to produce a reference fault condition;

— for cycles intended to produce satisfactory steam penetration.

F.2 Criteria for acceptability of test cycles

F.2.1 General

Use the conditions described below as the limiting conditions within which the temperature trace from the standard test pack shall lie. For each cycle run, these criteria shall be met. In addition by integrating the area between the chamber reference temperature and the test pack temperature for the hold period it is possible to calculate the reference integrated fault (RIF) which provides a simple means of comparing cycles [see F.2.2 j)].

F.2.2 Reference fault condition

For cycles to evaluate the ability of the indicator to detect inadequate steam penetration, the results of thermometric monitoring of the steam exposure apparatus and the standard test pack shall meet the following criteria as shown in Figure F.1.

a) The operating cycle, including the steam admission stage, shall meet all the criteria given in annex B.

b) The elapsed time between the chamber attaining the set operating pressure and the chamber reference temperature attaining the set temperature shall not exceed 5 s.

NOTE In a correctly functioning steam exposure apparatus, any difference should be due solely to the difference in response time of the pressure and temperature sensors.

c) At the time the chamber reference temperature (T_c) attains the set temperature (T_r), the temperature measured in the standard test pack (T_p) shall show a temperature depression ($T_c - T_p$) of 2 K or greater.

d) The temperature depression shall remain at 2 K or greater throughout the reference fault period.

e) The test equilibration time shall be 90 s.

f) The temperature depression ($T_c - T_p$) at the beginning of the reference fault period shall be not greater than 7 K.

g) The temperature depression ($T_c - T_p$) at the end of the reference fault period shall be not greater than 4 K.

h) The temperature depression ($T_c - T_p$) at the end of the minimum permitted equilibration time shall be not greater than 2 K.

i) The temperature depression ($T_c - T_p$) at the end of the exposure time, or 10 min, whichever is shorter, shall not be greater than 1 K.

j) The Reference Integrated Fault (RIF) determined as the area bounded by the trace of the chamber reference temperature (T_c) and the temperature at the centre of the standard test pack (T_p) shall be between 120 s·K and 525 s·K for a cycle at 134 °C for 3,5 min, and 120 s·K and 1 080 s·K for a cycle at 121 °C for 15 min.

k) The steam entry rate shall be 100 kPa/min to 250 kPa/min.

F.2.3 No Fault condition

A 'No Fault' cycle is one which all indicators would be expected to provide evidence of a satisfactory cycle.

For cycles to evaluate the ability of the indicator to detect adequate steam penetration the results of thermometric monitoring of the steam exposure apparatus and the standard test pack shall meet the following criteria as shown in Figure F.1.

a) The operating cycle, including the steam admission stage, shall meet all the criteria given in annex B.

b) The elapsed time between the chamber attaining the set operating pressure and the chamber reference temperature attaining the set temperature shall not exceed 5 s.

NOTE In a correctly functioning steam exposure apparatus, any difference should be due solely to the difference in response time of the pressure and temperature sensors.

c) At the time (t_1) at which the chamber reference temperature (T_c) attains the set temperature (T_r), the temperature measured in the standard test pack (T_p) shall show a temperature depression ($T_c - T_p$) of 1 K or less.

d) By the end of the reference fault period there shall be no detectable temperature difference between the centre of the test pack (T_p) and the chamber reference temperature (T_c) (within the limits of accuracy of the measuring equipment).

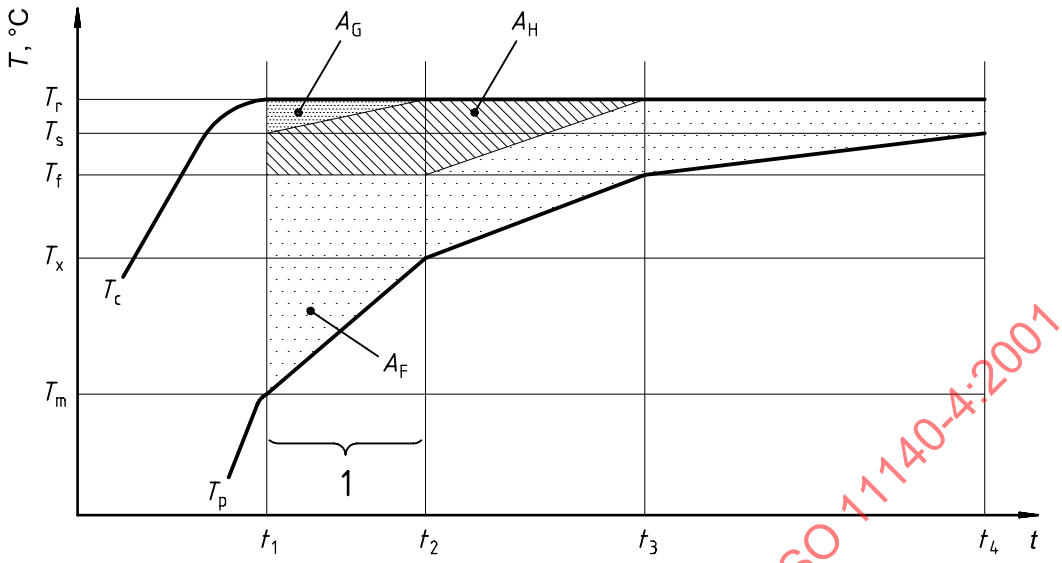
F.3 Statistical evaluation of reproducibility

F.3.1 Run 10 consecutive cycles on each of two days.

F.3.2 From the cycle records confirm that each cycle meets the acceptance criteria and determine the value of the Reference Integrated Fault. [See F.2.2 j).]

F.3.3 Calculate the mean and estimate of variance for the two sets of runs.

NOTE It is not necessary to assume, or demonstrate, that the data is normally distributed (Gaussian). Using the F statistic shows this is not critical since the test is robust.



Key

1 Reference fault period

T_r set temperature
T_s minimum value of T_p at the time t_1 ('NO FAULT' condition) ($T_r - 1$)
T_f maximum value of T_p at the time t_1 and t_2 ('FAULT' condition) ($T_r - 2$)
T_m minimum value of T_p at the time t_1 ('FAULT' condition) ($T_r - 7$)
T_x minimum value of T_p at the time t_2 ('FAULT' condition) ($T_r - 4$)
T_p temperature at the centre of the standard test pack
T_c chamber reference temperature
t_1 time at which the chamber reference temperature attains set temperature (T_r)
t_2 end of the reference fault period $= (t_1 + 30)$ s
t_3 end of the minimum permitted test equilibration time $= (t_1 + 90)$ s
t_4 end of the set exposure time
A_F area within which the plot of the test pack temperature (T_p) shall lie for a 'FAULT' condition
A_G area within which the plot of the test pack temperature (T_p) shall lie for a 'NO FAULT' condition
A_H area within which the plot of the test pack temperature (T_p) would indicate failure to obtain a correctly defined cycle for either 'FAULT' or 'NO FAULT' conditions.

Figure F.1 — Integral of the fault condition

F.3.4 Carry out the F test (variance ratio test):

$$F = (\text{estimate of variance}_{\text{day 1}})^2 \,/\, (\text{estimate of variance}_{\text{day 2}})^2 \qquad \text{(F.1)}$$

with $(n_1 - 1)$ and $(n_2 - 1)$ degrees of freedom.

NOTE Standard F tables are drawn up for 1-sided hypothesis, so for H_1 $(\text{variance}_{\text{day 1}}) = (\text{variance}_{\text{day 2}})$ and $\alpha = 0{,}05$, the upper limit is given by δ_1/δ_2 and $(n_1 - 1)$ and $(n_2 - 1)$ degrees of freedom, and the lower limit is given by δ_2/δ_1 and $(n_2 - 1)$ and $(n_1 - 1)$ degrees of freedom.

F.3.5 Compare the calculated value of F with the critical region obtained from the tabulated values of F.

NOTE The same tables can also be used to calculate the confidence interval.

Annex G
(normative)

Evaluation of indicator colour change on exposure to dry heat

G.1 Apparatus

G.1.1 **Dry heat oven**, capable of maintaining a steady temperature of (140 ± 2) °C.

The relative humidity in the oven should be less than 5 % throughout the period of the test.

G.2 Procedure

G.2.1 General

Carry out both tests three times for each of three separate production batches of the indicator system.

NOTE Several test samples may be exposed simultaneously.

G.2.2 Test 1

G.2.2.1 Pre-heat the oven to the operating temperature.

G.2.2.2 Place the indicator systems in the oven and subject them to dry heat as required in 6.3 and 6.4 respectively. Remove the samples and examine for colour change after the required exposure period.

G.2.3 Test 2

G.2.3.1 Fit the indicator system in combination with its specified test load (the indicator) with a temperature sensor to monitor the temperature of the indicator system and subject it to dry heat at (140 ± 2) °C to determine the time required for the indicator to reach 134 °C. This time is the heat-up time.

NOTE Indicators intended for use only at a sterilization temperature of 121 °C can be subjected to (130 ± 2) °C and the time required to attain 121 °C is determined.

G.2.3.2 Dry new samples of the indicator system in combination with its specified test load (the indicator) to constant mass at a temperature greater than 100 °C, or by equivalent means at a lower temperature using a suitable desiccant.

G.2.3.3 Transfer the dried indicators to the oven without undue delay and in a manner which will prevent rehydration of the indicator and subject them to dry heat at (140 ± 2) °C for $[(\text{heat-up time}) + 30]$ min.

NOTE Indicators intended for use only at a sterilization temperature of 121 °C can be subjected to dry heat at (130 ± 2) °C.

Annex H
(normative)

Demonstration of shelf life of product

H.1 The testing of the product for determination of the shelf life shall be performed in accordance with a written protocol which shall be established before the commencement of the study.

H.2 The samples of the product shall be stored in their normal packaging at or above the maximum temperature and relative humidity recommended for storage. These conditions shall be controlled and monitored.

H.3 All performance requirements shall be met during and on completion of the storage period and this shall be verified by testing.

H.4 All results of the storage trial shall be retained for a period of at least five years from completion of the trial. After this period, a summary report should be retained for as long as the product is commercially available.

Annex I
(normative)

Accelerated ageing of test samples

I.1 Place the indicator, or indicator system, in a desiccator in a horizontal position on a perforated plate above a saturated aqueous solution which provides a relative humidity of approximately 80 % at 65 °C.

The salts used in solution to control humidity should be selected from those which will not interact with the indicator reagent (for example, potassium chloride).

I.2 Use sufficient samples of the indicator, or indicator system, to allow the tests to demonstrate compliance with the requirements given in 5.1 b) and 6.1 to 6.6 inclusive to be tested.

I.3 Seal and place the desiccator in an oven in which the temperature is maintained uniformly throughout the interior, and heat for 7 days at a temperature of (65 ± 2) °C.

A mechanical convection oven with air circulated by a multi-blade centrifugal fan acting as a mixer and impeller, and having a continuous temperature-recording device, is recommended.

I.4 Prior to testing, remove the indicator system and condition it for 24 h (see 4.2).