

INTERNATIONAL STANDARD

ISO 18472

First edition
2006-06-01

Sterilization of health care products — Biological and chemical indicators — Test equipment

*Stérilisation des produits de santé — Indicateurs biologiques et
chimiques — Appareillage d'essai*

www.iso.org



Reference number
ISO 18472:2006(E)

© ISO 2006

PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

© ISO 2006

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

Foreword.....	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions.....	2
4 Performance requirements for resistometers	4
4.1 Intended use.....	4
4.2 Measurement and control capabilities	4
4.3 Test methods.....	4
4.4 Leak test	4
4.5 Steam resistometer performance requirements.....	4
4.6 Ethylene oxide resistometer performance requirements	7
4.7 Dry heat (heated air) resistometer performance requirements	9
4.8 Vaporized hydrogen peroxide process resistometer performance requirements.....	11
5 Recording systems.....	12
5.1 Measurement systems	12
5.2 Calibration	12
6 Documentation	12
6.1 General.....	12
6.2 Minimum information	13
Annex A (informative) Additional performance characterization — Steam	14
Annex B (informative) Additional performance characterization — Ethylene oxide.....	17
Annex C (informative) Additional performance characterization – Dry heat	20
Annex D (informative) Resistometer documentation and derivations.....	22
Bibliography	28

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 18472 was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

This first edition of ISO 18472 partially replaces ISO 11140-2.

Introduction

To test the performance of chemical and biological indicators, specific test equipment is required. This International Standard specifies the performance requirements for the test equipment to be used in order to establish the response of chemical and biological indicators to critical process variables. This International Standard does not apply to test equipment for irradiation indicators or low temperature steam and formaldehyde indicators.

Resistometers constitute test equipment designed to create precise and repeatable sterilizing environments, allowing the evaluation of their effect on biological inactivation kinetics, chemical reactions, material degradation and product bioburden. Resistometers allow precise variation of the environmental conditions and cycle sequences in order to produce controlled physical studies. When used with the defined test methods given in ISO 11138 for biological indicators and ISO 11140 for chemical indicators, the results of these studies can be used to demonstrate conformance of biological indicators and chemical indicators to these standards.

Resistometers differ from conventional sterilizers. Instrumentation selection and control requirements for resistometers are based upon mathematical models in which rates of reaction, measurement accuracy and process control requirements are evaluated to quantify the effects induced by test equipment-controlled variables. The requirements for accurate measurement, precise control, and rapid rates of change approach limits of commercially available process control and calibration instrumentation accuracy. The measurement and control requirements often prohibit practical validation of a resistometer using procedures that might be employed in a conventional heat or chemical sterilization system. Resistometers are considered test equipment rather than sterilizers; therefore, an understanding of instrumentation and process design is critical in clarifying requirements on precision and accuracy. Practical design has to consider the following:

- achievable measurement and control;
- acceptable equipment induced variation in test results;
- economic design (utilizing tight process controls only where required);
- test method correlation with intended use;
- historical knowledge applied to test procedures and an understanding of micro-environmental physical phenomena;
- testing and analysis alternatives, when accurate quantitative determinations exceed physical measurement/control limits.

Sterilization of health care products — Biological and chemical indicators — Test equipment

1 Scope

1.1 This International Standard specifies the requirements for test equipment to be used to test chemical and biological indicators for steam, ethylene oxide, dry heat and vaporized hydrogen peroxide processes for conformity to the requirements given in ISO 11140-1 for chemical indicators, or the requirements given in the ISO 11138 series for biological indicators. This International Standard also provides informative methods useful in characterizing the performance of biological and chemical indicators for intended use and for routine quality control testing.

ISO 11138-2, ISO 11138-3, ISO 11138-4, and ISO 11140-1 require the use of resistometers specified in this International Standard, and these resistometers are used in conjunction with the test methods specified in the appropriate parts of ISO 11138 and ISO 11140.

NOTE Resistometers for formaldehyde indicators are not included in this International Standard. Test methods using laboratory apparatus for steam-formaldehyde are included in ISO 11138-5, ISO 11140-3 and ISO 11140-4.

1.2 This International Standard does not address the methods used to demonstrate compliance of biological or chemical indicators to ISO 11138 and ISO 11140, as these are covered in the appropriate parts of these standards. Indicators used with combination processes, such as washer-disinfection, are not covered by this International Standard.

NOTE Test equipment and methods necessary for ISO 11140-3, ISO 11140-4 or ISO 11140-5 are specified in those standards.

1.3 This International Standard does not address safety aspects of the test equipment because these are usually covered by specific regional, national or local regulations.

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

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

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

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

ISO 11140-4¹⁾, *Sterilization of health care products — Chemical indicators — Part 4: Class 2 indicators as an alternative to Bowie and Dick test for detection of steam penetration*

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

IEC 60584 (all parts), *Thermocouples*

IEC 60751:1983, *Industrial platinum resistance thermometer sensors*

3 Terms and definitions

For the purpose of this document, the terms and definitions given in ISO 11138, ISO 11140 and the following apply.

3.1 biological indicator

test system containing viable microorganisms providing a defined resistance to a specified sterilization process

[ISO/TS 11139:2006, definition 2.3]

3.2 calibration

set of operations that establish, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards

[VIM 1993, definition 6.11]

3.3 chemical indicator non-biological indicator

system that reveals change in one or more predefined process variables based on a chemical or physical change resulting from exposure to a process

[ISO/TS 11139:2006, definition 2.6]

3.4 come-up period

time elapsed from the introduction of the sterilizing agent to the attainment of the minimum specified exposure conditions

3.5 come-down period

time elapsed from the termination of the exposure period to an established null reaction point

3.6 exposure period

time from the initial attainment of the minimum specified exposure conditions to the termination of the exposure period

NOTE This phase of the cycle includes the stabilization period and the steady state period

1) To be published. (Revision of ISO 11140-4:2001)

3.7**measurement accuracy**

closeness of the agreement between the result of a measurement and the true value of the measurement

NOTE 1 “Accuracy” is a qualitative concept.

NOTE 2 The term “precision” should not be used for “accuracy”.

3.8**null reaction point**

terminating set of conditions that are either specified in the document or have been established and demonstrated to produce no significant effect on the indicators

3.9**precision**

degree of reproducibility of a measurement

3.10**reference standard**

standard, generally having the highest metrological quality available at a given location or in a given organization, from which measurements are derived

3.11**resistometer**

test equipment designed to create defined combinations of the physical and/or chemical variables of a sterilization process

NOTE Resistometers were formerly referred to as a Biological Indicator Evaluator Resistometer (BIER) or Chemical Indicator Evaluator Resistometer (CIER) test systems.

3.12**response time**

time required for a 90 % change in sensor output when exposed to a step change in the variable being measured

NOTE It may be necessary to determine the sensor response time using a faster data sampling rate than the minimum for the equipment specified in this International Standard. Documentary evidence from the sensor manufacturer's stated response time is equally acceptable as proof of conformance.

3.13**saturated steam**

water vapour in a state of equilibrium between condensation and evaporation

3.14**stabilization period**

elapsed time from the attainment of the minimum specified exposure conditions until the end of the defined time to achieve steady state conditions

3.15**steady state period**

that portion of the exposure period which begins after the stabilization period and terminates at the end of the exposure period, during which time all control parameters are within the specified limits

4 Performance requirements for resistometers

4.1 Intended use

The resistometer is intended to be used to expose test samples under stated test conditions, and therefore shall be capable of producing cycle sequences as required for specific test methods. Depending upon the test methods defined in ISO 11138-2, ISO 11138-3, ISO 11138-4 and ISO 11140-1, the resistometer utilized need only verify those limits necessary to characterize the chemical or biological indicators.

NOTE The following specifications define the conditions to be achieved in the vessel in which the sample is to be placed, but the means by which these conditions are to be controlled are not addressed.

4.2 Measurement and control capabilities

The following performance requirements define the measurement and control capabilities for steam, ethylene oxide, dry heat, and vaporized hydrogen peroxide resistometers.

4.3 Test methods

The equipment specified in this International Standard shall be used with the detailed test methods given in the appropriate parts of ISO 11138 and ISO 11140.

NOTE The claimed performance tolerances for chemical and biological indicators are based on the specified exposure conditions without allowance for the operational tolerances of the test equipment.

4.4 Leak test

4.4.1 With the temperature stabilized and the chamber empty (except for fixed furniture and necessary monitoring sensors) start the test cycle. When the pressure in the chamber has reached or is below the value corresponding to the lowest operating vacuum of the test cycle air removal stages, close all the valves connected to the chamber and stop the vacuum pump. Observe and record the time, t_1 , and the absolute pressure, p_1 . Allow evaporation of condensation in the chamber for $300 \text{ s} \pm 10 \text{ s}$ and then observe and record the absolute pressure, p_2 , in the chamber and the time, t_2 . After a further $600 \text{ s} \pm 10 \text{ s}$, again observe and record the absolute pressure, p_3 , and the time, t_3 .

The resistometer may be fitted with a test cycle for air leakage that will carry out this procedure automatically and display the air leakage in kPa/min (mbar/min).

4.4.2 At the end of the test calculate the rate of pressure rise for the 600 s period.

NOTE 1 If the value of $(p_2 - p_1)$ is greater than 2 kPa (20 mbar), this could be due to the initial presence of excessive condensate in the sterilizer chamber.

NOTE 2 In a closed vessel at 4 kPa pressure, the pressure changes by approximately 0,1 kPa (1 mbar) for each 10 °C change in temperature; over the range 20 °C to 140 °C; at 7 kPa (70 mbar) the change is approximately 0,2 kPa (2 mbar). The test can be compromised if the temperature changes by more than 10 °C during the period in which the chamber pressure is monitored.

4.5 Steam resistometer performance requirements

4.5.1 Measurement accuracy

The sensors used to measure temperature, vacuum and pressure from within the steam resistometer shall have a response time as specified in Table 1. The system used to record time, temperature, vacuum and pressure from within the steam resistometer shall be capable of operation with a resolution and accuracy within the scale range specified in Table 1.

The measurement systems used may operate beyond the scale range specified as long as the limiting values within the scale range specified in Table 1 are attained.

Requirements shall apply to complete measurement chains including sensors and data processing.

Table 1 — Steam resistometer instrumentation requirements

Measurement	Unit	Scale range	Resolution	Accuracy (+/-) ^a	Sensor response time ms
Time	HH:MM:SS	Selectable	00:00:01	00:00:01	—
Temperature	°C	110 to 145	0,1	0,5	≤ 500
Vacuum	kPa	0 to 100	0,1	1,0	≤ 30
Pressure	kPa	100 to 420	0,1	3,5	≤ 30

^a Accuracy over the test condition range (see 4.1).

4.5.2 Recording interval

The measurement system shall have a recording interval for each of the measurements specified in Table 1 of not less than one data point per second.

The data may be electronically archived and the print interval can be at the user's discretion.

4.5.3 Process control

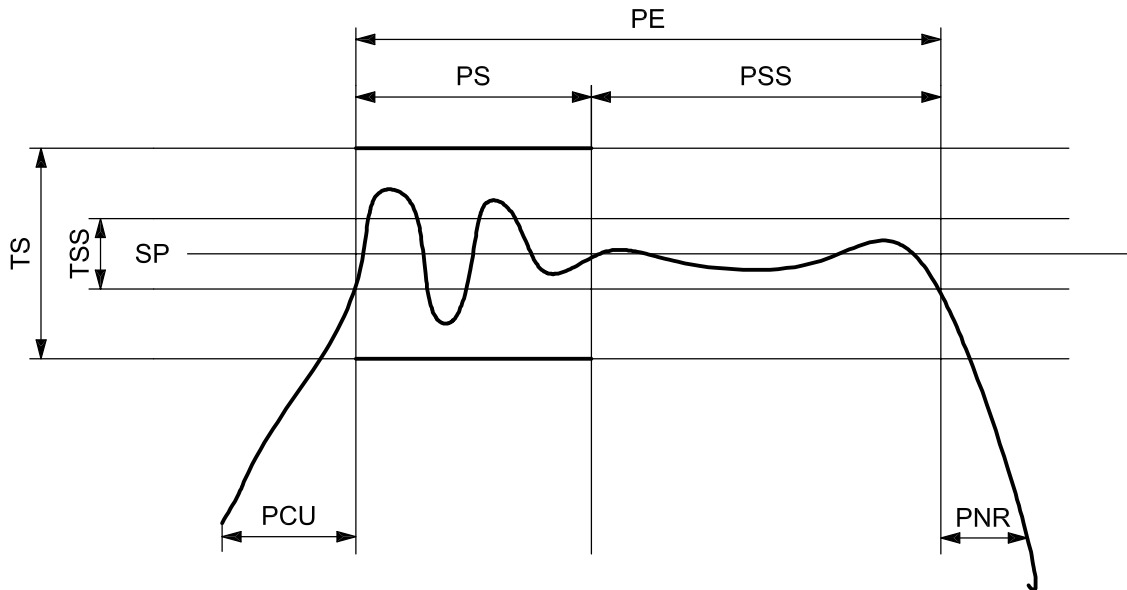
The steam resistometer process control shall be capable of producing the conditions given in Table 2.

Table 2 — Steam resistometer physical design/control specifications

Parameter	Unit	Range	Tolerance (+/-)
Time	HH:MM:SS	Selectable	00:00:01
Temperature	°C	110 to 145	0,5 ^a
Pressure	kPa	100 to 420	3,5 ^a
Vacuum	kPa	3 to 100	1,0
Time to achieve vacuum set-point	HH:MM:SS	≤ 00:02:00 ^b	—
Come-up period	HH:MM:SS	≤ 00:00:10	—
Come-down period	HH:MM:SS	≤ 00:00:10	—
Stabilization period		≤ 00:00:10 HH:MM:SS	1,0 °C

^a During steady state period (see Figure 1).

^b Some indicators can be adversely affected by prolonged exposure to dry heat and vacuum. The minimum practicable settings for evacuation should be used. The time taken should be as consistent as possible in order to minimize potential variability (e.g., desiccation can occur).



Key

PS	stabilization period	PNR	null reaction point
PSS	steady state period	SP	set point
PE	exposure period	TS	stabilization tolerance
PCU	come-up period	TSS	steady state tolerance

Figure 1 — Stabilization time for temperature for steam resistometer

4.5.4 General steam resistometer requirements

4.5.4.1 The chamber shall be supplied with saturated steam from a source external to the chamber. The steam supply shall meet the requirements of ISO 11140-4. Means shall be provided to ensure that the test items are not wetted by entrained water droplets in the steam supply.

4.5.4.2 Air admitted at the end of the cycle shall be filtered through a filter having the capability of removing not less than 99,5 % of 0,5 µm particles.

4.5.4.3 The sample holder shall allow the indicator to be exposed to the test conditions in the manner intended by the indicator manufacturer.

The various types of indicator may require customized sample holders. Sample holders might have to be constructed to hold test items in different vertical and horizontal attitudes to test performance differences. Consult the indicator manufacturer for guidance when verifying label claim performance.

4.5.5 Air leakage test

When determined by the method given in 4.4, the air leakage rate shall not be greater than $0,13 \text{ kPa/min} \times (54,8/V_c)$ where V_c is the chamber volume in litres.

4.5.6 Operation of steam resistometer

4.5.6.1 The chamber shall be designed such that the formation of condensate during any stage of the operating cycle does not affect the required test conditions. In order to avoid excessive condensate formation during the operating cycle it might be necessary to provide thermostatic control of the inner surfaces of the resistometer so that they can be maintained at a specified temperature (e.g. the exposure phase temperature).

4.5.6.2 Before initiating a test cycle, the inner surface of the chamber shall be heated to the test temperature.

4.5.6.3 The equipment shall be provided with means to evacuate the chamber to the vacuum set point to permit adequate air removal prior to admission of steam.

NOTE Steam admission to the chamber should not be used to effect air removal.

4.5.6.4 Temperature and absolute pressure shall be monitored, recorded and verified to be within the limits required in Table 2 for the selected exposure conditions as given in ISO 11138-3 or ISO 11140-1 (see 4.1).

4.6 Ethylene oxide resistometer performance requirements

4.6.1 Measurement accuracy

The EO resistometer shall be capable of measuring the variables listed in Table 3 within the limits there given.

Table 3 — EO resistometer instrumentation requirements (measurement and recording)

Measurement	Unit	Scale range	Resolution	Accuracy (+/-) ^a	Response time ms
Time constant	HH:MM:SS	Selectable	00:00:01	00:00:01	—
Temperature	°C	25 to 80	0,1	0,5	≤ 500
Vacuum	kPa	0 to 100	0,1	1,0	≤ 30
Pressure	kPa	100 to 200	0,1	3,5	≤ 30
Relative humidity ^b	% RH	20 to 90	1	5	15 000
Sterilizing agent concentration	mg/l	25 to 1 200	—	5 % of the concentration targeted	—

^a Accuracy over the test condition range (see 4.1).

^b If relative humidity is not determined by partial pressure.

4.6.2 Recording interval

The measurement system shall have a recording interval for each of the measurements specified in Table 3, of not less than one data point every 10 s.

The percentage relative humidity and sterilizing agent concentration shall be measured by direct measurement using a suitable sensor, or determined from pressure measurements during steady state stages of the operating cycle.

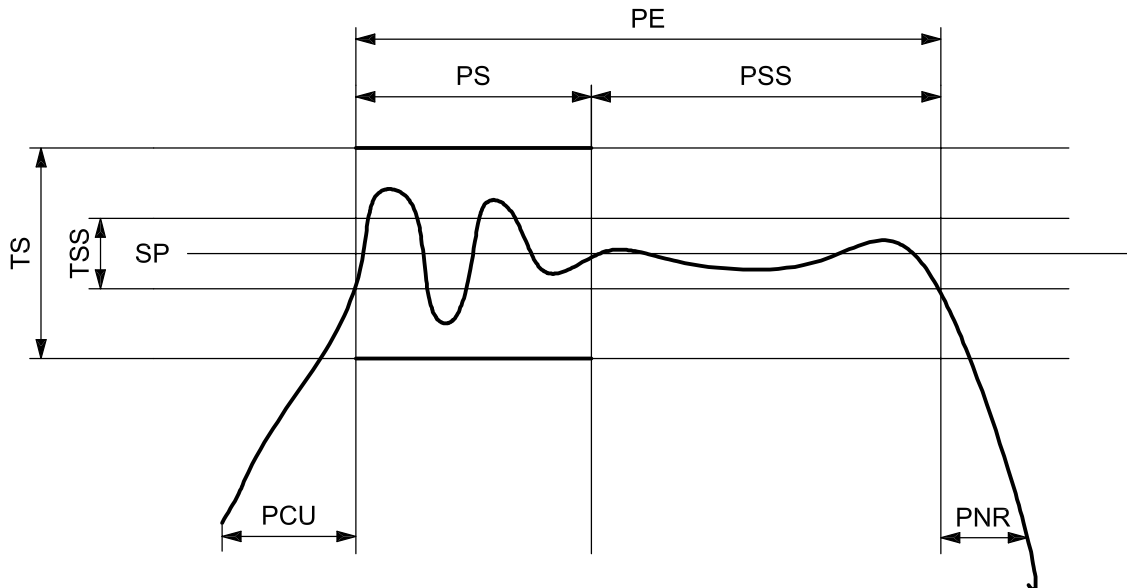
4.6.3 Process control

The EO resistometer process control shall be capable of producing the conditions given in Table 4.

Table 4 — EO resistometer physical design/control specifications

Parameter	Units	Range	Tolerance (+/-)
Time	HH:MM:SS	Selectable	00:00:01
Pressure	kPa	100 to 200	3,5
Sterilizing agent concentration	mg/l	200 to 1 200	5 % of the targeted
Temperature	°C	29 to 65	± 1 ^a
Relative humidity	% RH	30 to 80	10
Vacuum	kPa	4 to 100	1,0
Time to achieve vacuum set-point	HH:MM:SS	≤ 00:0 01:00	—
Come-up period	HH:MM:SS	≤ 00:01:00	—
Come-down period	HH:MM:SS	≤ 00:01:00	—
Stabilization period		≤ 00:02:00 HH:MM:SS	3,0 °C

^a During steady state period (see Figure 2).



Key

- PS stabilization period
- PSS steady state period
- PE exposure period
- PCU come-up period
- PNR null reaction point
- SP set point
- TS stabilization tolerance
- TSS steady state tolerance

Figure 2 — Stabilization time for temperature for ethylene oxide resistometer

4.6.4 General ethylene oxide resistometer requirements

4.6.4.1 Means shall be provided to ensure that test samples are not contacted by liquid ethylene oxide or particles of polymer entering the chamber. Due to the potential for stratification, a mixing device may be used to facilitate homogeneous conditions.

4.6.4.2 The test system including the chamber and door shall be provided with a means of maintaining the temperature of the inner surfaces above the dew point for the test temperature and relative humidity. The chamber environment shall be at thermal equilibrium control conditions before a cycle is initiated.

4.6.4.3 Air admitted at the end of the cycle shall be filtered through a filter having the capability of removing not less than 99,5 % of 0,5 µm particles.

4.6.4.4 The sample holder should allow the indicator to be exposed to the test conditions in the manner intended by the indicator manufacturer.

The various types of indicator may require customized sample holders. Sample holders might have to be constructed to hold test items in different vertical and horizontal attitudes in order to test performance differences. Consult the indicator manufacturer for guidance when verifying label claim performance.

4.6.5 Air leakage test

When determined by the method given in 4.4, the air leakage rate shall not be greater than $0,13 \text{ kPa/min} \times (54,8/V_c)$ where V_c is the chamber volume in litres.

4.6.6 Operation of ethylene oxide resistometer

4.6.6.1 The equipment shall be provided with a means of evacuating the chamber to less than the vacuum set point to permit adequate air removal prior to admission of water vapour and ethylene oxide.

4.6.6.2 Before initiating a test cycle, the inner surface of the chamber shall be heated to the test temperature.

4.6.6.3 Temperature and absolute pressure shall be monitored, recorded and verified to be within the required limits given in Table 4 for the selected exposure conditions as given in ISO 11138-2 or ISO 11140-1 (see 4.1).

4.7 Dry heat (heated air) resistometer performance requirements

4.7.1 Measurement accuracy

The dry heat resistometer shall be capable of measuring time and temperature within the limits given in Table 5.

Table 5 — Dry heat resistometer instrumentation requirements (measurement and recording)

Measurement	Unit	Scale range	Resolution	Accuracy (+/-) ^a	Response time ms
Time	HH:MM:SS	Selectable	00:00:01	00:00:01	–
Temperature	°C	120 to 200	0,1	0,5	≤ 500

^a The accuracy over the test condition range (see 4.1).

4.7.2 Recording interval

The measurement system shall have a recording interval for each of the measurements specified in Table 5, of not less than one data point every 10 s.

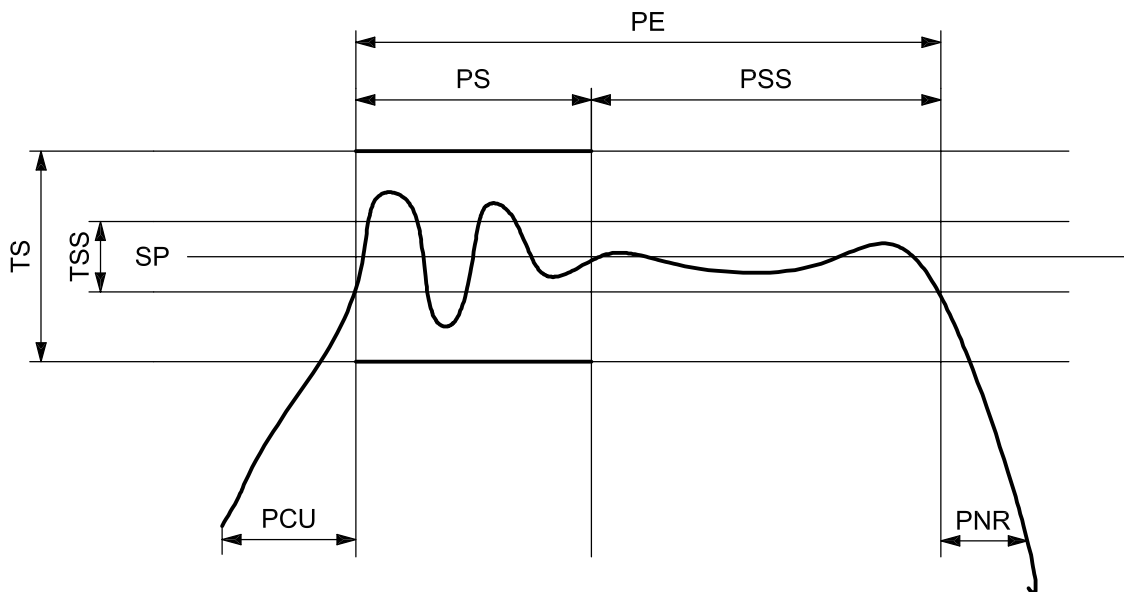
4.7.3 Process control

The dry heat resistometer process control shall be capable of producing the conditions given in Table 6.

Table 6 — Dry heat resistometer physical design/control specifications

Parameter	Units	Range	Tolerance (+/-)
Time	HH:MM:SS	Selectable	00:00:02
Temperature	°C	120 to 200	2,5 ^a
Come-up period	HH:MM:SS	≤ 00:01:00	—
Come-down period	HH:MM:SS	≤ 00:01:00	—
Stabilisation period		≤ 00:02:00 HH:MM:SS	4,0 °C

^a After a 2 min stabilization time (see Figure 3).



Key

- PS stabilisation period
- PSS steady state period
- PE exposure period
- PCU come-up period
- PNR null reaction point
- SP set point
- TS stabilisation tolerance
- TSS steady state tolerance

Figure 3 — Stabilization time for temperature tolerance for dry heat resistometer

4.7.4 General dry heat (heated air) resistometer requirements

4.7.4.1 Test samples shall be loaded on to a suitable sample holder. The sample holder shall not adversely affect the performance of the indicator.

4.7.4.2 The test gas shall be of the type for the indicator's intended use (generally air).

4.7.5 Operation of dry heat (heated air) resistometer

4.7.5.1 Before initiating a test cycle, the inner surface of the chamber shall be heated to the test temperature.

This can require thermostatic control of the inner surfaces of the chamber and/or door to be at the selected operating temperature.

4.7.5.2 Temperature shall be monitored, recorded and verified to be within the required limits given in Table 6 for the selected exposure conditions as given in ISO 11138-4 or ISO 11140-1 (see 4.1).

4.8 Vaporized hydrogen peroxide process resistometer performance requirements

4.8.1 Measurement accuracy

The H₂O₂ resistometer shall be capable of measuring the following conditions within the limits given in Table 7.

Table 7 — Vaporized hydrogen peroxide resistometer instrumentation requirements (measurement and recording)

Measurement	Unit	Scale range	Resolution	Accuracy (+/-)	Response time ms
Time	HH:MM:SS	Selectable	00:00:01	00:00:01	—
Temperature	°C	20 to 60	0,1 °C	1,0 °C	≤ 500
Pressure	kPa	0 to 102	0,01	0,25 %	≤ 30
Hydrogen peroxide	mg/l	1 to 10	0,1	0,3	—

4.8.2 Recording interval

The measurement system shall have a recording interval for each of the measurements specified in Table 1 of not less than 1 one data point per second.

The sterilizing agent concentration shall be measured by direct measurement using a suitable sensor or determined from pressure measurements during steady state stages of the operating cycle.

4.8.3 Process control

A vaporized hydrogen peroxide resistometer shall be capable of producing the conditions given in Table 8.

Table 8 — Vaporized hydrogen resistometer physical design/control specifications

Measurement	Unit	Limit	Resolution	Accuracy (+/-)
Time	HH:MM:SS.S	Selectable	00:00:01	00:00:00,5
Temperature	°C	20 to 60	0,1 °C	1,0 °C
Pressure	kPa	0 to 102	0,01	0,25 %
Hydrogen Peroxide	mg/l	1 to 5	0,1	0,3

4.8.4 General vaporized hydrogen peroxide resistometer requirements

4.8.4.1 Air admitted at the end of the cycle shall be filtered through a filter having the ability to remove not less than 99,5 % of 0,5 µm particles.

4.8.4.2 The sample holder should allow the indicator to be exposed to the test conditions in the manner intended by the indicator manufacturer.

The various types of indicator may require customized sample holders. Sample holders may have to be constructed to hold test items in different vertical and horizontal attitudes in order to test performance. Consult the indicator manufacturer for guidance when verifying label claim performance.

4.8.4.3 The test system, including the chamber and the door, shall be provided with means to maintain the temperature of the inner surfaces above the dew point for the test temperature. The chamber environment shall be at thermal equilibrium with control set-points before a cycle shall be initiated.

4.8.4.4 The contact surface of the chamber and the sample holder shall be constructed from materials that do not interfere with the sterilizing agent concentration.

4.8.5 Air leakage test

When determined by the method given in 4.4, the air leakage rate shall not be greater than $0,5 \times 10^{-2}$ kPa/min (0,05 mbar/min).

4.8.6 Operation of vaporized hydrogen peroxide resistometer

4.8.6.1 The equipment shall be provided with a means of evacuating the chamber to less than the vacuum set point in order to permit air and sterilizing agent removal.

4.8.6.2 Before initiating a test cycle, the inner surface of the chamber shall be heated to the test temperature.

4.8.6.3 Temperature, sterilizing agent concentration and absolute pressure shall be monitored, recorded and verified to be within the required limits given in ISO 11138-4 and ISO 11140-1 (see 4.1).

5 Recording systems

5.1 Measurement systems

Temperature sensors shall be either platinum resistances that comply with class A of IEC 60751:1983 or thermocouples that comply with IEC 60584 or be another sensor system having an equivalent performance.

5.2 Calibration

Calibration shall be carried out using a working or reference standard that is traceable to the national standard or primary standard.

NOTE Calibration procedures are provided in ISO 10013.

6 Documentation

6.1 General

A test cycle consists of a sequence of phases and steps. This can be graphically described by plotting against time, one or more variables relevant to a desired test cycle. Phases are parts of a cycle that can be uniquely described (e.g. pre-vacuum, preconditioning, steam charge, exposure, post vacuum, air vent ...etc.). Steps are parts of a phase which also have uniquely described functions that may be repeated as a sequence within a phase a selected number, *n*, of times (e.g., 1[steam-pulse, vacuum] 2[steam-pulse, vacuum] ... *n*[steam-pulse, vacuum]). Cycle documentation is used to record that the events comprising a test cycle have occurred. Phase and step duration times indicate the time in the respective phase or step. The cycle duration indicates the total time required to perform a test cycle.

6.2 Minimum information

For every test cycle run, the following minimum information shall be documented:

- a) the phase/step description and associated set point(s);
- b) the date and time the cycle started;
- c) the cycle number and resistometer identification number;
- d) the data recorded from the resistometer instrumentation;
- e) operator.

NOTE Annex D shows typical documentation formats.

Annex A (informative)

Additional performance characterization — Steam

A.1 General

There are many factors that can influence the performance of the biological and chemical indicators used to evaluate different types of steam sterilization processes. Tests are performed on a routine basis as prescribed in ISO 11138-2 and ISO 11140-1 to characterize indicator performance. In addition to these normative tests, other tests also may be used to verify that indicators are suitable for their intended applications.

If the chamber temperature is higher than the pressure corresponding to saturated steam, a condition of superheated steam is present.

Superheated steam is a recognized fault condition in steam sterilization. Superheated steam can be intentionally generated in a resistometer to assess the ability of chemical and biological indicators to detect this condition.

Figure A.1 graphically depicts the typical sequence of the steam resistometer used in saturated steam exposure processes.

A.2 Chamber air removal

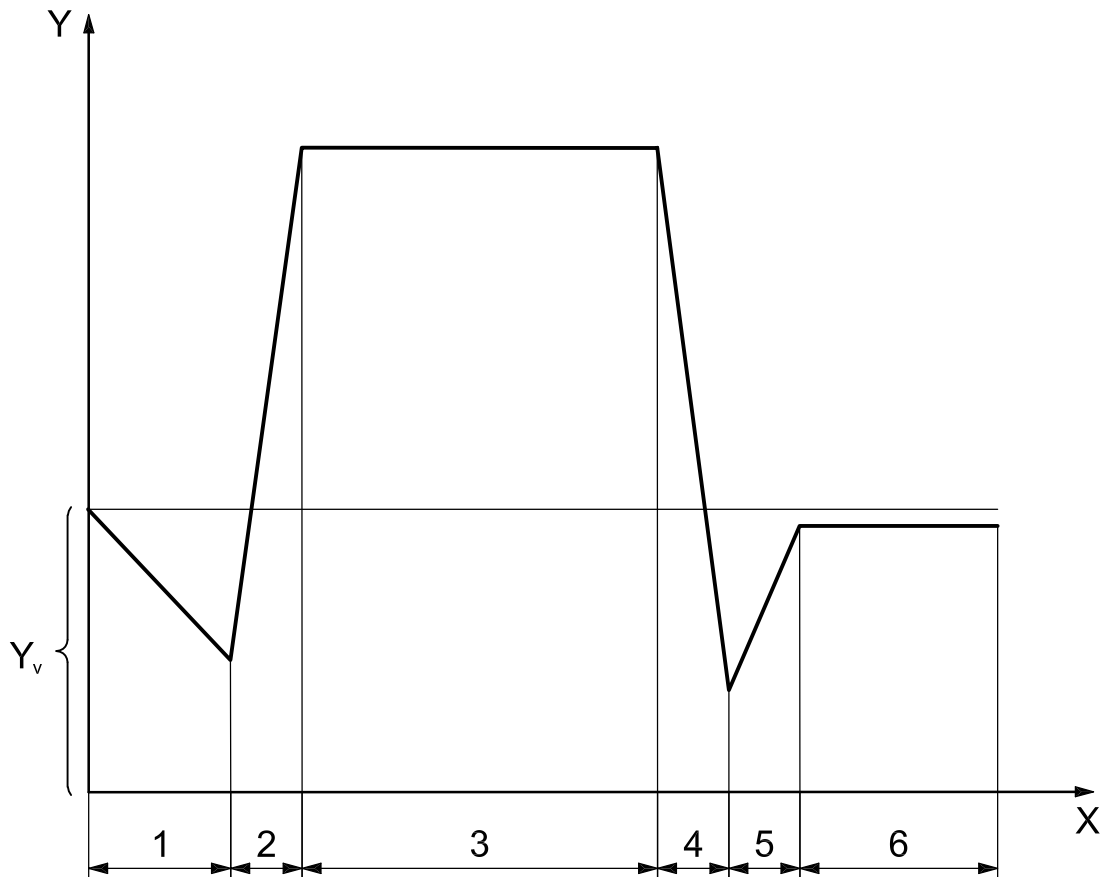
There are demonstrable differences in indicator performance based upon residual air in the test system/indicator device. Varying the level of chamber air removal provides information relative to the change in performance expected for indicators with processes that use different vacuum levels and types of air removal prior to come-up to the selected sterilization temperature. If this range of tests is used, a range of depths of vacuum should be tested in order to characterize product performance.

A.3 Vacuum/pressurization rate

Alteration of the vacuum/pressurization rate can be utilized to evaluate device damage and resultant performance change related to the rate at which pressure changes in sterilization processes. During resistometer testing, pressure changes occur at rates significantly faster than expected in normal applications. If the resistometer is capable of slower pressure changes, indicators may be tested using slower pressurization and evacuation phases. Observations that have been reported to produce changes in indicator performance might include:

- a) indicating chemicals migrating by capillary action rather than wicking along the intended path;
- b) ballooning of the primary package resulting in inconsistent synergistic inactivation characteristics (air/steam mixtures);
- c) delamination;
- d) dehydration/desiccation and sublimation;
- e) rates of chemical reactions.

NOTE An observation view port or camera is helpful for visual observation of physical changes of the indicator that could affect performance of the indicator.



Key

X time
 Y steam pressure
 Y_v vacuum

- 1 air removal: air is removed from the test chamber by evacuation to a selected vacuum level
- 2 come-up: steam is injected into the test chamber until the selected test temperature is attained
- 3 exposure time: the test chamber is maintained at the selected test temperature for the selected exposure time
- 4 come-down: the test chamber is exhausted to a selected level

NOTE As the chamber pressure drops to atmospheric pressure or below, the chamber temperature drops below temperatures that contribute to indicator kinetics. A post-vacuum further cools samples.

- 5 air vent: the test chamber is vented to atmospheric pressure
- 6 cycle complete: test samples are manually removed from the chamber

Figure A.1 — Steam test sequence

A.4 Specific variable response

Indicators that are designed to react to multiple variables or parameters may react partially or completely on exposure to only one of the critical process parameters. Indicators may be exposed to different combinations of these variables in order to observe the indicator response. This may include exposing the indicators to temperatures or conditions slightly outside the specified values.

A.5 Range characterization

Indicators may be tested over the application range specified by the manufacturer in order to quantify performance characteristics. This type of testing will generally be as prescribed by the applicable normative standards for a given type of indicator, and can be performed whenever there is a change to the indicator design or manufacturing process.

© ISO 2006 – All rights reserved

Annex B (informative)

Additional performance characterization — Ethylene oxide

B.1 General

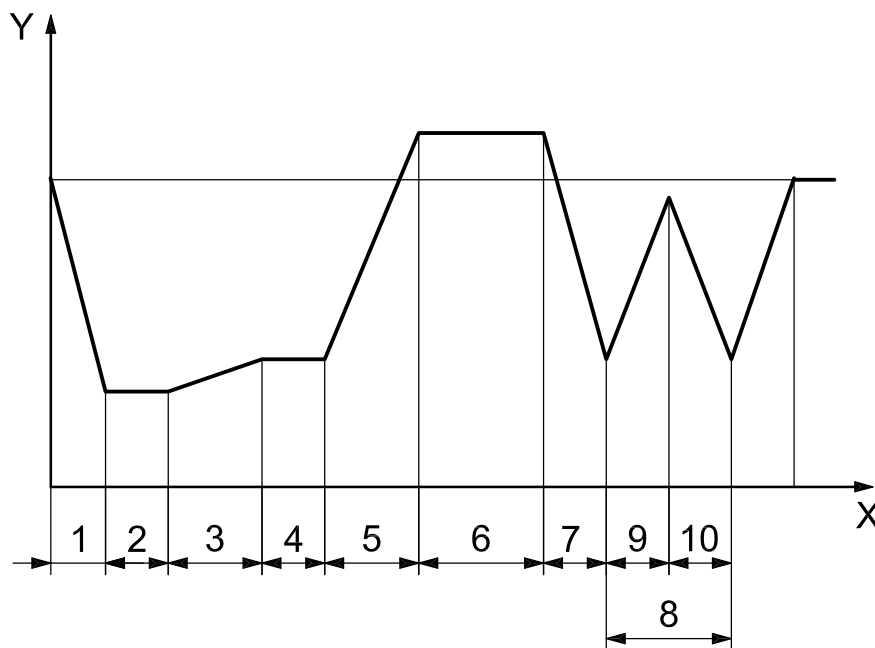
There are many factors that can influence the performance of the biological and chemical indicators used to evaluate different types of ethylene sterilization process. Tests are performed on a routine basis as prescribed in ISO 11138-2 and ISO 11140-1 to characterize indicator performance. In addition to these normative tests, other tests may also be used to verify that the indicators are suitable for their intended applications.

Figure B.1 graphically depicts the typical sequence of an ethylene oxide resistometer used in a pure ethylene oxide process.

B.2 Chamber air removal

Residual air in the test system/indicator device can result in demonstrable differences in indicator performance. The chamber air removal test provides information relative to the change in performance expected for indicators with processes that use different vacuum levels and mechanical air removal techniques prior to admission of ethylene oxide. Results are compared to the performance requirements for the respective indicators.

- a) Air removal: air is removed from the test chamber by evacuation to a selected vacuum level.
- b) Vacuum hold: the sterilizing chamber is held at the pre-vacuum level for a selected time to allow the environmental temperature to return to the set chamber temperature that decreased as a result of pre-vacuum cooling.
- c) Humidification: sub-atmospheric steam is injected into the test chamber until the selected test humidity is attained.
- d) Humidity dwell: the test chamber is maintained at the selected humidification level for a selected time to allow the test sample to equilibrate with the environmental test condition.
- e) Come-up: the test chamber is pressurized with vaporized ethylene oxide (sterilizing agent) until a selected sterilizing agent partial pressure (sterilizing agent concentration) is attained.
- f) Exposure time: the selected temperature, humidity and ethylene oxide (sterilizing agent) concentration are maintained for a selected time.
- g) Come-down: the test chamber is evacuated to a selected vacuum level to remove the majority of sterilizing agent.
- h) Air vent: the test chamber is vented to atmospheric pressure.
- i) Cycle complete: test materials are manually removed from the test chamber.



Key

- X time
- Y absolute pressure
- 1 pre-vacuum: see B.2 a)
- 2 vacuum hold: see B.2 b)
- 3 humidification: see B.2 c)
- 4 humidity dwell: see B.2 d)
- 5 sterilizing agent charge: see B.2 e)
- 6 exposure: see B.2 f)
- 7 exhaust/post vacuum: see B.2 g)
- 8 air wash: the test chamber is pressurized with air to a selected level (9) and evacuated to a selected vacuum level (10) for a selected number of times
- 9 air vent: see B.2 h)
- 10 cycle complete: see B.2 i)

Figure B.1 — Sequence of ethylene oxide resistometer used in ethylene oxide exposure processes

B.3 Vacuum/pressurization rate

Alteration of the vacuum/pressurization rate can be utilized to evaluate device damage and resultant performance change related to the rate at which pressure changes in sterilization processes. During resistometer testing, pressure changes occur at rates significantly faster than expected in normal applications. If the resistometer is capable of slower pressure changes, indicators may be tested using slower pressurization and evacuation phases. Observations that have been reported to produce changes in indicator performance might include:

- ballooning of the primary package;
- delamination.

NOTE An observation view port is helpful for visual observation of physical changes of the indicator device that could affect performance of the indicator.

B.4 Specific variable response

Indicators that are designed to react to multiple variables or parameters could react partially or completely on exposure to only one of the critical process parameters. Indicators may be exposed to different combinations of these variables to observe the indicator response. This may include exposing the indicators to temperatures or gas concentrations slightly outside of the specified values.

B.5 Range characterization

Indicators may be tested over the application range specified by the manufacturer to quantify performance characteristics. This type of testing will generally be as prescribed by the applicable normative standards for a given type of indicator, and can be performed whenever there is a change to the indicator design or manufacturing process.

Annex C (informative)

Additional performance characterization — Dry heat

C.1 General

There are many factors that can influence the performance of the biological and chemical indicators used to evaluate different types of dry heat sterilization processes. Tests are performed on a routine basis as prescribed in ISO 11138-4 and ISO 11140-1 to characterize indicator performance. In addition to these normative tests, other tests may also be used to verify that the indicators are suitable for their intended applications.

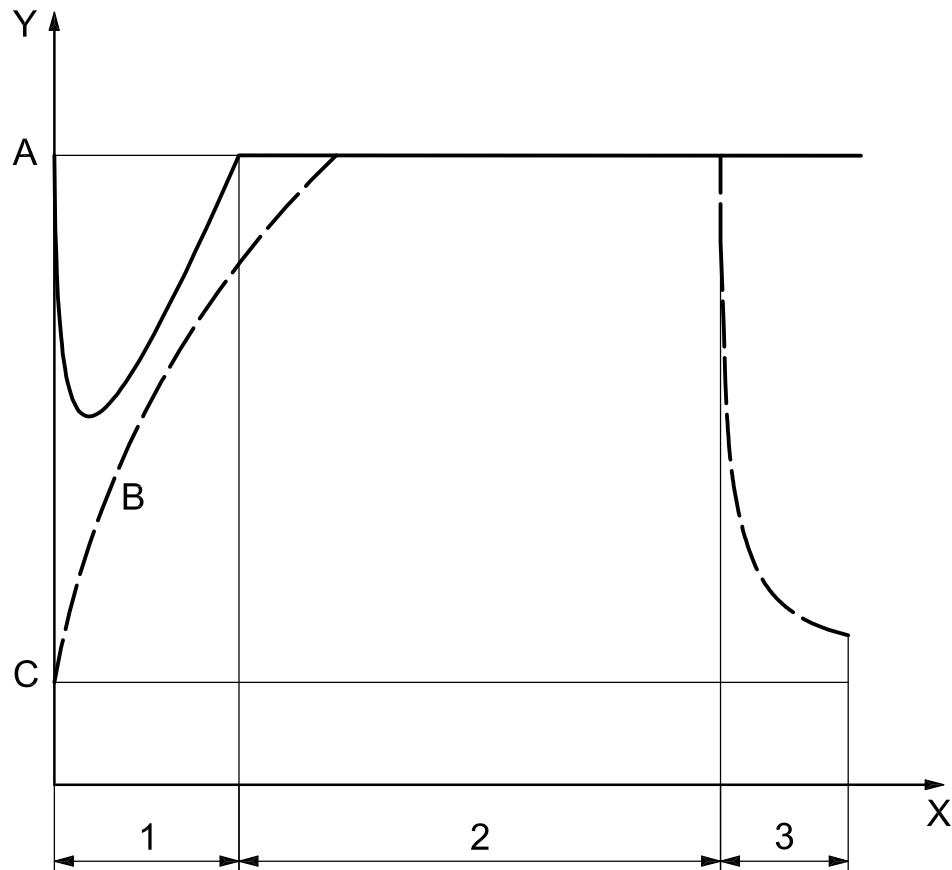
Figure C.1 graphically depicts the typical sequence of the resistometer used in a dry heat exposure process.

C.2 Specific variable response

Indicators that are designed to react to multiple variables or parameters may react partially or completely on exposure to only one of the critical process parameters. Indicators may be exposed to different combinations of these variables in order to observe the indicator response. This may include exposing the indicators to temperatures or conditions slightly outside of specified values.

C.3 Range characterization

Indicators may be tested over the application range specified by the manufacturer in order to quantify performance characteristics. This type of testing will generally be as prescribed by the applicable normative standards for a given type of indicator, and may be performed whenever there is a change to the indicator design or manufacturing process.



Key

X time

Y temperature

A exposure temperature

B sample temperature

C ambient temperature

- 1 sample placement: the test samples are placed in a preheated test environment that is designed to recover to the selected test temperature within the times specified in the normative references
- 2 exposure time: the test chamber is maintained at the selected test temperature for the selected exposure time.
- 3 cycle complete: test samples are manually removed from the test environment

NOTE An observation view port or camera is helpful for visual observation of physical changes of the indicator that could affect performance of the indicator.

Figure C.1 — Sequence of dry heat resistometer used in dry heat exposure processes

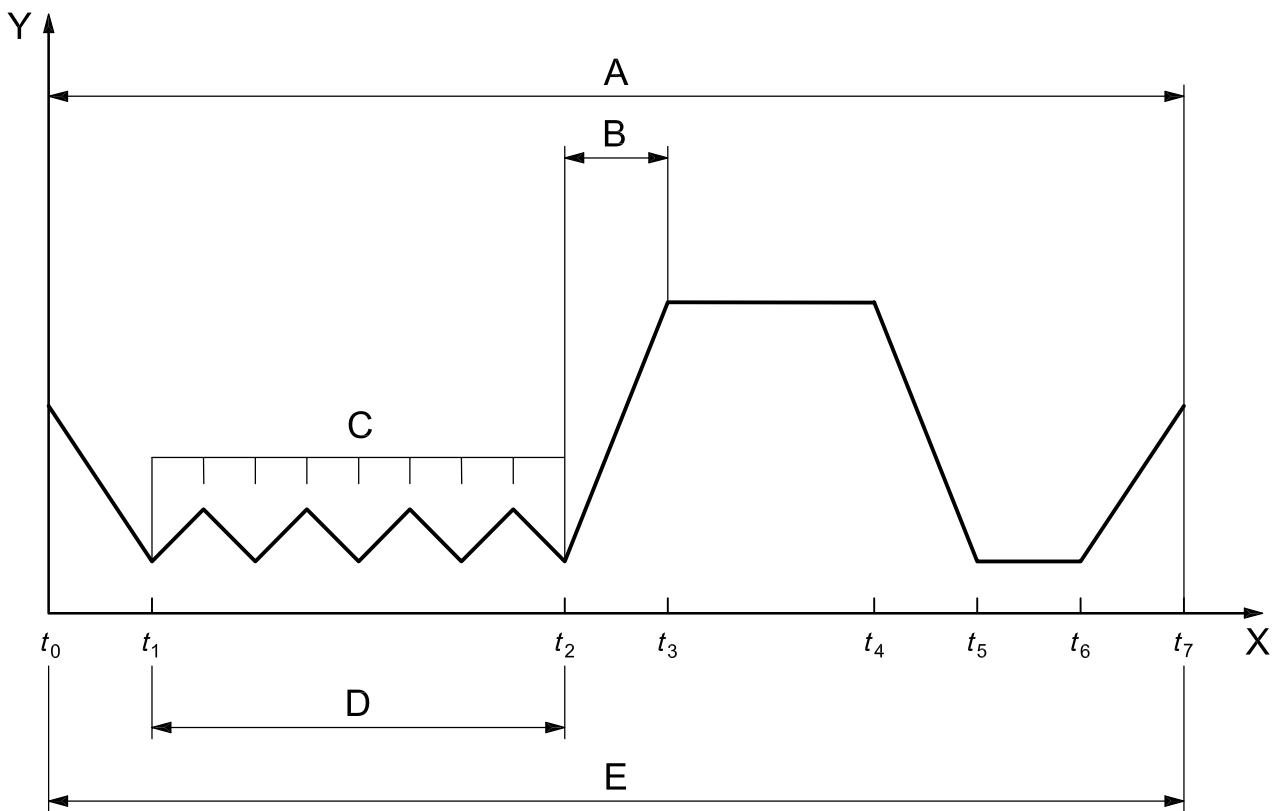
Annex D
(informative)

Resistometer documentation and derivations

D.1 General

Resistometer documentation presents cycle information from each cycle that is performed. These data may be presented digitally or graphically, provided they meet the requirements of Clause 6.

Figure D.1 presents an example of a resistometer exposure cycle.



Key

- X time
- Y process variable measured
- A cycle (process)
- B phases
- C steps
- D phase duration time = $(t_2 - t_1)$
- E cycle duration time = $(t_7 - t_0)$

Figure D.1 — Example of standard resistometer cycle

D.2 Calculation of relative humidity concentration

D.2.1 Relative humidity is the ratio of the mass or partial pressure in an environment to the mass or partial pressure that a saturated environment could hold at a given temperature, by definition. This ratio is usually expressed as percent relative humidity (% RH) by multiplying the ratio by 100. Measurement of relative humidity is typically determined by measuring the partial pressure of water vapour in an enclosed environment. Humidity determinations of ± 5 % RH are reasonably obtained. Mathematically this is expressed as follows:

$$\% \text{ RH} = \frac{\text{Actual partial pressure of water vapour (at test temperature)}}{\text{Saturation vapour pressure of water (at test temperature)}} \times 100 \quad (\text{D.1})$$

EXAMPLE 1

Test temperature: 54,4 °C

Saturation pressure at 54,4 °C (steam table): 15,3 kPa

Measured partial pressure (humidity added): 8 kPa

$$\% \text{ RH} = \frac{8 \text{ kPa}}{15,3 \text{ kPa}} \times 100 \% = 52,3 \% \text{ RH}$$

D.2.2 Primary measurement of relative humidity is best performed by direct measurement of physical properties using instrumentation that is easy to calibrate and that maintains accuracy, such as temperature and pressure measurement devices. Direct measurement of humidity is typically performed by measurement of partial pressure at a temperature as described above or by dew point measurement.

Dew point is typically used for environmental monitoring, as the measurement/response time is usually too slow for control of fast processes. Dew point measurement uses a mirror that is chilled to a temperature at which the environmental humidity condenses. The formation of condensate on the surface of the mirror is detected using a photo sensor and light emitting source combination. The condensation temperature represents the saturation temperature or dew point. A dew point can be expressed in terms of relative humidity using the same format as above, but substituting the dew point pressure from a steam table for the measured partial pressure of humidity. Accuracies are typically within 1,5 % RH provided contaminants do not shift the dew point measurement.

EXAMPLE 2

Environmental temperature: 54,4 °C

Measured dew point temperature: 40,5 °C

Saturation pressure at 54,4 °C: 15,3 kPa

Dew point saturation pressure at 40,5 °C: 7,6 kPa

$$\% \text{ RH} = \frac{7,6 \text{ kPa}}{15,3 \text{ kPa}} \times 100 \% = 49,7 \% \text{ RH}$$

D.2.3 Secondary relative humidity monitoring devices are:

- electrical relative humidity sensors;
- electro-hygrometric sensors;
- spectroscopic (infrared/ultraviolet) hygrometers;
- gas chromatographs (for water content analysis).

These devices rely on generation of calibration maps that describe condition and signal characteristics for each variable condition, which are subject to error. Calibration errors are related to an accumulation of errors associated with the primary standard, the uniformity and precision of the calibration transfer environment, interpolation between test points and the native accuracy, linearity and stability of the device under calibration, and number of parameters being calibrated to provide the measurement of the specific quantity in question. Measurement systems that rely upon fewer rather than more calibrated parameters are preferred for this reason.

D.3 Calculation of ethylene oxide concentration

D.3.1 Introduction

The theoretical calculation of the concentration of ethylene oxide in a sterilizer, after the initial charge of gas and at temperature equilibrium, is based on the ideal gas law ($pV = nRT$)². This idealized relationship does not provide an exact determination of the gas concentration; however, in the range of temperatures and pressures encountered in ethylene oxide sterilization, determination of the ideal gas law is considered an adequate method of producing consistent test conditions. The assumptions given below are made.

- a) The mixture of ethylene oxide, water vapour, and air (and the diluent gas when used) behaves as an ideal gas.
- b) There is no selective loss of a component of the mixture, e.g., by means of absorption or adsorption, i.e., the initial composition of the mixture remains unchanged throughout the cycle.
- c) The label information on the cylinders containing the gas is accurate, and the percentage by weight of the mixture of gas remains constant during admission to the sterilizer.
- d) Pressure readings are in absolute units.

D.3.2 Calculations

The ethylene oxide concentration is calculated from the difference in total pressure resulting from the addition of ethylene oxide plus carrier or diluent gas, and the sterilizer chamber temperature.

The difference in total pressure due to the addition of ethylene oxide and diluent can be expressed as:

$$pV = nRT \tag{D.2}$$

Rearranging the above gas law facilitates the calculation of ethylene oxide concentration, using the following equation:

$$C = \frac{Kp}{RT} \tag{D.3}$$

where

- C is the ethylene oxide concentration in milligrams per litre;
- K is the constant for a given diluent (see Equation D.4);
- p is the difference in total pressure due to ethylene oxide and diluent;
- R is the gas constant (see Table D.3);
- T is the absolute temperature of ethylene oxide/diluent gas mixture giving pressure p .

2) p represents pressure; V represents volume; n represents the number of moles; R is the gas constant; T represents temperature.

$$K = \frac{4,4 \times 10^4 M_w}{M_w + 44 (100 - w)} \quad (\text{D.4})$$

where

M is the average molecular mass of diluent gas (multicomponent diluent gas) or molecular mass of diluent gas (single component diluent gas);

w is the mass fraction of ethylene oxide in diluent mixture.

Table D.1 and Table D.2 list constants and molecular mass of some common ethylene oxide/diluent formulations.

Table D.1 — EO/diluent constants, K

EO/diluent	Diluent molecular mass Average molecular mass	K mg/g mol ^a
8,5 % EO/91.5 % CO ₂	44	$3,74 \times 10^3$
10 % EO/63 % HCFC-124/27% HCFC-22	121,49	$9,989 \times 10^3$
8,6 % EO/91.4 % HCFC-124	136,5	$9,942 \times 10^3$
20 % EO/80 % CO ₂	44	$8,80 \times 10^3$
100 % EO	N/A	$4,40 \times 10^4$

^a Use in Equation 3 when calculating mg/l.

NOTE Gas cylinders containing mixtures of carbon dioxide and ethylene oxide, which are intended for multiple cycle gas charges, will not consistently deliver the same concentration of ethylene oxide per charge. Therefore, pressure measurements are not an accurate indicator of ethylene oxide gas concentration, and some other method must be used to determine this parameter.

Table D.2 — Molecular mass

Gas	Molecular mass
Ethylene oxide (EO)	44,0
HCFC-124	136,5
HCFC-22	86,47
70 % HCFC-124 + 30 % HCFC-22	121,49
Carbon dioxide	44,0

D.3.3 Example calculations/Determining ethylene oxide concentration

EXAMPLE 3

Assume a process that uses 10 % ethylene oxide and 90 % HCFC-124/HCFC-22. After gas injection the rise in pressure is 155,74 kPa. If the temperature at the end of gas injection is 55 °C, then:

$$p = 155,74 \text{ kPa}$$

$$T = 55 \text{ °C} = 328 \text{ K}$$

$$R = 8,314 \frac{\text{J}}{\text{mol}\cdot\text{K}}$$

ISO 18472:2006(E)

See Table D.3 for gas constants.

$$K = 9,989 \times 10^3 \text{ mg/g mol}$$

Using Equation 3, the ethylene oxide concentration is:

$$C = \frac{Kp}{RT} = \frac{9,889 \times 10^3 \times 155,74}{8,314 \times 328} = 570,5 \text{ mg/l}$$

EXAMPLE 4

Assume a process that uses 100 % ethylene oxide. After gas injection the rise in pressure is 29,43 kPa. If the temperature at the end of gas injection is 55 °C, then:

$$p = 29,43 \text{ kPa}$$

$$T = 55 \text{ °C} = 328 \text{ °K}$$

$$R = 8,314 \frac{\text{J}}{\text{mol} \cdot \text{K}}$$

See Table D.3 for gas constants.

$$K = 4,40 \times 10^4 \text{ mg/g mol}$$

Using Equation D.3, the ethylene oxide concentration is:

$$C = \frac{Kp}{RT} = \frac{4,40 \times 10^4 \times 29,43}{8,314 \times 328} = 474,9 \text{ mg/l}$$

D.3.4 Derivation of Equation D.4

Because most operations record the pressure change during ethylene oxide gas injection, Equation D.4 was derived to allow the calculation of ethylene oxide concentration from the pressure rise due to ethylene oxide gas injection, with or without a single diluent gas such as carbon dioxide or HCFC-124. The purpose of this equation is to provide a simple and rapid method for calculating ethylene oxide concentration for production sterilizers as well as for experimental facilities.

The pressure rise can be expressed as:

$$P = P_{\text{Exposure}} - P_{\text{Humidification}}$$

See Equation D.1 for the pressure rise due to humidification. $P_{\text{Humidification}}$ is the absolute pressure at which humidification takes place, which is the pressure rise due to humidification plus the last prevacuum pressure achieved (in absolute units such as psi, kPa, or mbar).

Using this value for the pressure and, to obtain the concentration for ethylene oxide in mg/l for any gas mixture, Equation D.3 is generalized using Equation D.4 to include the molecular masses and mass fractions of the ethylene oxide and diluent gases in the mixture:

$$C = \frac{4,4 \times 10^4 M_w p}{[M_w + 44(100 - w)]RT}$$

where

M is the average molecular mass of the diluent;

w is the mass fraction of ethylene oxide in the mixture.

To calculate M , use the following expression for the average molecular mass:

$$M = \sum_i M_i w_i$$

where

M_i is the molecular mass of diluent gas component i ;

w_i is the mass fraction of diluent component i in the diluent (not the ethylene oxide mixture).

Using this in the equation for the concentration,

$$C = \frac{4,4 \times 10^4 w p \sum_i M_i w_i}{E \sum_i [M_i w_i + 44(100 - w)] RT}$$

Table D.3 — Gas constants, R

Pressure	Volume	Temperatures	R
atm	cm ³	K	82,057
atm	l	K	0,082 05
bar	l	K	0,083 14
kg/m ²	l	K	847,80
kg/cm ²	l	K	0,084 78
kPa	l	K	8,312
kPa	m ³	K	0,008 312

NOTE

1 atm = 760 mmHg = 29,92 inHg = 14,70 psi = 1,013 bar = 1,033 kg/cm² = 101,3 kPa (kN/m²);

1 l = 1 000 cm³ = 0,035 32 ft³;

1 m³ = 1 000 l;

K = °C + 273,15.

Bibliography

- [1] ISO 10013:1995, *Guidelines for developing quality manuals*
- [2] ISO 11138-5, *Sterilization of health care products — Biological indicators — Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization processes*
- [3] ISO 11140-3, *Sterilization of health care products — Chemical indicators — Part 3: Class 2 indicator systems for use in the Bowie and Dick steam penetration test*
- [4] ISO 11140-5, *Sterilization of health care products — Chemical indicators — Part 5: Class 2 indicators for Bowie and Dick air removal test sheets and packs*
- [5] BLOCK, S.S. *Disinfection, sterilization, and preservation*, 3d ed., Philadelphia, Lea and Febiger, 1983, p. 774
- [6] *International Vocabulary of Basic and General Terms in Metrology, (VIM)*, BIPM/IEC/IFCC/ISO/IUPAC/IUPAP/OIML, 2nd ed., 1993
- [7] JOSLYN, L.J. Biological Indicator Evaluator Resistometer (BIER) Laboratory Test System — Equipment Manual, *Basic Principles of Resistometers*, STERIS Corporation
- [8] MCCULLOCH, E.C. *Disinfection and sterilization*, 2nd ed., Philadelphia, Lea and Febiger, 1945
- [9] MORRISSEY, R.F. and PHILLIPS, G.B. *Sterilization technology: A practical guide for manufacturers and users of health care products*, Van Norstrand Reinhold, 1993
- [10] MOSLEY, G.A. and GILLIS, J.R. Operating Precision of Steam BIER Vessels and the Interactive Effects of Varying Z Values on the Reproducibility of Listed D Values, *PDA Journal of Pharmaceutical Science and Technology*, 2002, **56**, (6), pp. 318-331
- [11] MOSLEY, G.A., Estimating the Effects of EtO BIER-Vessel Operating Precision on D-Value Calculations, *Medical Device and Diagnostic Industry*, **24**, (4), pp. 46-56
- [12] OXBORROW, G.S., TWOHY, C.W. and DEMITRIUS, C. Determining the variability of BIER vessels for EtO and steam, *Medical Device and Diagnostic Industry*, May 1990, **12**, (5), pp. 78-83
- [13] PERKINS, J.J. *Principles and methods of sterilization in health sciences*, 2nd ed. Springfield, IL, Charles C. Thomas, 1969, pp. 286-310
- [14] PFLUG, I.J. and ESSELEN, W.B. Development and application of apparatus for study of thermal resistance of bacterial spores and thiamine at temperatures above 250°F, *Food Technol.*, 1953, **7**, pp. 237-241
- [15] U.S. FOOD AND DRUG ADMINISTRATION, *Guideline on the general principles of process validation*, Rockville, MD, FDA, May 1987
- [16] U.S. FOOD AND DRUG ADMINISTRATION, *Preproduction quality assurance planning: recommendations for medical device manufacturers*, Rockville, MD, FDA/CDRH, 1990
- [17] WEXLER, A. (ed.), *Humidity and moisture: measurement and control in science and industry*. 4 vols. New York, Reinhold Publishing, 1965

.....

ICS 11.080.01

Price based on 28 pages