American National Standard

ANSI/AAMI ST44:2002

Resistometers used for characterizing the performance of biological and chemical indicators

AAMI Association for the Advancement of Medical Instrumentation

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Despite periodic review and revision (at least once every five years), a standard or recommended practice is necessarily a static document applied to a dynamic technology. Therefore, a standards user must carefully review the reasons why the document was initially developed and the specific rationale for each of its provisions. This review will reveal whether the document remains relevant to the specific needs of the user.

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American National Standard

ANSI/AAMI ST44:2002
(Combined revision of ANSI/AAMI ST44:1992
and ANSI/AAMI ST45:1992)

Resistometers used for characterizing the performance of biological and chemical indicators

Developed by
Association for the Advancement of Medical Instrumentation

Approved 12 September 2002 by
American National Standards Institute, Inc.

Abstract: This standard establishes minimum safety and performance requirements for biological indicator-evaluator resistometer (BIER) and chemical indicator-evaluator resistometer (CIER) vessels.

Keywords: dry heat, EO, ethylene oxide, steam, sterilization equipment, thermal

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AAMI Sterilization Standards Committee

This standard was developed by the AAMI Indicator-Evaluator Resistometer Working Group under the auspices of the Sterilization Standards Committee of the Association for the Advancement of Medical Instrumentation. Working group approval of the standard does not necessarily imply that all working group members voted for its approval.

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NOTE—Participation by federal agency representatives in the development of this American National Standard does not constitute endorsement by the federal government or any of its agencies.

Foreword

This American National Standard was developed by the Association for the Advancement of Medical Instrumentation (AAMI) Indicator-Evaluator Resistometer Working Group under the auspices of the AAMI Sterilization Standards Committee. The objective of this standard is to provide performance requirements, terminology, equipment specifications, and guidance on resistometers that characterize the performance of biological and chemical indicators intended for use with sterilization processes using steam, ethylene oxide (EO), and dry heat.

This standard is a combined revision of ANSI/AAMI ST44:1992, *BIER/EO gas vessels*, and ANSI/AAMI ST45:1992, *BIER/steam vessels*, and is based in part on these two documents as well as ISO 11140-2:1998, *Sterilization of health care products—Chemical indicators—Part 2: Test equipment and methods*.

Recognizing that a resistometer vessel can be used for characterization of either biological or chemical indicators, this standard has taken this commonality into consideration. This standard combines the previously separate standards for ethylene oxide (ST44) and steam (ST45) resistometers, and also includes specifications for dry heat resistometers. This standard was written with the knowledge and understanding of the advancement in available technology necessary to create resistometers capable of accurately and consistently characterizing biological and chemical indicators. One significant change to this standard as compared to the ST44 and ST45 standards is that specific resistometer exposure parameters for characterizing indicators have been taken out of this standard and can be found in the respective standards for biological and chemical indicators.

The concepts incorporated into this document should not be considered inflexible or static. To remain relevant, this standard must be reviewed and updated periodically to assimilate progressive technological developments. This standard reflects the conscientious efforts of those individuals and organizations substantially concerned with its scope and provisions to develop a standard for those performance levels that can reasonably be achieved at this time.

As used within the context of this document, “shall” indicates requirements strictly to be followed to conform to the recommended practice. “Should” indicates that among several possibilities, one is recommended as particularly suitable, without mentioning or excluding others, or that a certain course of action is preferred but not necessarily required, or that (in the negative form) a certain possibility or course of action should be avoided but is not prohibited. “May” is used to indicate that a course of action is permissible within the limits of the standard. “Can” is used as a statement of possibility and capability. Finally, “must” is used only to describe “unavoidable” situations, including those mandated by government regulation.

Suggestions for improving this standard are invited. Comments and suggested revisions should be sent to Technical Programs, AAMI, 1110 N. Glebe Road, Suite 220, Arlington, VA 22201-4795.

NOTE—This foreword is not part of the American National Standard *Resistometers used for characterizing the performance of biological and chemical indicators* (ANSI/AAMI ST44:2002), but it does provide important information about the development and intended use of the document.

Introduction

Resistometers are test instruments designed to evaluate the effects of sterilizing environments on biological inactivation kinetics, chemical reactions, material degradation, and product bioburden. The laboratory resistometer test systems allow precise variation of the environmental conditions and cycle sequences in order to produce controlled physical studies. The results of those studies are used in research and quality control programs to establish appropriateness of biological and chemical indicators for applications.

Resistometer test systems differ from conventional sterilizers. Instrumentation selection and control requirements for resistometers are based on 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 laboratory test system by using procedures that might be employed in a conventional heat or chemical sterilization system. Resistometer systems are test instruments rather than sterilizers; thus, an understanding of instrumentation and process design is critical in clarifying requirements for precision and accuracy. Practical design has to consider:

— Achievable measurement and control,

— Acceptable equipment induced variation in test results,

— Economic design (using 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 phenomenon, and

— Testing and analysis alternatives when accurate quantitative determinations exceed physical measurement and control limits.

Diverse requirements for biological and chemical indicators are used for evaluating sterilization processes. Chemical indicators are evolving that are intended to emulate the capabilities of biological evaluation methods for verifying efficacy. This requires characterization of biological inactivation characteristics as well as substantiation of chemical indicator characteristics. Additionally, numerous variations in cycles may affect applicability of indicators for the intended use. Testing the performance of biological and chemical indicators requires specific equipment. This ANSI/AAMI standard specifies the performance requirements and test capabilities for test equipment able to:

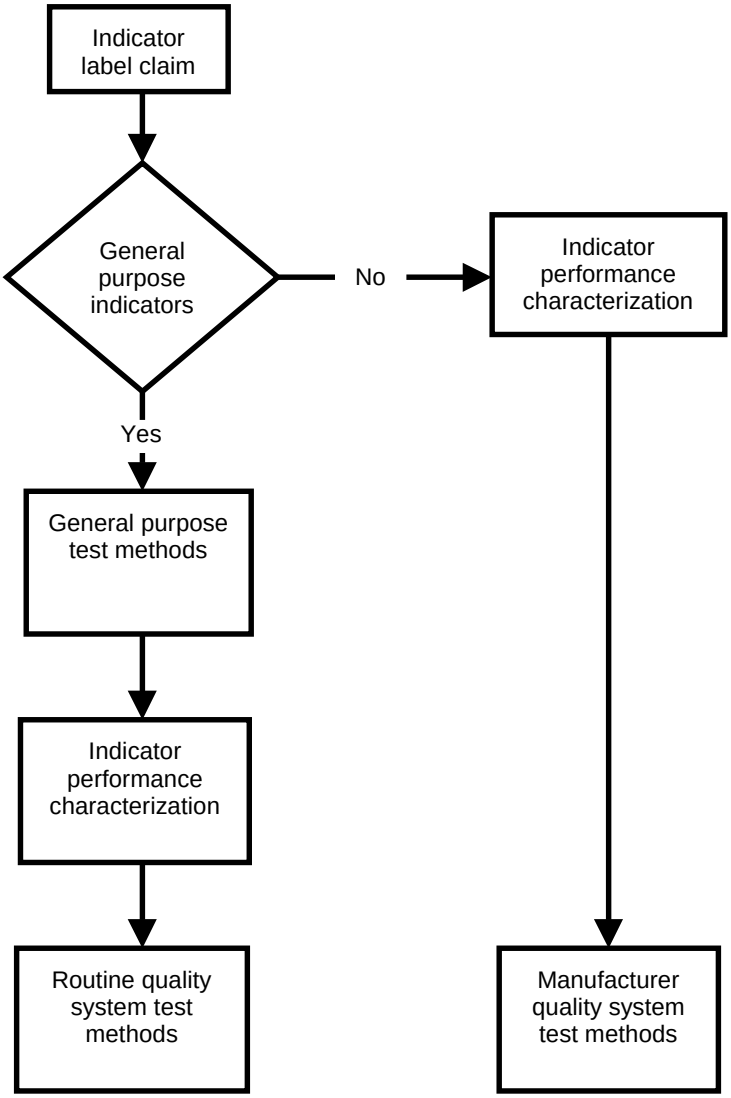
— Characterize the response of biological and chemical indicators to the critical physical parameters encountered during exposure to steam, ethylene oxide, and dry heat sterilization processes;

— Evaluate applicability of the biological and chemical indicators for the intended use; and

— Perform quality control testing on biological and chemical indicators used to monitor processes.

Figure 1 shows a typical sequence for evaluating biological and chemical indicators used in monitoring sterilization processes.

Figure 1—Typical sequence for evaluating biological and chemical indicators used in monitoring sterilization processes



Explanation of Figure 1

Routine quality system test methods are based on testing required in the applicable provisions of the normative references for biological and chemical indicators (Section 2—Normative references). Indicator performance characterization relates to information that may be needed to apply an indicator at points other than the test points required in the normative references. The following definitions apply to Figure 1.

Indicator label claim: Commercial performance declarations about indicators distributed by device manufacturers.

General purpose indicators: Indicator intended for use in multiple types of processes, where the type of process does not substantially change the ability of the indicator to quantify delivered lethality for the sterilization medium within defined limits. An indicator is not considered to be a general purpose indicator if the indicator label claim specifically limits its application to one type of process relative to the variety of processes for a sterilization medium. For example, an indicator is not a general purpose indicator if it applies only to gravity sterilization versus all steam sterilization processes (e.g., vacuum-assisted), because using it for such processes will result in significant changes in performance or damage the indicator.

Indicator performance characterization: Qualitative and quantitative attributes that qualify the device as an indicator.

General purpose test methods: Series of tests performed to emulate conditions that might change an indicator's native expected performance characteristics when the indicator is intended for use in multiple types of processes using a given sterilization medium. The tests selected are used to quantify whether a variation in a sterilization process is likely to substantially change the ability of the indicator to quantify delivered lethality relative to defined performance limits. The tests selected may be used to verify that indicators are not physically damaged or adversely affected by unrecognized anomalies when they are applied in accordance with recommendations for intended use. For example, different air removal techniques, such as gravity displacement, or prevacuum or steam-flush pressure-pulsing, may shift the indicator's performance characteristics outside the required minimum process lethality limits. An indicator appropriate for a gravity sterilization process may not work properly in a prevacuum or steam-flush pressure-pulsing process. An indicator may be physically damaged with deep vacuums or multiple pulse pressure excursions.

Routine quality system test methods: Tests performed in accordance with consensus standard requirements or agency-approved methods that are designed to standardize safety, efficacy, and quality systems on the basis of the best available knowledge. The number of tests required for routine release testing may depend on the support information derived during performance characterization and general purpose performance testing. For example, an indicator that exhibits minimal performance-shift characteristics when tested under several process variation conditions and that exhibits predictable characteristics within defined limits over the intended-use range may only require testing at one test condition for routine release, with periodic testing at other test conditions within the intended-use range.

Manufacturer quality system test methods: Test methods specifically designed and approved to meet quality system requirements for indicators that have limited, specific, or new application claims. Indicators may not comply, or consensus standards may not exist for the indicator application.

Resistometers used for characterizing the performance of biological and chemical indicators

1 Scope

1.1 Inclusions

This standard specifies the requirements for resistometers that characterize the performance of biological and chemical indicators used in steam, ethylene oxide (EO), and dry heat processes. This standard also provides informative methods that help characterize the performance of biological and chemical indicators for their intended use and routine quality control testing.

1.2 Exclusions

This standard does not consider equipment and methods used to characterize biological or chemical indicators exposed to γ and β irradiation, steam-formaldehyde or sterilization processes, or combination processes such as washer disinfectors.

This standard does not specify testing, performance, or acceptance requirements that are specifically covered in the normative references.

This standard does not address safety aspects of the test equipment because specific federal, state, or local regulations govern such requirements.

2 Normative references

The following publications contain provisions that, through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the publications indicated below. The American National Standards Institute maintains a register of currently valid American National Standards.

2.1 ISO/TR 10013:2001, *Guidelines for quality management system documentation*.

2.2 ANSI/AAMI/ISO 11134:1993, *Sterilization of health care products—Requirements for validation and routine control—Industrial moist heat sterilization*.

2.3 KEMPER CA and PFLUG IJ. *Microbiology and engineering of sterilization processes*, 10ed., 1999. Chapter 12, “Temperature measurement in the gathering of design data or during validation of equipment and processes.”

3 Definitions

For the purposes of this American National Standard, the following definitions apply.

3.1 absolute pressure: Pressure that is measured when the reference baseline is 0 kPa (0 psia) and is not atmospheric pressure.

3.2 accuracy: Degree to which a measurement, or an estimate based on measurements, represents the true value of the attribute that is being measured.

3.3 biological indicator (BI): Microbiological test system providing a defined resistance to a specified sterilization process.

3.4 calibration: Set of operations that establish, under specified conditions, the relationship between values indicated by a measuring system, or values represented by a material measure or reference material, and the corresponding values of that quantity obtained from a reference standard.

3.5 carrier: Supporting material on which test organisms are deposited.

3.6 chemical indicator: System that reveals change in one or more predefined process variables on the basis of a chemical or physical change resulting from exposure to a process.

3.7 come-up time: Time elapsed from the commencement of the selected exposure condition to the attainment of the selected exposure conditions.

3.8 critical parameters: Quantifiable parameters that influence the efficacy of a sterilization process.

3.9 D-value; D10-value; decimal reduction value: Time or radiation dose required to achieve inactivation of 90 % of a population of the test microorganism under stated exposure conditions.

3.10 endpoint: Defined change that occurs after an indicator has been exposed to certain predefined physical conditions and represents successful attainment of the conditions intended to cause the indicated change.

3.11 exposure time: Period for which the process parameters are maintained within their specified tolerances.

3.12 gauge: Instrument or means of measuring a physical condition.

3.13 general purpose indicators: Indicators that exhibit appropriate performance characteristics when used in a variety of conditions with a given sterilizing agent.

3.14 general purpose test methods: Test methods used to demonstrate appropriate indicator performance in different types of sterilizing processes using a given sterilization medium.

3.15 inactivation: Loss of ability of microorganisms to grow or multiply.

3.16 label claim: Commercial performance declarations about indicators that are distributed by device manufacturers.

3.17 nominal population: Stated number of microorganisms.

NOTE—The actual number of microorganisms will differ from the nominal population of microorganisms as a result of the accuracy of the inoculation technique, recovery and enumeration measurement, and other determination methods.

3.18 performance characterization: Qualitative and quantitative attributes that qualify the device as an indicator.

3.19 precision: Relative tightness of the distribution of measurements of a quantity about their mean value, expressed in terms of standard deviation.

3.20 quality system test methods: Specific tests that show a device is being consistently produced.

3.21 reference standard: Standard of the highest order of accuracy in a calibration system that establishes the basic accuracy values for that system.

3.22 resistometer: Test instrument designed to rapidly produce and precisely control critical parameters associated with a given sterilization process.

NOTE 1—In addition to routine quality system testing of indicator performance consistency, a resistometer is used to characterize cause-and-effect relationships associated with the given sterilization process and devices used to evaluate the efficacy of the sterilization process.

NOTE 2—Resistometers were formerly referred to as biological indicator-evaluator resistometer (BIER) or chemical indicator-evaluator resistometer (CIER) test systems.

3.23 saturated steam: Water vapor in a state of equilibrium between the liquid and gaseous states.

3.24 self-contained biological indicator: Inoculated carrier enclosed in a primary pack along with an incubation medium that allows growth of organisms on the carrier when the indicator is placed in an incubator.

3.25 time-constant (response time): Time required for a variety of sensors to produce a change in signal output equal to 63.2 % of the final signal output that will result in stabilization to a step in a physical condition. Five time-constants are required for the signal to indicate the actual physical condition. Temperature sensor comparisons are typically based on the rate of signal change on placement of the sensor transverse in water flowing across the sensor at 0.2 m/s. Some manufacturers and technical references also use a 50 % or 90 % step change, rather than a 63.2 % step change for describing the time-constant (response time) in specifications.

3.26 z-value: Change in exposure temperature that corresponds to a 10-fold change in D-value.

4 Performance requirements for resistometers

Test systems shall be capable of producing cycle sequences as required for specific test methods. Test methods, phase-control requirements, and test rationale are shown in annexes A through F.

The following specifications define the measurement and control capabilities for steam, ethylene oxide, and dry heat resistometers used for routine quality system testing.

NOTE—Testing for an indicator may require only part of the functionality described in this document, depending on the intended use and on the normative reference requirements.

4.1 Performance requirements for steam resistometers

The following represents the performance requirements for resistometers intended for use with saturated steam.

4.1.1 Measurement accuracy

The steam resistometer shall be capable of measuring the conditions shown in Table 1 within the limits given.

Table 1—Steam resistometer instrumentation requirements (measurement and recording)

Measurement	Unit	Range	Resolution	Accuracy (+/–)	Time-constant milliseconds
Time	hh:mm:ss	Selectable	00:00:01	00:00:02	–
Temperature	°C	110 to 145	0.1	0.5	1,500 to 2,000 [a)]
Pressure	kPa/psia	0 to 420/0 to 60	0.1	3.5/0.5	≤ 30

[a)] Water time-constant rating.

NOTE—Temperature probes should be selected to have approximately the same time-constant referenced for consistency in measurement and control from one system to the next (approximately 0.125 in [3 mm] diameter stainless steel sheath sensor).

4.1.2 Process control

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

Table 2—Steam resistometer physical design/control specifications

Parameter	Units	Limit(s)	Control (+/–)
Time	hh:mm:ss	Selectable	00:00:01
Pressure vessel	kPa/psia	0 to 420/0 to 60	–
Temperature control	°C	110 to 145	0.5 [a)]
Pressure control	kPa/psia	4.5 to 420/0.65 to 60	3.5/0.5
Vacuum level	kPa/psia	4.5/0.65	3.5/0.5
Chamber prevacuum time	hh:mm:ss	< 00:02:00	–
Steam charge time (100 °C to test temperature)	hh:mm:ss	< 00:00:10	–
Postvacuum time (to atmospheric pressure)	hh:mm:ss	< 00:00:10	–

[a)] After a 10 s stabilization time.

4.1.3 Requirements for general steam resistometers

4.1.3.1 The chamber shall be supplied with saturated steam from a source external to the chamber. The steam supply shall meet the requirements of ANSI/AAMI/ISO 11134 (see normative reference 2.2), and means must be provided to ensure that test items are not wetted by steam supply water particles.

4.1.3.2 Air admitted at the end of the cycle shall pass through a filter capable of removing not less than 99.9 % of 0.5 μm particles.

4.1.3.3 The sample holder should allow the indicator to be exposed to the test conditions in the manner intended by the indicator's manufacturer. The various types of indicators may require customized sample holders. Sample holders may have to be constructed to hold test items in different vertical and horizontal attitudes to test performance differences.

4.1.3.4 Means shall be provided to evacuate the test chamber to the selected vacuum levels.

4.1.4 Test methods

There are a variety of quality control and performance evaluation test methods that may be applicable. Annex A (normative) describes test sequence quality system practices currently employed for evaluating biological and chemical indicators. Annex B (informative) describes test sequences that may be helpful in characterizing biological and chemical indicators.

4.2 Performance requirements for ethylene oxide resistometers

The following represents the performance requirements for resistometers intended for use with ethylene oxide (EO).

4.2.1 Measurement accuracy

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

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

Measurement	Unit	Range	Resolution	Accuracy (+/–)	Time-constant milliseconds
Time	hh:mm:ss	Selectable	00:00:01	00:00:02	–
Temperature	°C	0 to 65	0.1	0.5	1,500 to 2,000 [a]
Pressure	kPa/psia	0 to 420/0 to 60	0.1	3.5/0.5	≤ 30
Relative humidity [b]	% RH	0 to 90	1	5	15,000
Sterilizing agent concentration	mg/liter	0 to 1,200	–	25	–

[a] Water time-constant rating.

[b] If relative humidity is not determined by partial pressure.

4.2.2 Process control

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

Table 4—EO resistometer physical design/control specifications

Parameter	Units	Limit(s)	Control (+/–)
Time	hh:mm:ss	Selectable	00:00:01
Pressure vessel	kPa/psia	0 to 450/0 to 65	3.5/0.5
Sterilizing agent concentration	mg/l	200 to 1,200	30
Temperature control	°C	30 to 60	0.5 [a, b)]
Humidity	% RH	20 to 90	10
Vacuum level	kPa/psia	4.5/0.65	3.5/0.5
Maximum chamber prevacuum time	hh:mm:ss	00:01:00	–
Maximum sterilizing agent charge time	hh:mm:ss	00:00:60	–
Maximum postvacuum time (to design limit)	hh:mm:ss	00:01:30	–

[a)] After a 30 s stabilization time.

[b)] A 0.5 °C level of control is necessary when testing some higher classes of chemical indicators. Otherwise, a 1.0 °C level of control is acceptable.

4.2.3 Requirements for general ethylene oxide resistometers

4.2.3.1 The chamber shall be supplied with steam from a source external to the chamber. The steam supply shall meet the requirements of ISO 11134.

4.2.3.2 Air admitted at the end of the cycle shall pass through a filter capable of removing not less than 99.9 % of 0.5 μm particles.

4.2.3.3 The sample holder should allow the indicator to be exposed to the test conditions in the manner intended by the indicator's manufacturer. Different indicators may require different designs of sample holders. Consult the indicator's manufacturer for guidance when verifying label claim performance. Sample holders may have to be constructed to hold test items in different vertical and horizontal attitudes to test performance differences.

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

4.2.3.5 Means shall be provided to ensure that test samples are not contacted by liquid ethylene oxide or particles of polymers entering the chamber.

4.2.3.6 Means shall be provided to evacuate the test chamber to the selected vacuum levels.

4.2.4 Test methods

A variety of quality control and performance evaluation test methods may apply. Annex C (normative) describes test sequence quality system practices currently used for evaluating biological and chemical indicators. Annex D (informative) describes test sequences that may be helpful in characterizing biological and chemical indicators.

4.3 Performance requirements for dry heat resistometers

4.3.1 Measurement accuracy

The dry heat resistometer shall be capable of measuring the conditions shown in Table 5 within the limits given.

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

Measurement	Unit	Range	Resolution (+/–)	Accuracy (+/–)	Time-constant milliseconds
Time	hh:mm:ss	Selectable	00:00:01	00:00:02	–
Temperature	°C	120 to 200	0.1	0.5	1,500 to 2,000

4.3.2 Process control

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

Table 6—Dry heat resistometer physical design/control specifications

Parameter	Units	Limit(s)	Control (+/–)
Time	hh:mm:ss	Selectable	–
Temperature control	°C	120 to 200	1.5 [a)]
Temperature uniformity	°C	–	1
Maximum temperature recovery time	hh:mm:ss	00:02:00	–
Maximum temperature cool down time <100 °C	hh:mm:ss	00:01:00	–

[a)] After a 2 min stabilization time.

4.3.3 General requirements for dry heat resistometers

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

4.3.3.2 The test environment shall be a gas of the type required (generally air) for the intended use of the indicator.

4.3.3.3 Temperature shall be monitored and recorded throughout the test cycle. The temperature recorded shall have a sampling and recording rate relevant to the process dynamics.

4.3.4 Test methods

A variety of quality control and performance evaluation test methods may apply. Annex E (normative) describes test sequence quality system practices that are currently used for evaluating biological and chemical indicators. Annex F (informative) describes test sequences that may be helpful in characterizing biological and chemical indicators.

5 Calibration

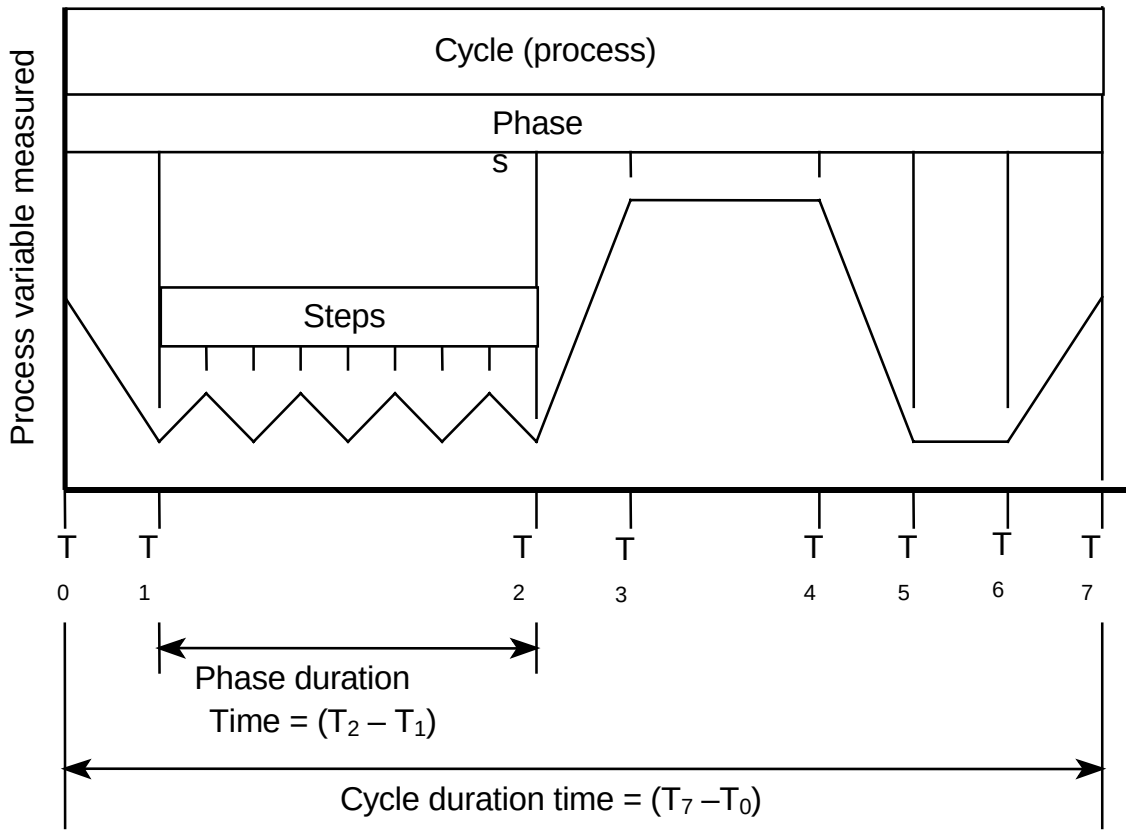
Calibration shall be carried out by using working references or standards that are traceable to the national standard or a physical constant.

NOTE—Sample calibration procedures are provided in ISO 10013:2001, *Guidelines for quality management system documentation* (normative reference 2.1), and chapter 12, "Temperature measurement in the gathering of design data or during validation of equipment and processes," in Kemper and Pflug's *Microbiology and engineering of sterilization processes* (normative reference 2.3).

6 Documentation

A test cycle can be viewed as a sequence of phases and steps plotted against one or more variables relevant to a desired test cycle. Phases are parts of a cycle that can be uniquely described (i.e., prevacuum, preconditioning, steam charge, exposure, postvacuum, air vent, etc.). Steps are parts of a phase that also have uniquely described functions that may be repeated as a sequence within a phase a selected number (n) of times (i.e., 1 [steam-pulse, vacuum], 2 [steam-pulse, vacuum], … n [steam-pulse, vacuum]). Cycle documentation is used to verify that the events that make up a test cycle have occurred. Phase and step duration times indicate the time in the respective phase or step. The cycle duration indicates the accumulative time required to perform a test cycle.

Figure 2—Cycle documentation



For each test method, the following minimum information shall be documented (G.1 shows typical documentation formats):

a) The phase or steps description and associated set points,

b) The date and time the cycle started, and

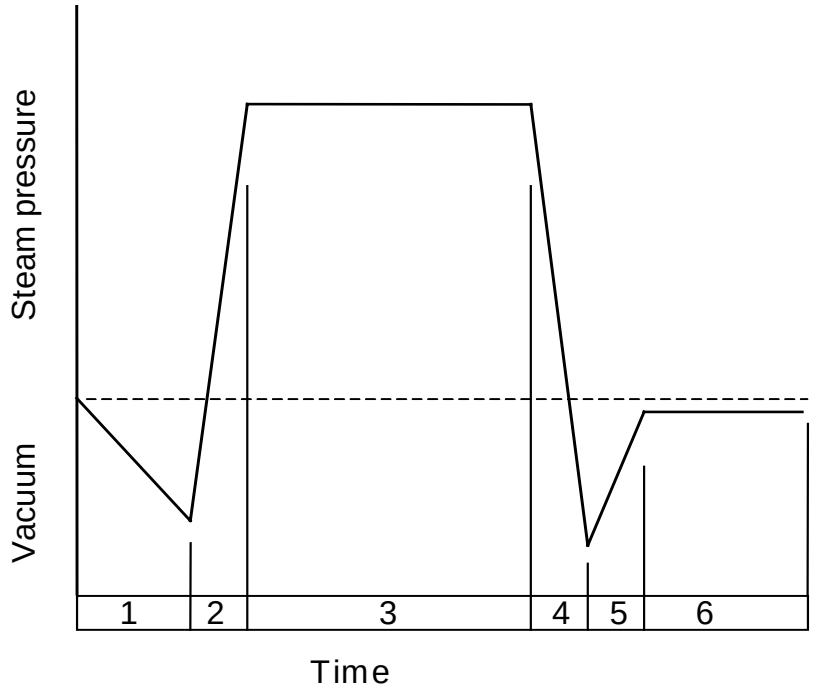
c) The cycle number and sterilization equipment identification number.

Annex A
(normative)

Test sequence—Steam

This steam test sequence is performed on a routine basis as prescribed in applicable normative standards. Figure A.1 graphically depicts the sequence of the steam resistometer used in saturated steam exposure processes.

Figure A.1—Test sequence pressure/time diagram



Phase	Description
Air removal (Phase 1)	Air is removed from the test chamber by evacuation to a selected vacuum level. Subatmospheric steam may be injected into the test chamber so that the partial pressure remaining in the test chamber is steam.
Steam charge (Phase 2)	Steam is injected into the test chamber until the selected test temperature is attained.
Exposure time (Phase 3)	The test chamber is maintained at the selected test temperature for the selected exposure time.
Exhaust/ postvacuum (Phase 4)	The test chamber is vented to atmospheric pressure. Alternatively, the test chamber may be evacuated below atmospheric pressure to a selected level. NOTE—As the chamber drops to atmospheric pressure or below, the chamber temperature drops below temperatures that contribute to indicator kinetics. A postvacuum further cools samples. Exhaust/ postvacuum time, for the purpose of the test method, is the time required to reach atmospheric pressure.
Air vent (Phase 5)	The test chamber is vented to atmospheric pressure.
Cycle complete (Phase 6)	Test samples are manually removed from the chamber within 15 s following completion of the cycle. NOTE—Care must be exercised with samples such as sealed vials, which may break and release stored energy.

Annex B
(informative)

Steam intended-use characterization

Many factors can influence the performance of the biological and chemical indicators used to evaluate different types of steam sterilization processes. The following are some specific test methods that have been found to be helpful in understanding expected results when used to characterize the performance of the variety of devices available. These test methods can be helpful for quantifying performance and application recommendations. Other tests, in addition to those identified below, can be used to characterize indicator performance, such as superheat.

B.1 Chamber air removal

Demonstrable differences in indicator performance are based on residue air in the test system or indicator device. 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 before pressurization with steam in a sterilization process. Results are compared to the performance requirements for the respective indicators. A range of depths of vacuum should be tested to characterize product performance (see Air removal [Phase 1], item a).

B.2 Vacuum/pressurization rate

The vacuum/pressurization rate test is performed to evaluate device damage and resultant performance change related to the rate at which pressure changes in the sterilization processes. During resistometer testing (see annex A or B.1), pressure changes occur at rates significantly faster than are expected in normal applications. Test items can be observed during pressurization and evacuation phases. Observations that have been reported to produce changes in indicator performance might include:

— Indicating chemistry migration by capillary action rather than wicking along the intended path;

— Ballooning of the primary package, resulting in inconsistent synergistic inactivation characteristics (air and steam mixtures);

— Delamination;

— Dehydration or desiccation and sublimation; and

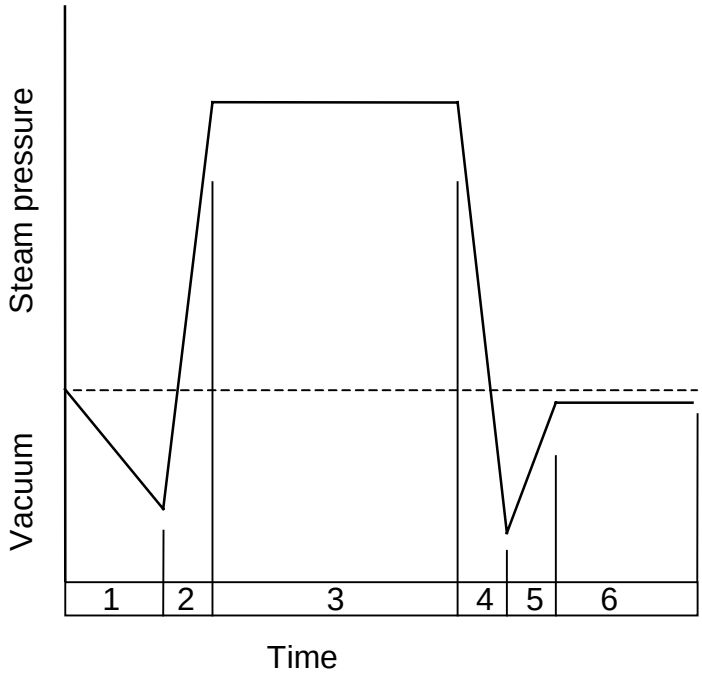
— Chemical reactions or rates of chemical reactions.

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

B.3 Vacuum dwell

The vacuum dwell test is performed to examine the effect of uneven test sample preheating caused by radiant and convective heat rate differences that might exist between the center, outside, top, and bottom samples. Uneven preheating may be produced by proximity to heated pressure vessel walls in addition to differential temperatures caused by the specific gravity with different air temperatures. Test sample locations have to be mapped to locations within the test chamber, and inactivation kinetics have to be analyzed comparing locations. For this test, the standard cycle sequence is performed; however, the vacuum rate is programmed for different prevacuum times. Localized differences in inactivation kinetics indicate that the test samples are susceptible to effects of preheating associated with the time to pull the prevacuum.

Figure B.1—Test sequence pressure/time diagram



Phase	Description
Air removal (Phase 1)	Air is removed from the test chamber by evacuation to a selected vacuum level with no injection of steam during evacuation. The partial pressure remaining in the test chamber is air. a) Atmospheric pressure—no vacuum (gravity displacement) b) 10 kPa (1.5 psia) c) 4.5 kPa (0.65 psia)
Steam charge (Phase 2)	Steam is injected into the test chamber until the selected test temperature is attained.
Exposure time (Phase 3)	The test chamber is maintained at the selected test temperature for the selected exposure time. The test chamber is purged with steam while air and steam are vented from the test chamber so that the air is removed from the test chamber.
Exhaust/ postvacuum (Phase 4)	The test chamber is vented to atmospheric pressure. Alternatively, the test chamber may be evacuated below atmospheric pressure to a selected level.
Air vent (Phase 5)	The test chamber is vented to atmospheric pressure.
Cycle complete (Phase 6)	Test samples are manually removed from the chamber within 15 s following the cycle completion. NOTE—Care must be exercised with samples such as sealed vials, which may break and release stored energy.

B.4 Parametric verification

Indicators that are designed to react to multiple parameters may react partially or completely on exposure to only one of the critical process parameters. Indicators are placed in the test chamber with the resistometer set to control the environmental temperature at or slightly above recommended application temperatures for the indicators. The indicators are observed after extended times at temperatures under nonprocess conditions to determine the effect

on the indicators relative to immediate indications. Subsequently, test samples may be tested per the standard test methods to evaluate changes in performance.

B.5 Range characterization

Indicators may be tested over the application range recommended by the manufacturer to quantify performance characteristics. This test should be repeated in part or entirely as prescribed by applicable normative standards for a given type of indicator, or whenever there is a change to the indicator design or manufacturing process. One should test enough time and temperature combinations to fully characterize the kinetic response over the application range.

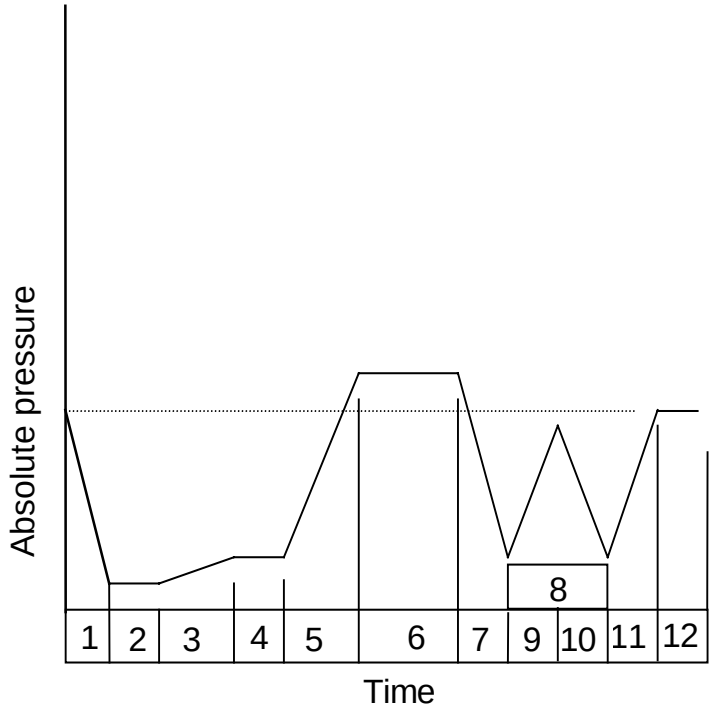
Performance requirements for indicators define minimum requirements to demonstrate minimum delivered process lethality. Additionally, upper limits are defined to minimize false positives in standardized sterilization processes. There will be variation between different types of indicators or within different manufacturing lots of indicators. Indicators should operate within the limits defined in the various normative references. Some indicators require higher lethality and extended exposure time at specific temperature regions before producing an indication of effective sterilization. This requirement does not mean that the indicator is not appropriate for monitoring a process; however, it requires user information to prevent misapplication resulting in false positives.

Annex C
(normative)

Test sequence—Ethylene oxide

This ethylene oxide test sequence is performed on a routine basis as prescribed in applicable normative standards. Figure C.1 depicts the sequence of the ethylene oxide resistometer used in ethylene oxide exposure processes.

Figure C.1—Test sequence pressure/time diagram



Phase	Description
Prevacuum (Phase 1)	Air is removed from the test chamber by evacuation to a selected vacuum level.
Vacuum vold (Phase 2)	The sterilizing chamber is held at the prevacuum level for a selected time to allow the environmental temperature to return to the set chamber temperature that decreased as a result of prevacuum cooling.
Humidification (Phase 3)	Subatmospheric steam is injected into the test chamber until the selected test humidity is attained.
Humidity dwell (Phase 4)	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.
Sterilizing agent charge (Phase 5)	The test chamber is pressurized with vaporized sterilizing agent until a selected sterilizing agent partial pressure (sterilizing agent concentration) is attained.
Exposure (Phase 6)	The selected temperature, humidity, and sterilizing agent concentrations are maintained for a selected time.

Exhaust/ postvacuum (Phase 7)	The test chamber is evacuated to a selected vacuum level to remove most of the sterilizing agent.
Air wash (Phase 8)	The test chamber is pressurized with air to a selected level (Phase 9) and evacuated to a selected vacuum level (Phase 10) for a selected number of times.
Air vent (Phase 11)	The test chamber is vented to atmospheric pressure.
Cycle complete (Phase 12)	Test materials are manually removed from the test chamber within 30 s following the completion of the cycle.

Annex D
(informative)

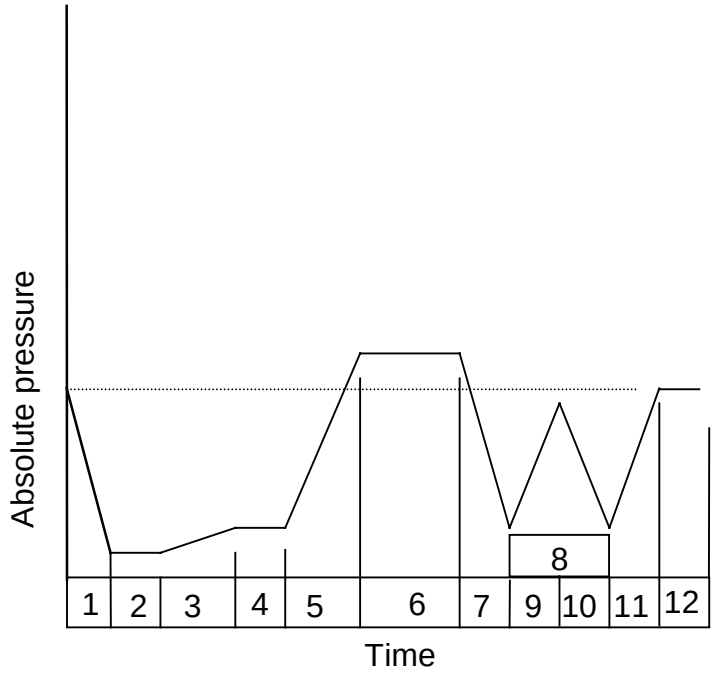
Ethylene oxide intended-use characterization

Many factors can influence the performance of the biological and chemical indicators used to evaluate different sterilization processes. The following are some specific test methods that have been found to be helpful in understanding expected results when used to characterize the performance of the variety of devices available. These test methods can be useful for quantifying results for quality system test procedures.

D.1 Baseline population

The baseline population test is used to establish the log reduction by the sterilizing agent versus physical effects that reduce the test population as a result of other preconditioning variables and organism states. Test samples are exposed to the test shown in Figure D.1; however, no sterilizing agent is injected into the sterilizing chamber during the sterilizing agent charge (Phase 5 of the sequence). Air may be vented into the sterilizing chamber to more closely emulate the conditions in the test sequence when using the sterilizing agent. The exposure time may be set from 0 s to 10 s. The test sample population is compared with the initial population.

Figure D.1—Test sequence pressure/time diagram



Phase	Description
Prevacuum (Phase 1)	Air is removed from the test chamber by evacuation to a selected vacuum level: a) 6.89 kPa (1 psia), b) 13.79 (2 psia), or c) 20.68 (3 psia).
Vacuum hold (Phase 2)	The sterilizing chamber is held at the prevacuum level for a selected time to allow the environmental temperature to return to the set chamber temperature that decreased as a result of prevacuum cooling.

Humidification (Phase 3)	Subatmospheric steam is injected into the test chamber until the selected test humidity is attained.
Humidity dwell (Phase 4)	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.
Sterilizing agent charge (Phase 5)	The test chamber is pressurized with vaporized sterilizing agent until a selected sterilizing agent partial pressure (sterilizing agent concentration) is attained.
Exposure (Phase 6)	The selected temperature, humidity, and sterilizing agent concentrations are maintained for a selected time.
Exhaust/ postvacuum (Phase 7)	The test chamber is evacuated to a selected vacuum level to remove most of the sterilizing agent.
Air wash (Phase 8)	The test chamber is pressurized with air to a selected level (Phase 9) and evacuated to a selected vacuum level (Phase 10) for a selected number of times.
Air vent (Phase 11)	The test chamber is vented to atmospheric pressure.
Cycle complete (Phase 12)	Test materials are manually removed from the test chamber within 30 s following completion of the cycle.

D.2 Chamber air removal

Demonstrable differences in biological indicator D-values are based on residue air in the test system or indicator device. 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 before pressurization with steam in a sterilization process. Results are compared with the performance requirements for the respective indicators.

D.3 Vacuum/pressurization rate

The vacuum/pressurization rate test is performed to verify that there is no device damage and resultant performance change related to the rate at which pressure changes in the sterilization processes. During resistometer testing (see annex C or D.1), pressure changes occur at rates significantly faster than are expected in normal applications. Test items can be observed during pressurization and evacuation phases. Observations that have been reported to produce changes in indicator performance include:

— Ballooning of the primary package, and

— Delamination.

NOTE—An observation view port is needed for visual observation of physical changes of the indicator device that could affect operation of the indicator.

D.4 Parametric verification

Indicators that are designed to react to multiple parameters may react partially or completely on exposure to only one of the critical process parameters. Indicators are placed in the test chamber with the resistometer set to control the environment by selectively reducing or eliminating one or more of the required process parameters. The indicators are observed after extended times under such conditions to determine the effect on the indicators relative to immediate indications. Subsequently, test samples may be tested per the standard test method to determine changes in performance.

D.5 Range characterization

Indicators shall be tested over the application range recommended by the manufacturer to demonstrate a predictable performance characteristic for an indicator. This test may be repeated in part or entirely as prescribed by applicable normative standards for a given type of indicator or whenever there is a change to the indicator design or manufacturing process.

Performance requirements for indicators define minimum requirements to demonstrate minimum delivered process lethality. Additionally, upper limits are defined to minimize false positives in typical sterilization processes. There will

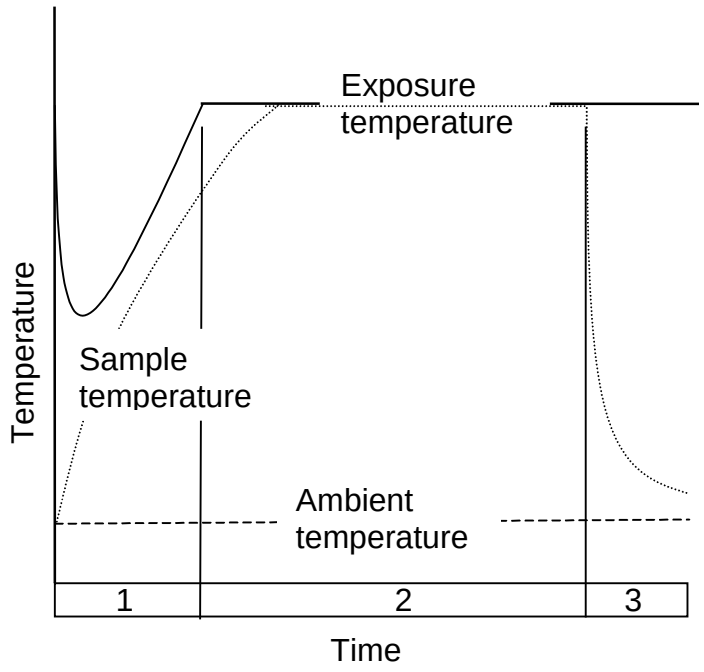
be variation between different types of indicators or within different manufacturing lots of indicators. Generally, indicators should operate within the limits defined in the various normative references. Some indicators may need higher lethality before producing an indication of effective sterilization. This requirement does not mean that the indicator is not appropriate for monitoring a process; however, user information is necessary to prevent misapplication resulting in false positives.

Annex E
(normative)

Test sequence—Dry heat

The dry heat test sequence is performed on a routine basis as prescribed in applicable normative standards. Figure E.1 depicts the sequence of the dry heat resistometer used in dry heat exposure processes.

Figure E.1—Test sequence temperature/time diagram



Phase	Description
Sample injection (Phase 1)	The test samples are injected into a preheated test environment that is designed to recover to the selected test temperature within the times specified in the normative references.
Exposure time (Phase 2)	The test chamber is maintained at the selected test temperature for the selected exposure time.
Cycle complete (Phase 3)	Test samples are manually removed from the test environment.

Annex F
(informative)

Dry heat intended-use characterization

F.1 Many factors can influence the performance of the biological and chemical indicators used to evaluate different sterilization processes. The following is a specific test method that has been found to be helpful in understanding expected results when used to characterize the performance of the various devices available. This test method can be useful for quantifying results for quality system test procedures.

F.2 The environmental gas test quantifies the inactivation kinetics or indicator performance characteristics in environments other than air. Testing is performed as in the standard test method, except that the test system is inside an environmental chamber containing the test gas. Alternatively, the environmental gas may be heated to the appropriate temperature and used to displace air from the test system. Following testing, the test sample characteristics may be compared with the characteristics in an air environment.

Annex G
(informative)

Example of resistometer documentation

G.1 Resistometer documentation

Figure G.1 depicts what might be considered a fairly standard resistometer exposure cycle consisting of the following different phases: a pre-vacuum phase, an exposure phase, and a post-exposure phase. The duration of each phase can be determined by calculating the difference between each step of the phase. Figure G.2 represents what might be considered a fairly standard resistometer printout providing additional details associated with each step and each phase of the cycle.

Figure G.1—Example of standard resistometer cycle

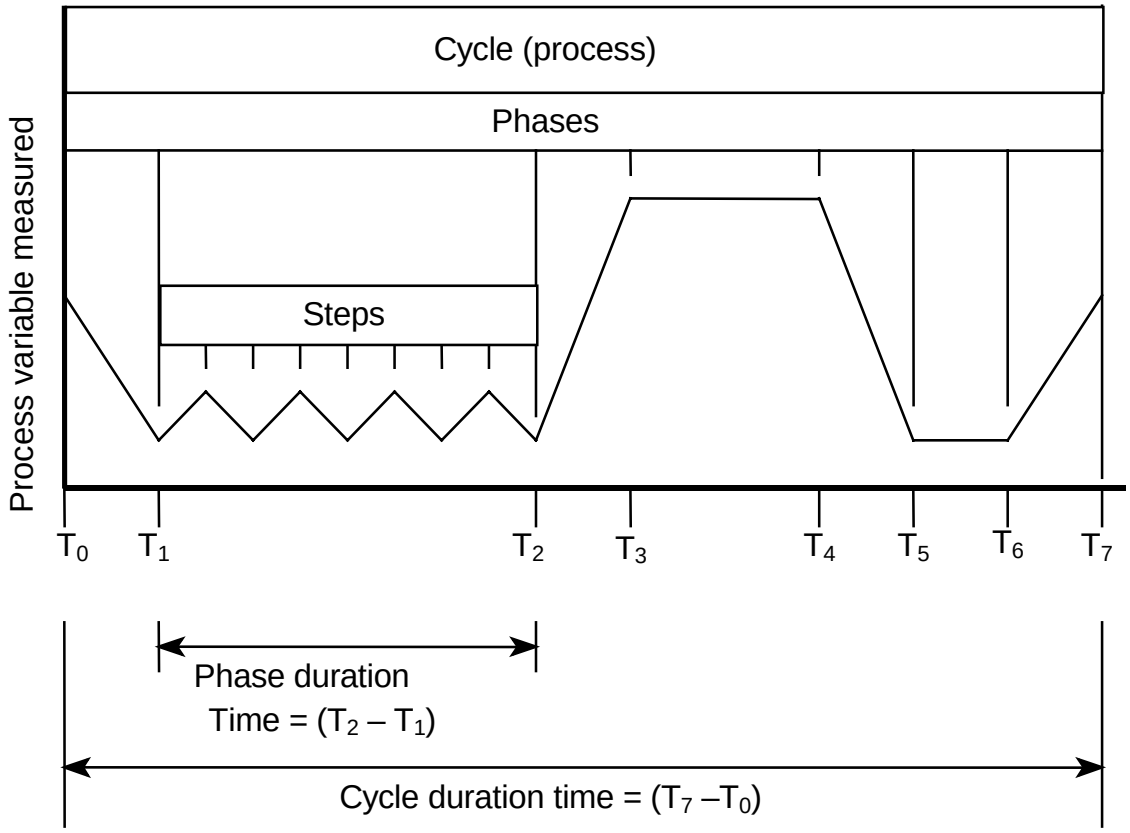


Figure G.2—Example of a standard resistometer printout providing additional details associated with each step and each phase of the cycle

Operator identification: _____
Equipment identification: _____
Run number: _____
Date: _____ (mm/dd/yy)
Time: _____ (hh:mm:ss)

Cycle/test name: XYZ

Phase (set points) and Step (set points)	Time (hh:mm:ss)	Chamber pressure (psia)	Chamber temperature (°C)	Duration (hh:mm:ss)
Phase 1	09:11:43	38.8	131.0	
	09:13:13	22.2	129.2	00:01:30
Phase 2				
Step 1	09:13:13	22.2	129.2	
	09:14:05	41.6	132.0	00:00:52
Step 2	09:14:05	41.6	132.0	
	09:14:16	14.9	114.2	00:00:11
Step 3	09:14:16	14.9	114.2	
	09:15:01	15.67	115.8	00:00:45
Step 1	09:15:01	15.67	115.8	
	09:15:50	41.6	132.0	00:00:49
Step 2	09:15:50	41.6	132.0	
	09:16:03	14.9	112.4	00:00:13
Step 3	09:16:03	14.9	112.4	
	09:16:48	15.5	126.9	00:00:45
Step 1	09:16:48	15.6	115.8	
	09:17:37	41.6	132.0	00:00:49
Step 2	09:17:37	41.6	132.0	
	09:17:51	14.9	109.9	00:00:14
Step 3	09:17:51	14.9	109.9	
	09:18:36	15.3	126.2	00:00:45
	Phase duration time: Sequence repeats			
Phase 3	09:18:36	15.3	126.2	
	09:19:31	42.5	134.1	00:00:55
Exposure 4	09:19:31	42.5	134.1	
	09:22:31	42.5	134.7	00:03:00
	Maximum [1)]			
	Minimum [1)]			
Phase 5	09:22:31	42.5	102.7	
	09:22:48	14.1	103.0	00:00:17
Cycle complete	Total cycle time:			00:11:05

[1)] Process parameter electrical noise or control spike values that are less than 1 s in duration may be digitally filtered from max/min determination, and analog/digital excursions on recorded data may be ignored.

G.2 Relative humidity calculation or measurement

Relative humidity, by definition, is the ratio of the mass or partial pressure in an environment versus the mass or partial pressure that a saturated environment can hold at a given temperature. That ratio is usually multiplied by 100 and is expressed as a percent relative humidity (% RH). Measurement of the water vapor mass is very complicated; therefore, relative humidity is typically determined by measuring the partial pressure of water vapor in an enclosed environment. Humidity determinations of ± 5 % RH are reasonably obtained. Mathematically, this measurement is expressed as follows:

$$\%\ RH = \frac{\text{Actual partial pressure of water vapor (at the test temperature)}}{\text{Saturation vapor pressure of water (at the test temperature)}} \times 100\ \% \qquad \text{(Equation 1)}$$

Example:

Test temperature: 54.4 ˚C (130 ˚F)

Saturation pressure at 54.4 ˚C (steam table): 2.223 psia

Measured partial pressure (humidity added): 1.159 psia

$$\%\ RH = \frac{1.159\ \text{psia}}{2.223\ \text{psia}} \times 100\ \% = 51.14\ \%\ RH$$

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

Dew point measurement is typically used for monitoring, because the measurement/response time is 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 with a combination of a photo sensor and a light-emitting source. The condensation temperature represents the saturation temperature or dew point. A dew point can be expressed in terms of RH using the same format as above, but substituting the dew point pressure from a steam table for the measurement of partial pressure of humidity. Accuracies are typically within 1.5 % RH, provided that contaminants do not shift the dew point measurement.

Example:

Environmental temperature: 54.4 ˚C (130 ˚F)

Measured dew point temperature: 40.5 ˚C (105 ˚F)

Saturation pressure at 54.4 ˚C (130 ˚F): 2.223 psia

Dew point saturation pressure at 40.5 ˚C (105 ˚F): 1.102 psia

$$\%\ RH = \frac{1.102\ \text{psia}}{2.223\ \text{psia}} \times 100\% = 49.57\ \%\ RH$$

Secondary relative humidity-monitoring devices are electrical relative humidity sensors, electrohygrometric sensors, spectroscopic (infrared or ultraviolet) hygrometers, and gas chromatographs (for water content analysis). Those devices rely on the generation of calibration maps that describe condition and signal characteristics for each variable condition. 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.

G.3 Calculation of ethylene oxide concentrations

G.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*). This simple relationship does not provide an exact determination of the gas concentration. However, for the application, determinations are considered an adequate method of consistently producing the same test conditions. The following assumptions are made:

a) The mixture of ethylene oxide, water vapor, and air (and the diluent gas when used) behaves as a gas.

b) There is no selective loss of a component of the mixture (e.g., by means of absorption or adsorption).

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) Gauge readings are absolute pressure readings.

G.3.2 Calculations

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

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

$PV = nRT$

Rearranging the Ideal Gas Law allows for the calculation of ethylene oxide concentration using the following equation:

$$C = \frac{KP}{RT} \qquad \text{(Equation 2)}$$

where:

C = Ethylene oxide concentration (mg/L);

R = Gas constant (see Table G.2);

P = Difference in total pressure resulting from EO and diluent;

T = Absolute temperature of EO and diluent gas mixture giving pressure P;

K = Constant for a given diluent (see equation 3);

and where K is calculated as:

$$K = \frac{4.4 \times 10^4 \, ME}{ME + 44\,(100 - E)} \qquad \text{(Equation 3)}$$

where:

M = Molecular weight of diluent gas; and

E = Weight percentage of EO in diluent mixture.

Table G.1 lists constants and molecular weights of some common ethylene oxide/diluent combinations.

Table G.1—EO/diluent constants and molecular weights

EO/diluent	K (mg/gm mole) [a]	K (lb/lb mole) [b]
10 % EO/90 % CO_2	4.40×10^3	4.40
12 % EO/88 % CFC12	1.20×10^4	1.20×10^1
20 % EO/80 % CO_2	8.80×10^3	8.80
100 % EO	4.40×10^4	4.40×10^1

Molecular weight

Ethylene oxide (EO)	44.0
CFC12	120.9
Carbon dioxide	44.0

[a] Use when calculating mg/L.
[b] Use when calculating lb/ft^3.

G.3.3 Example calculations

G.3.3.1 Determining ethylene oxide concentration in terms of pounds per cubic foot (lb/ft^3)

Assume a process that uses 10 % ethylene oxide and 90 % CO_2. After gas injection, the rise in pressure was 283.411 kPa (41.105 psia).

NOTE—This calculation does not include pressure rise resulting from moisture preconditioning.

If the temperature at the end of gas injection was 134 °F, then:

P = 283.411 kPa = 2.80 atm

T = 134 °F = 56 °C = 329 °K

$$R = 1.3140 \ \frac{\text{atm ft}^3}{\text{lb mole °K}}$$

(see Table G.2 for gas constants)

$$K = 4.40 \ \frac{\text{lb}}{\text{lb mole}}$$

(see Table G.1)

Using equation 2, the ethylene oxide concentration is:

$$C = \frac{KP}{RT} = \frac{4.40 \times 2.80}{1.314 \times 329} = 0.0285 \text{ lb/ft}^3$$

G.3.3.2 Determining ethylene oxide concentration in terms of mg/L

Assume a process that uses 12 % ethylene oxide and 88 % CFC12. After gas injection, the rise in pressure is 137.133 kPa (19.894 psia). If the temperature at the end of gas injection is 55 °C, then:

P = 137.133 kPa = 1.35 atm

T = 55 °C = 328 °K

$$R = 0.08205 \ \frac{\text{l atm}}{\text{gm} - \text{mole °K}}$$

(see Table G.2 for gas constants)

$K = 1.20 \times 10^4$ mg/gm mole

Using equation 2, the ethylene oxide concentration is:

$$C = \frac{KP}{RT} = \frac{1.2 \times 10^4 \times 1.35}{0.08205 \times 328} = 602.5 \text{ mg/L}$$

G.3.3.3 Derivation of equation 3

Because most operations record the pressure change during ethylene oxide gas injection, equation 3 was derived to allow the calculation of ethylene oxide concentration from the pressure rise caused by ethylene oxide gas injection, with or without a single diluent gas such as carbon dioxide or CFC12. The purpose of this equation is to provide a simple and rapid method for calculating ethylene oxide concentration for production sterilizers and experimental facilities.

The pressure rise can be rewritten as:

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

See Equation 1 for the pressure rise due to humidification. $P_{Humidification}$ 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 psia, kPa, or mbar).

Using this value for the pressure, and to obtain the concentration for ethylene oxide in mg/L for any gas mixture, the equation

$$C = \frac{KP}{RT}$$

is generalized using equation 3 to include the molecular weights and weight percentages of the ethylene oxide and diluant gases in the mixture:

$$C = \frac{4.4 \times 10^4 MEP}{[ME + 44(100 - E)]RT}$$

M = the average molecular weight of the diluant

E = the weight percent of ethylene oxide in the mixture

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

$$M = \sum_i M_i E_i$$

M_i = the molecular weight of diluant gas component i

E_i = the weight percent of diluant component i in the diluant, not the ethylene oxide mixture

Using this in the equation for the concentration,

$$C = \frac{4.4 \times 10^4 EP \sum_i M_i E_i}{E \sum_i M_i E_i + 44(100 - E)]RT}$$

Table G.2—Gas constants (R)

Pressure	Volume	Temperatures	R
atm	cc	°K	82.057
atm	liters	°K	0.08205
atm	ft^3	°K	1.3140
bar	liters	°K	0.08314
kg/m^2	liters	°K	847.80
kg/cm^2	liters	°K	0.08478
mmHg	liters	°K	62.361
mmHg	ft^3	°K	998.90
in Hg	liters	°K	2.4549

NOTE 1—1 atm = 760 mmHg = 29.92 in Hg = 14.70 psia = 1.013 bar = 1.033 kg/cm^2 = 101.3 kPa (kN/m^3)
1 liter = 1000 cc = 0.03532 ft^3

NOTE 2—°K = °C + 273.15

Annex H
(informative)

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