Kirk-Othmer Encyclopedia of Chemical Technology.

STERILIZATION TECHNIQUES

Sterilization, the act of sterilizing or the condition of being sterilized, is defined as rendering a substance incapable of reproduction. Whereas this is often taken to mean total absence of living organisms, a more accurate representation is that the substance is free from living microorganisms with a probability previously agreed to be acceptable.

Sterilization technology is of primary importance in industries as diverse as food processing (qv) and space exploration. Generally, however, it is more readily associated with the health-care profession and industry or with the electronics industry. Many of the former industries employing sterilization technology are regulated by some federal agency. In the United States, the introduction of foods, pharmaceuticals (qv), and medical devices into interstate commerce is regulated by the FDA. The registration of chemical sterilants is regulated by the EPA in the United States.

The U.S. market in hospital sterilizing equipment is ca $\$70 \times 10^6$. Manufacturers of various types of equipment are given in Table 1.

A distinction must be made between sterilization and certain other processes often called sterilization as a result of popular misconception. Methods and procedures less rigorous than sterilization, such as disinfection, sanitization, and the use of antiseptics and bacteriostats (see Disinfectants and antiseptics; Food processing; Industrial antimicrobial agents), are often applied to render the object safe for certain applications. In most instances, a judgment on the suitability of a sterilization or a substitute process can only be made by a microbiologist. Well-known exceptions are the common practice of boiling infants' feeding bottles under ambient atmospheric pressure for 5–15 min, being the aim to sterilize them, or the practice of soaking objects in 70% alcohol (1). Boiling at atmospheric pressure does not result in sterilization, nor does soaking in alcohol. Neither procedure kills bacterial spores.

It is necessary to establish a criterion for microbial death when considering a sterilization process. With respect to the individual cell, the irreversible cessation of all vital functions such as growth, reproduction, and in the case of viruses, inability to attach and infect, is a most suitable criterion. On a practical level, it is necessary to establish test criteria that permit a conclusion without having to observe individual microbial cells. The failure to reproduce in a suitable medium after incubation at optimum conditions for some acceptable time period is traditionally accepted as satisfactory proof of microbial death and, consequently, sterility. The application of such a testing method is, for practical purposes, however, not considered possible. The cultured article cannot be retrieved for subsequent use and the size of many items totally precludes practical culturing techniques. In order to design acceptable test procedures, the kinetics and thermodynamics of the sterilization process must be understood.

1. Kinetics

An overwhelming body of evidence, starting with the earliest investigations (2), supports the contention that the rate of destruction of microorganisms is logarithmic, ie, first order with respect to the concentration of

Table 1. Manufacturers of Steam, Ethylene Oxide Sterilizers, and Dry Heat Sterilizers

Manufacturer	Location
Amsco/American Sterilizer Co.[a]	Erie, Pa.
H. W. Anderson Products, Inc.	Oyster Bay, N.Y.
Bard International CR	Durham, U.K.
Baumer Equipment Medico Hospitalar S/A	Sao Paulo, Brazil
Be Venue Laboratories, Inc.	Bedford, Ohio
British Sterilizer Co., Ltd.	Essex, U.K.
Britains Hospital Supplies, Ltd.	Cheddleton, U.K.
MDT-Castle Co.	Rochester, N.Y.
Consolidated Stills & Sterilizers	Boston, Mass.
Danspital, Ltd., Turn-Key Hospitals	Roedovre, Denmark
Dent & Hellyer, Ltd.	Andover, U.K.
Downs Surgical, Ltd.	Surrey, U.K.
Drayton Castle, Ltd.	Middlesex, U.K.
Electrolux Wascator	Alingas, Sweden
Environmental Tectonics Corp.	Southampton, Pa.
Getinge AG	Getingen, Sweden
Harsanyi Labor Mim	Budapest, Hungary
Intermed	Stafford, U.K.
Labotal, Ltd.	Jerusalem, Israel
MMM Munchener Medizin Mechanik	Munich, Germany
O.C.R.A.S. Zambelli S.A.S.	Torino, Italy
Nishimoto Sangyo Co., Ltd.	Osaka, Japan
Royal Adlinden	Wyndyecht, the Netherlands
Sakura Finetechnical Co., Ltd.	Tokyo, Japan
Scardi Construzioni Sanitario	Milano, Italy
Surgical Equipment Supplies, Ltd.	London, U.K.
3M (Minnesota Mining and Manufacturing Co.)	St. Paul, Minn.
Vacudyne Altair	Chicago Heights, Ill.
Webecke & Co.	Bad Schwartau, Germany
Atomic Energy of Canada, Ltd.[b]	Ottawa, Canada

[a]As of 1996, purchased by Steris Corp. (Ohio).
[b]Radiation sterilizer.

microorganisms. The process can be described by the following expression:

$$\frac{N_o}{N_t} = e^{-kt} \qquad (1)$$

in which N_t = the number of organisms alive at time t, N_o = the initial number of organisms, and k = the kinetic rate constant. It can be seen that N_t approaches zero as t approaches infinity. Absolute sterility, accordingly, is impossible to attain.

First-order kinetics yield linear plots on semilogarithmic graphs when plotted against time. It has been found convenient to express the rate of microbial kill in terms of a decimal reduction rate or D-value. The D-value represents the time of exposure (at given conditions for a given microorganism) required for a 10-fold decrease in the viable population. This principle has found great utility in the health-care industry as well as in food processing (3). The practical significance of the D-value is that it simplifies the design of sterilization cycles. A sufficiently large D for any process results in a negative log N_t which, in a practical sense, represents the probability of survival of the last remaining microorganism. In the health sciences, a 10^{-6} residual concentration of microorganisms is generally regarded as an acceptable criterium for sterility.

Exceptions in the direction of higher or lower values do exist, however, depending on the type of sterilization process used, or the purpose of the sterile product.

2. Thermodynamics

The Arrhenius rate theory, an empirical derivation, holds for the sterilization process:

$$k = Ae^{-\Delta E/RT} \qquad (2)$$

where k = kinetic constant, R = gas − law constant, T = absolute temperature, ΔE = the activation energy, and A = the Arrhenius rate constant.

The Eyring equation for the theory of absolute reaction rates is more accurate:

$$k = \frac{k_B T}{h} e^{(T\Delta S - \Delta H/RT)} \qquad (3)$$

where k_B = Boltzman′s constant, h = Plank′s constant, and ΔS and ΔH are the standard entropy and enthalpy changes, respectively. Determinations of ΔE or ΔH and ΔS usually yield values of 167–335 J/mol (40–80 cal/mol). Such values are often characteristic of protein denaturation. Microbial death may involve irreversible denaturation of some or even all of the cell's proteins.

The relationship between the D-value and k can be derived by considering the meaning of D:

$$D = \frac{t_2 - t_1}{\log_{10} N_1 - \log_{10} N_2} \qquad (4)$$

where $N_1 = 10N_2$.

Substituting into equation 1:

$$D = 2.3/k \qquad (5)$$

Of great practical value is the derivation for the effect of temperature, the z-value, defined as the temperature change necessary to effect a 10-fold reduction ($1D$):

$$z = \frac{T_2 - T_1}{\log_{10} D_1 - \log_{10} D_2} \qquad (6)$$

A convenient multiple of D is the thermal death time F_o. It is the exposure time required for less than 1×10^{-6} probability of survival (4, 5). The relationship between F_o and z becomes equation 7:

$$z = \frac{T_2 - T_1}{\log_{10} F_o^1 - \log_{10} F_o^2} \qquad (7)$$

The comparison of the effectiveness of sterilization cycles at different temperatures becomes possible. For example, for steam sterilization,

$$F_o^{121} = t \Big/ \text{antilog} \frac{121 - t}{z} \qquad (8)$$

$F^{121}{}_0$, the thermal death time at 121°C, is accepted to be 18 min (6) and the z-value used is 10°C. Substituting into equation 8, an equivalent thermal death time t of 1.35 min at 132°C or 0.75 min at 135°C is obtained.

3. Testing and Monitoring

Direct testing for sterility by culturing is a destructive test method, ie, the product is rendered useless for food or medical purposes. Indirect testing methods usually rely on a statistically valid sampling (qv) pattern for a product. In the case of sterilization, where the desired outcome has to demonstrate a $<10^{-6}$ probability of failure, even a sample size of 500,000 cultures could only provide a 50% chance of detecting a failure in the process. Accordingly, product monitoring for sterilization using a test sample is a limited value. Product monitoring is only utilized if no other information is available for the particular process cycle for a given lot of products, eg, a screening test. Because sterilization is a highly reproducible and well-understood process, it has been found that process monitoring is far more suitable for purposes of sterility assurance. Process monitoring can be accomplished by measurement of individual parameters, each known to be critical for success, or by methods that are capable of integrating all critical conditions into a single display which can be observed or measured.

3.1. Biological Monitoring

Biological indicators are preparations of specific microorganisms particularly resistant to the sterilization process they are intended to monitor. The organisms are inoculated onto filter paper specified in the *U.S. Pharmacopeia* for specific purposes. Organisms considered to be particularly suitable are specified in appropriate sections of the ISO standards and the *U.S. Pharmacopeia, European Pharmacopeia*, or pharmacopeias of other nations. It is possible to prepare indicators using organisms other than those, or to inoculate product or packaging samples selected to resemble as closely as possible the actual product being sterilized.

When designing industrial sterilization cycles, the bioburden or bioload is determined first. The bioload, the average number of organisms present on or in an article that is to be sterilized, has been found to be highly reproducible from lot to lot for mass-produced items. An appropriate number of biological indicators is added to the load, reflecting the bioload and the desired degree of safety. If the bioload were small, eg, 10^2, the number of organisms on the biological indicator would have to be 10^6. All of the organisms must be killed during the process to indicate that the process produced the 10^{-6} concentration considered necessary.

The carriers containing the biological indicators are retrieved following exposure, transferred aseptically into sterile culture media, and incubated for the required length of time, usually two to seven days. Some unexposed indicators are also incubated to prove that the spores were viable. If no growth is observed while the viability control displays the required growth, the conclusion is made that the sterilization cycle was successful. In order for the biological indicator concept to work, it is necessary to place the indicators into that portion of the sterilizer load considered the most difficult for the sterilant to reach. The lot of products is quarantined to prevent dissemination in case of incomplete sterilization.

Hospital and health-care institutions face a different problem. The sterilizer loads are diverse and generally prepared manually. Therefore, the bioburden varies and it is impossible to determine it for each load. Some prior assumptions are made about the bioburden when designing hospital cycles, and design includes a sufficient degree of additional safety factors. A problem is the inability of hospitals to quarantine sterilized supplies, both for lack of an adequate number of instruments and storage space. For hospital sterilization, biological indicators are considered inadequate and the measurement of physical parameters is essential.

Spores of *Bacillus stearothermophilus* are frequently used for testing steam sterilization because of their high resistance to this type of sterilization. *Bacillus subtilis* is used for dry-heat, ethylene oxide, or other types of vapor/gas sterilization. For radiation, *Bacillus pumilus* is used. The viability of any spore preparation is

Table 2. Performance Characteristics of Some Biological Indicators[a]

Culture spores	Sterilization process	Approximate *D*-value
Bacillus subtilis	ethylene oxide at 50% rh and 54°C	
	600 mg/L	3 min
	1200 mg/L	1.7 min
Bacillus stearothermophilus	saturated steam at 121°C	1.5 min
Bacillus pumilus	gamma-radiation	
	wet preparations	2×10^{-6} Gy[b]
	dry preparations	1.5×10^{-6} Gy[b]
Bacillus subtilis	dry heat at 121–170°C	60–1 min
Clostridium sporogenes	saturated steam at 112°C	3.5–0.7 min

[a]Ref. 6.
[b]To convert Gy to rad, multiply by 100.

Table 3. Minimum Exposure Periods for Sterilization of Hospital Supplies[a]

Load	Temperature, °C	Holding time, min	Air removal method	Heat-up time, min	Safety factor	Exposure time, min
hard goods						
unwrapped	121–123	12	gravity	1	2	15
	133–135	2	gravity[b]	<1	0.5	3
	133–135	2	pulsing	<1	1	4
	133–135	2	prevacuum	1	1	4
wrapped	121–123	12	gravity	5	3	20
	133–135	2	gravity	7	1	10
	133–135	2	pulsing	<1	1	4
	133–135	2	prevacuum	1	1	4
fabrics, packs	121–123	12	gravity	12	6	30
	133–135	2	pulsing	<1	1	1
	133–135	2	prevacuum	1	1	4
	141–142	0.5	pulsing	<1	0.5	2
liquids	121–123	12	gravity	[c]	[c]	[c]

[a]Ref. 7. Courtesy of Charles C. Thomas, Publisher.
[b]High speed.
[c]Depending on liquid volume and container.

known to change with time, and such preparations have limited shelf lives. It is always advisable to test the viability of a given lot of spore preparations by culturing an unexposed sample from the same lot alongside the exposed samples. The labeling of commercially prepared products usually includes information on the number of organisms present, the *D*-values, performance characteristics, instructions for culturing, and the expiration date.

Typical performance characteristics for some of the most widely used biological indicators are given in Table 2.

According to the *D*-values shown in Table 2, a 6-*D* reduction using the 1.5-min requirement for steam at 121°C results in a 9-min cycle. Yet a minimum 12-min exposure is recommended, and with the safety factor, an 18-min steam contact is required (Table 3) for hospital cycles at 121°C.

Culturing is time-consuming and quarantining of packages sterilized in the hospital is not feasible. Under such circumstances, it is not possible to obtain information about a specific sterilizer load by means of biological indicators before the contents of that load are used. Nevertheless, authoritative sources such as the Joint Commission on Accreditation of Hospitals (JCAH) (8), the Sterilization Standards Committee of the

Association for the Advancement of Medical Instrumentation (AAMI) (9), and the Association of Operating Room Nurses (AORN) (10) recommend that biological testing of all sterilizers be conducted at least once a week, but preferably every day for steam sterilizers, every cycle for ethylene oxide sterilizers, and in every cycle when implantable devices are sterilized. If possible, the implantables should be quarantined until the results of the biological test become available. These tests are carried out by placing the biological indicators in test packs of specific constructions, and placing the test packs in otherwise normally loaded sterilizers.

Hospital sterilizer loads vary in composition, thus the challenge presented to the test organism can vary considerably, depending on the type and contents of packages in which they are placed. The benefits of a standardized test-pack construction and test protocol are obvious, and such recommendation is made by AAMI for steam and ethylene oxide sterilizers (11). More recently in European (CEN) and International (ISO) standards, biological indicators are considered as additional information supplemental to the measurement of physical parameters. Indeed, for sterilization using moist heat (steam), the biological indicator information is not considered to be relevant.

3.2. Monitoring by Electromechanical Instrumentation

According to basic engineering principles, no process can be conducted safely and effectively unless instantaneous information is available about its conditions. All sterilizers are equipped with gauges, sensors (qv), and timers for the measurement of the various critical process parameters. More and more sterilizers are equipped with computerized control to eliminate the possibility of human error. However, electromechanical instrumentation is subject to random breakdowns or drifts from calibrated settings and requires regular preventive maintenance procedures.

An inherent problem is the location of the sensors. It is not possible to locate the sensors inside the packages which are to be sterilized. Electromechanical instrumentation is, therefore, capable of providing information only on the conditions to which the packages are exposed; but cannot detect failures as the result of inadequate sterilization conditions inside the packages. Such instrumentation is considered a necessary, and for dry and moist heat sterilization, a sufficient, means of monitoring the sterilization process.

3.3. Chemical Monitoring

Chemical indicators are devices employing chemical reactions or physical processes designed in such a way as to permit observation of changes in a physical condition, such as color or shape, and to monitor one or more process parameters. Chemical indicators can be located inside the packages, and the results are observable immediately when the package is opened for use. Most available chemical indicator types, however, are not capable of fully integrating all the critical process parameters. They are, therefore, not accepted as a guarantee of sterility, although some can indicate if conditions were adequate for sterilization.

A particular advantage of chemical indicators is the manufacturer's ability to formulate a relatively large, homogeneous batch of reagent mixture that can be deposited on inexpensive substrates, such as paper, by high speed printing techniques, resulting in relatively low unit costs. Because hospital sterilization cycles are standardized, hospitals can benefit considerably from the use of chemical indicators. Moreover, the use of chemical indicators makes it easy to distinguish those materials which have gone through a sterilization procedure from those that have not.

Industrial sterilization cycles tend to vary considerably, not only from manufacturer to manufacturer, but often from product type to product type, depending on the bioburden present on a given load. Chemical indicators have historically been used only to differentiate between sterilized and nonsterilized packages. More recent developments have resulted in the availability of chemical dosimeters of sufficient accuracy to permit their application either as total monitors or as critical detectors of specific parameters.

4. Dry-Heat Sterilization

Dry-heat sterilization is generally conducted at 160–170°C for ≥ 2 h. Specific exposures are dictated by the bioburden concentration and the temperature tolerance of the products under sterilization. At considerably higher temperatures, the required exposure times are much shorter. The effectiveness of any cycle type must be tested. For dry-heat sterilization, forced-air-type ovens are usually specified for better temperature distribution. Temperature-recording devices are recommended.

It is an axiom of sterilization technology that appropriate conditions must be established throughout the material to be sterilized. The time-at-temperature conditions are critical when considering any sterilization method using heat.

Chemical indicators for dry-heat sterilization are available either in the form of pellets enclosed in glass ampuls, or in the form of paper strips containing a heat-sensitive ink. The former displays its end point by melting, the latter by a color change (see Chromogenic materials).

5. Steam Sterilization

Steam (qv) sterilization specifically means sterilization by moist heat. The process cannot be considered adequate without assurance that complete penetration of saturated steam takes place to all parts and surfaces of the load to be sterilized (Fig. 1). Steam sterilization at 100°C and atmospheric pressure is not considered effective. The process is invariably carried out under higher pressure in autoclaves using saturated steam. The temperature can be as low as 115°C, but is usually 121°C or higher.

Great care is needed in the design of autoclaves and sterilization cycles because of the requirement for the presence of moisture. The autoclave must be loaded to allow complete steam penetration to occur in all parts of the load before timing of the sterilization cycle commences. The time required for complete penetration, the so-called heat-up time, varies with different autoclave construction and different types of loads and packaging materials. The time may not exceed specific limits in order to guarantee reproducibility and, for porous loads, saturated steam. The volume of each container has a considerable effect on the heatup time whenever fluids are sterilized. Thermocouples led into the chamber through a special connector are often employed to determine heatup times and peak temperatures. The pressure is relieved at the end of each sterilization cycle. Either vented containers must be used or specific provisions be made to allow for the safe removal of nonvented, hermetically sealed containers from the autoclave.

The elimination of air from the chamber and complete steam penetration of the load is of critical importance. This may be accomplished by gravity displacement or prevacuum techniques.

The gravity-displacement-type autoclave relies on the relative nonmiscibility of steam and air to allow the steam that enters to rise to the top of the chamber and fill it. The air is pushed out through the steam-discharge line located at the bottom of the chamber. Gravity-displacement autoclaves are utilized for the sterilization of liquids and for unwrapped nonhollow medical instruments at 134°C.

The prevacuum technique, as its name implies, eliminates air by creating a vacuum. This procedure facilitates steam penetration and permits more rapid steam penetration. Consequently this results in shorter cycle times. Prevacuum cycles employ either a vacuum pump/steam (or air) ejector combination to reduce air residuals in the chamber or rely on the pulse-vacuum technique of alternating steam injection and evacuation until the air residuals have been removed. Pulse-vacuum techniques are generally more economical; vacuum pumps or vacuum-pump–condenser combinations may be employed. The vacuum pumps used in these systems are water-seal or water-ring types, because of the problems created by mixing oil and steam. Prevacuum cycles are used for fabric loads and wrapped or unwrapped instruments (see Vacuum technology).

In prevacuum autoclaves, problems are created by the removal of air and the air-insulation systems. A specific test called the Bowie & Dick test (12), was developed to evaluate the ability of prevacuum sterilizers to

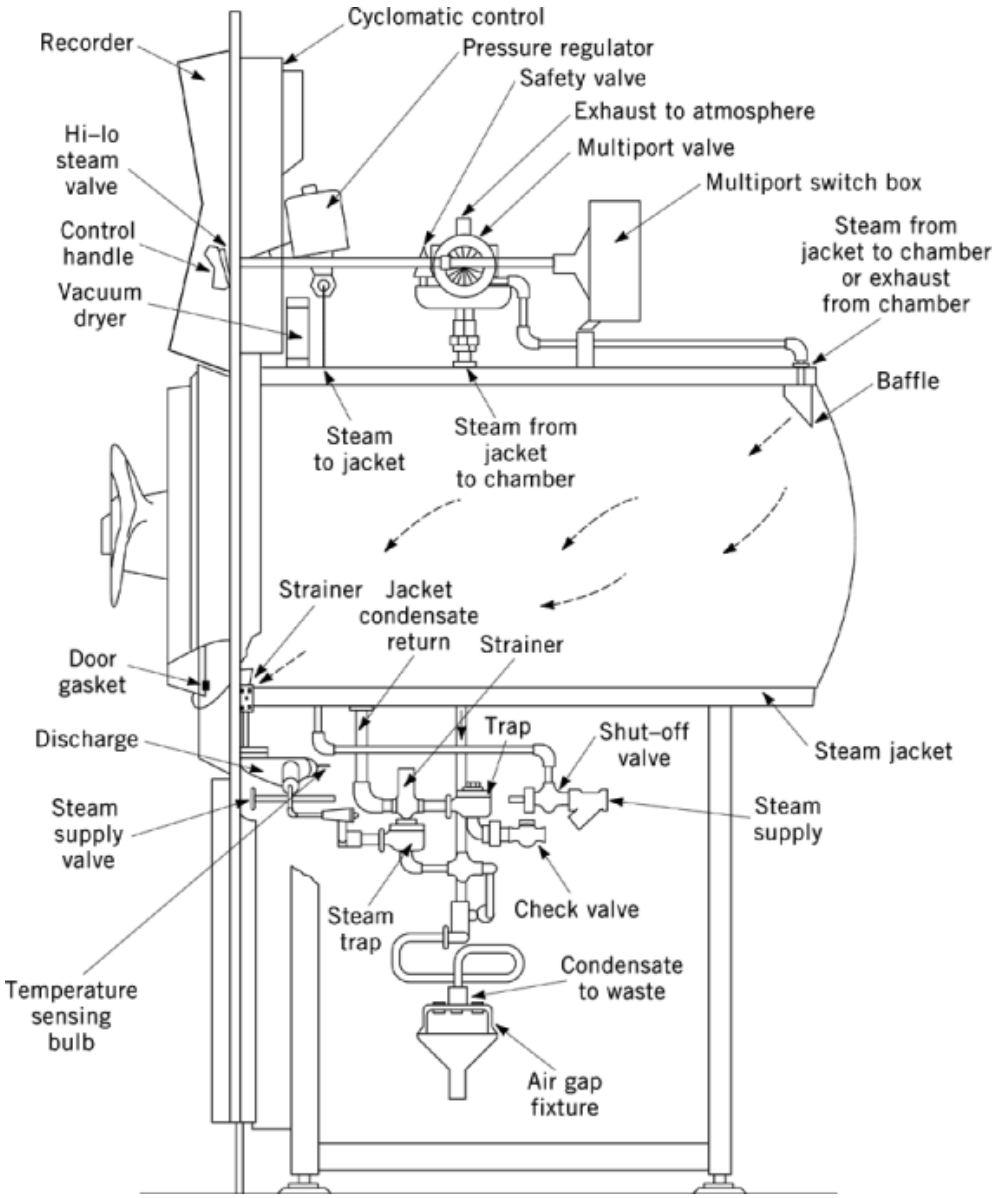

Fig. 1. Longitudinal cross section of steam sterilizer (1).

provide rapid and even steam penetration which includes the ability to eliminate air from the chambers, and prevent air from leaking back during the prevacuum phase. The test utilizes a pack of specific construction (or its proven equivalent) placed in the empty chamber and exposed to specific test conditions (9, 12). The pack contains a chemical indicator sheet. A correctly functioning sterilizer produces a uniform color change. A nonuniform color change indicates poor steam penetration and the possible presence of air which requires the attention of a qualified mechanic. The daily testing of all porous load sterilizers is recommended (1, 9, 10).

The critical parameters of steam sterilization are temperature, time, air elimination, steam quality, and the absence of superheating. Temperature and time are interrelated, as shown in equation 8. The success of steam sterilization is dependent on direct steam contact which can be prevented by the presence of air in the chamber. The ability of steam to heat a surface to a given temperature is considerably reduced by the presence of air. Air elimination, therefore, is regarded as an absolute parameter. If the required amount of air has not been eliminated from the chamber and the load, no combination of time and temperature results in complete sterilization.

The term steam quality refers to the amount of dry steam present relative to liquid water in the form of droplets. The steam delivered from the boiler usually contains some water. Excessive amounts can result in air entrapment, drying problems following exposure, and unacceptable steam levels ($>3\%$ water or $<97\%$ quality steam). Excessive amounts of water deposits dissolve boiler chemicals onto the load to be sterilized. Boiler chemicals are used to prevent corrosion in the lines. Inappropriate boiler chemicals, also called boiler amines, may introduce toxicity problems (see Corrosion and corrosion control).

Superheated steam results when steam is heated to a temperature higher than that which would produce saturated steam. The equilibrium between liquid and vapor is destroyed, and the steam behaves as a gas. It loses its ability to condense into moisture when in contact with the cooler surface of the article to be sterilized. This process resembles dry-heat sterilization more than steam sterilization and, under ordinary time–temperature conditions for steam sterilization, does not produce sterility.

The selection of an appropriate steam-sterilization cycle must be made after a careful study of the nature of the articles to be sterilized, the type and number of organisms present, type and size of each package, and type of packaging material used. Cycle-development studies may be conducted using full autoclave loads.

Because hospital loads are not uniform, certain assumptions were made by the manufacturers of sterilizers in arriving at recommended cycles. These recommendations include a safety factor, as well as allowance for heatup time. Exposure recommendations for various types of articles are available (12).

Biological indicators for steam sterilization utilize *Bacillus stearothermophilus*. In monitoring industrial cycles, a sufficient number of preparations each having a known degree of resistance are added to the load and retrieved after exposure, and cultured.

Electromechanical monitors for steam sterilization include pressure, temperature, time-recording charts, and pressure–vacuum gauges. Most recording charts are also capable of displaying pressure–vacuum values. The temperature sensor is generally located in the chamber-drain line, considered to be the coolest area in the autoclave because air exits from the chamber via the drain line. There is no way of locating the sensors inside the packages being sterilized except under specialized test conditions.

Chemical indicators for steam sterilization can be classified into four categories. Some indicators integrate the time–temperature of exposure. Some of these operate throughout the temperature range utilized for steam sterilization (121–141°C); others function in specific time–temperature cycles. Some are capable of monitoring the safety factor in the exposure; others only minimal conditions. All are capable of indicating incomplete steam penetration. The results are indicated by a color change. Certain types change from a specific initial color through a series of intermediate shades to a specific final color. An incomplete color change is an indication of incomplete processing. Other types function by having a color column advancing along the length of the test strip, in a manner reminiscent of paper chromatography. Inadequate processing is indicated by an advance that falls short of a predesignated finish line.

Some indicators can determine whether a specific temperature has been achieved. Because the entrapment of large amounts of air can result in the lowering of steam temperatures, these indicators react to some critical defect in sterilization conditions. For each different temperature, a different indicator must be used.

Indicators can determine if uniform steam penetration has been achieved during a Bowie & Dick-type test. Produced in the form of sheets (23×30 cm), chemical indicators are capable of uniform color change over their entire surface when exposed to pure saturated steam under test conditions. Nonuniform color development is

an indication of failure of the test. U.S. and international stands for the performance and accuracy of chemical indicators have been published (13, 14).

Indicators can be utilized to distinguish packages that have been processed from those that have not been processed. These are external indicators that do not have the capability to detect critical shortcomings in cycle parameters because they are not located inside the packages. A well-known example of this type is autoclave tape, which is also used to hold together packages wrapped in muslin or other kinds of wrap-type packaging materials (qv).

6. Gas Sterilization

When articles that cannot withstand the temperatures and moisture of steam sterilization or exposure to radiation require sterilization, gaseous sterilants that function at relatively low temperatures offer an attractive alternative. Although many chemical compounds or elements can be considered sterilants, an obvious practical requirement is that the gas selected should allow safe handling, and that any residue should volatilize relatively quickly if absorbed by components of the article sterilized. When properly applied, ethylene oxide [75-21-8] satisfies most of these requirements, and is the most frequent choice (1, 6, 15–17) (Fig. 2). Because it is highly flammable, ethylene oxide (qv) must be used in a carefully controlled manner. It is either dispensed from a single-use cartridge or diluted with inert gases until no longer flammable. The most frequently used diluents are hydrofluorocarbon (HCFC) gases and carbon dioxide. The HCFCs have been declared acceptable through the year 2035. Industrial ethylene oxide sterilizers are equipped with recovery systems whether they use 100% ethylene oxide or the HCFC-diluted material.

It is necessary to determine the bioburden and make cycle verification studies when ethylene oxide sterilization is used, as it is for other sterilization methods. The manufacturer of hospital sterilization equipment provides cycle recommendations based on the expected bioburden and the consideration of an appropriate safety factor. In ethylene oxide sterilization, it is necessary to determine if residues of the sterilant are absorbed by the sterilized article, and to examine the possible formation of other potentially toxic materials as a result of reaction with ethylene oxide.

The critical parameters of ethylene oxide sterilization are temperature, time, gas concentration, and relative humidity. The critical role of humidity has been demonstrated by a number of studies (11, 18, 19). Temperature, time, and gas concentration requirements are dependent not only on the bioburden, but also on the type of hardware and gas mixture used. If cycle development is not possible, as in the case of hospital sterilization, the manufacturer's recommendations should be followed.

Provisions must be made for allowing residues of the sterilant absorbed by the product to dissipate by using aeration cabinets that have forced-air circulation at elevated temperatures. The amount of remaining absorbed sterilant should be determined before releasing the sterilized articles. If, as in the case of hospital sterilization, such studies are not feasible, the recommendations of the manufacturers of the articles sterilized or of the aeration equipment should be obtained. The permissible residue concentrations are 10–250 ppm, depending on the type of article and on its intended use.

Biological monitoring of ethylene oxide sterilization is essential and is conducted using spores of *Bacillus subtilis*. See Table 2 for the required performance characteristics. Chemical indicators for ethylene oxide sterilization are usually of the color-change type and are capable of indicating the presence of ethylene oxide under some minimal conditions of temperature, time, and gas concentration. A few types are also capable of indicating the absence of moisture. External processing indicators similar to autoclave tape, but containing ethylene oxide-responsive chemicals are also available.

General recommendations for instrumentation include monitoring gas concentration, temperature, time, and the moisture content of the chamber. Hospital sterilizers are not usually equipped with instrumentation providing direct display of gas concentration and moisture content. These rely instead on a specific sequence

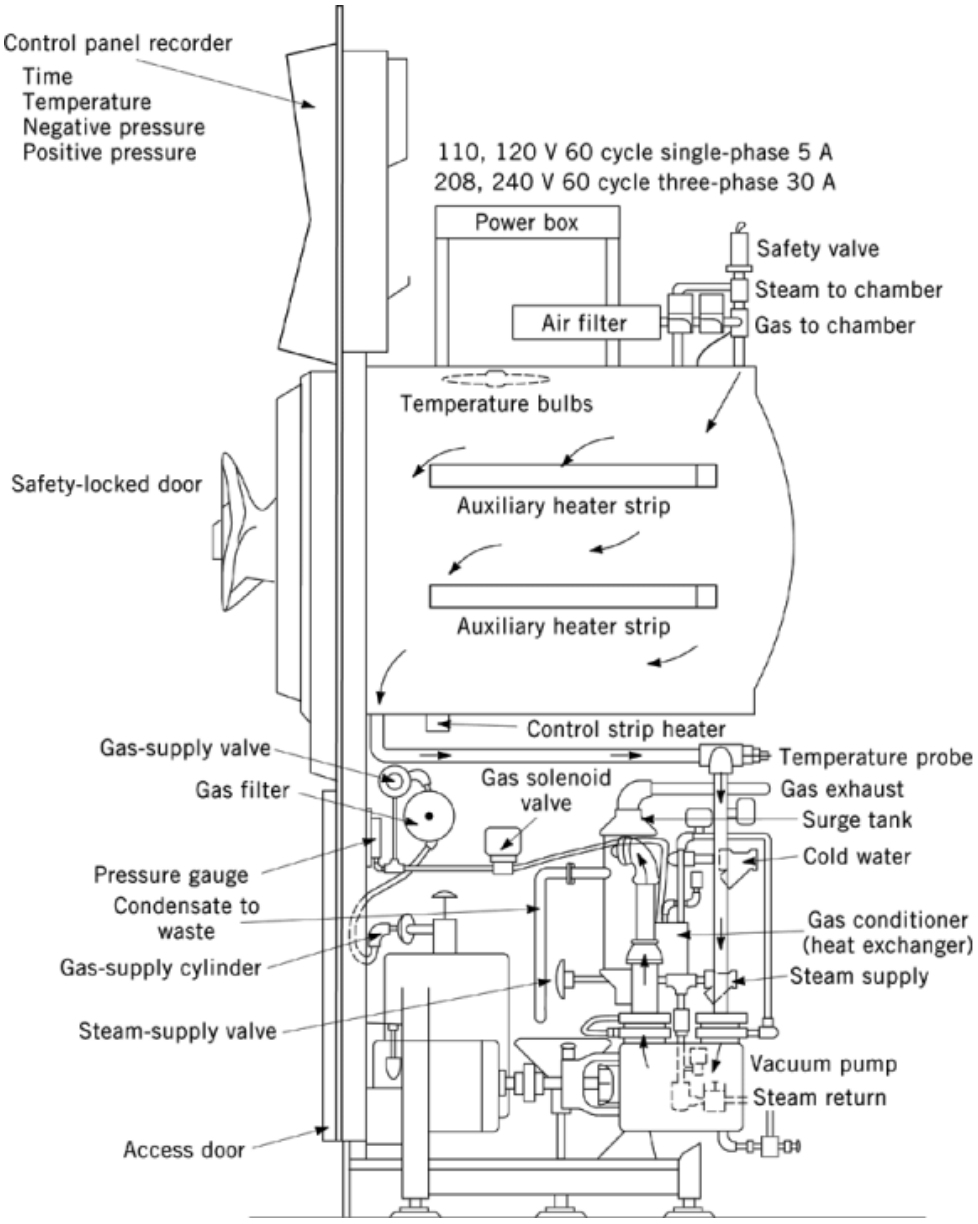

Fig. 2. Schematic of ethylene oxide sterilizer (1).

of steps performed automatically and the recording of pressure which when 100% ethylene oxide is used is a perfect measure for the concentration of this gas.

7. Other Sterilization Techniques

7.1. Ionizing Radiation

Radiation sterilization, as practiced in the 1990s, employs electron accelerators or radioisotopes (qv). Electrons have relatively low penetration ability, and the use of accelerators requires careful control. Gamma-radiation sterilization usually employs ^{60}Co and occasionally ^{137}Cs as the radioisotope source. A wide range of packaging materials can be used because gamma-rays possess a considerably greater penetrating ability. However, the materials must not be degraded to the point where the quality of the aseptic barrier is compromised.

Materials tend to undergo chemical changes when exposed to gamma-radiation. Therefore it is generally recommended that the effect of the radiation on any material be determined before gamma-ray sterilization is attempted. Exposure requirements are measured in terms of the delivered dose of radiations and the procedure becomes time independent. Bioburden determinations should be carried out to establish the effective dose. A dose of 2.5×10^4 Gy (2.5 Mrad) is selected for many articles, although lower doses are probably adequate. Radiation exposure can be monitored using counters and electronic measuring devices.

Chemical dosimeters based on ferrous sulfate, ferrous cupric sulfate, or ceric sulfate are generally used. Color-change process indicators are also used, but these cannot measure the radiation dose, only the extent of sterilization.

7.2. Filtration

The filtration process depends on the physical retardation of microorganisms from a fluid by a filter membrane or similarly effective medium. The effectiveness of this process is also influenced by the bioburden (6). Hollow-fiber membranes (qv) are most often employed. The resultant filtrate should be sterile, relatively free of particles, and not lose its effectiveness or safety. Filtration may be used for removal of both bacteria and viruses.

7.3. Liquid Sterilants

Formalin, a solution of methanal (formaldehyde (qv)) in water, has sterilizing properties (20), as does glutaraldehyde and hydrogen peroxide (qv) (21). The sterilizing action of these liquids depends on complete contact with the surface of the article to be sterilized, which can be prevented by grease, smudges, fingerprints, or other impurities. Liquid sterilants cannot penetrate a greasy layer, and careful precleaning is required before processing. The time of exposure is also critical. Although some sterilants can be used at temperatures higher than ambient, stability can be affected adversely. Changes in the pH of solutions may have similar adverse effects. The instructions of the sterilant manufacturer must be followed faithfully if liquid sterilants are to be effective.

Liquid sterilants are known to corrode the metal parts of articles and instruments that are to be sterilized, although articles composed exclusively of glass or certain type of corrosion-resistant metal alloys can be safely processed. Because the degree of corrosion is related to length of exposure, many articles are merely disinfected in a shorter exposure time. Disinfection may be suitable for certain applications. The safety of using liquid sterilants must be judged by a qualified microbiologist.

Other chemicals used historically as liquid sterilants include a solution based on hydrogen peroxide (qv). This was once offered commercially.

Processing in liquid sterilants results in wet products which require highly specialized packaging. Therefore, liquid sterilization should only be considered if the sterilized article is to be used almost immediately. Liquid sterilants or their residues can be harmful to living tissues. Therefore it is always necessary to rinse articles with sterile water or saline solution following treatment. Whereas liquid sterilization is an extremely

useful method for articles that cannot withstand the conditions of steam sterilization, the problems associated with its use limit its application.

7.4. Other Sterilants

Sterilization methods, developed in response to the requirements of a low temperature, noncorrosive sterilant and rapid turnaround time required by most hospitals, include use of hydrogen peroxide vapor, hydrogen peroxide plasma, and peroxy acetic acid. Acceptance of these methods was not universal as of this writing (ca 1996).

Other chemical agents or physical conditions that destroy microbial cells have limitations to general use. Ultraviolet light has sterilizing properties, but cannot penetrate many materials, whereas some substances are destroyed by exposure to a sterilizing dose. Some chemicals, such as 3-hydroxy-2-oxetanone (α-hydroxy-*O*-propiolactone), used in the past, have been found to be carcinogenic. Effectiveness of untested sterilants should be determined according to some acceptable test standard such as those listed in the Official Methods of the Association of Official Analytical Chemists (22). The manufacture and sale of chemical sterilants and disinfectants is regulated in the United States.

8. Sterilization Packaging

In rare instances it is possible to sterilize an article at the time and place of its use. However, sterilization generally takes place at one location prior to use of an article at another location. The main purpose of packaging (qv) is to protect the sterility of the contents. When an article is placed in its protective container and subsequently sterilized, the process is called terminal sterilization. When it is sterilized first and then placed in a presterilized container, the process is called sterile filling.

A sterile-filling operation requires an environment that excludes or diminishes the possibility of recontamination before the sterile product is sealed. Completely sealed units, such as glove boxes, are suitable. Specially constructed clean hoods or rooms which utilize laminar air flow having highly filtered air can be used for sterile filling. Personnel working in such environments have to wear special protective garments as well as masks, hair covers, etc. The packaging (qv) materials used in sterile filling can be of types that provide a hermetically sealed environment, such as glass, metal cans, or metal foil.

Packaging material used for terminal sterilization must permit full sterilant penetration as well as provide a microbial barrier. Consideration must also be given to the conditions to which the sterile package is to be exposed until used, such as storage, transportation, or frequency of handling.

Storage time by itself is not expected to affect the maintenance of sterility. However, longer storage times may increase the incidence of potentially harmful conditions. Frequent handling, wetness, and possible deterioration of the packaging material are typical examples of conditions that may compromise sterility and limit the shelf life of a package. The package contents may have a specific shelf life as well.

Most industrially prepared, presterilized packages contain inserts having statements that sterility is guaranteed only if the package is not opened or damaged. There is a wide choice of packaging materials and methods available for industrial processes. Using the appropriate packaging, sterilization methods, materials, and handling procedures, sterility can be protected for an indefinite length of time.

Hospital sterilization is more limited in the availability of sterilization methods and of packaging materials. Microbial invasion can occur particularly when articles are wrapped in traditional fabrics such as muslin (140-thread-count cotton). The expected shelf life of hospital-wrapped and sterilized articles is considered to be ca 21–30 days when a double-wrapping technique is used. Double-wrapping requires two successive wraps, each having a layer or layers of an approved packaging material.

Another type of hospital packaging system employs peel-open packages. These are constructed by heat sealing two webs of packaging material around the edges. One layer is usually a plastic film of composite construction, the other is a surgical-grade kraft paper designed to give an effective microbial barrier. Shelf life is extended to a time determined by need rather than sterility protection.

For any packaging method, provision must be made for opening of the package and retrieval of the sterilized article in a manner that does not compromise its sterility.

9. Related Techniques

Procedures less thorough than sterilization may be used for the preparation of foods and medical supplies. Some of these processes are capable of rendering an object microbiologically safe for a given purpose when employed using proper safeguards. For example, the cooking of food usually results in the reduction of spoilage because some of the potentially harmful organisms are destroyed, even though the process temperatures seldom exceed 100°C. Whereas the boiling of baby formula is not a sterilization procedure, it suffices in most cases.

Pasteurization, the heating of certain fluids, frequently milk or dairy products (see Milk and milk products), destroys potentially harmful organisms such as mycobacteria, *M. tuberculosis*, *M. bovis*, or *M. avium*. Pasteurization, carried out at 62°C for 30 min or at 72°C for 15 s, is not a sterilization procedure.

Disinfection destroys pathogenic organisms. This procedure can render an object safe for use. Disinfectants include solutions of hypochlorites, tinctures of iodine or iodophores, phenolic derivatives, quaternary ammonium salts, ethyl alcohol, formaldehyde, glutaraldehyde, and hydrogen peroxide (see Disinfectants and antiseptics). Effective use of disinfected materials must be judged by properly trained personnel.

Tyndalization, or fractional sterilization, is no longer considered acceptable for sterilization. Spores of vegetative organisms are the most difficult entities to destroy. In this procedure, rather than destroying the spores, spores are prompted to germinate and then destroyed by boiling water.

Bacteriostasis is the process of preventing the growth and reproduction of microorganisms. When the bacteriostat is removed or its power is exhausted, however, the organisms can resume growth. Bis(2-hydroxy-3,5,6-trichlorophenyl)methane (hexachlorophene) is a bacteriostatic agent.

Sanitization is a cleaning procedure that reduces microbial contaminants on certain surfaces to safe or relatively safe levels, as defined by the EPA or public health authorities. The article is usually cleaned with hot water and various germicidal detergents. Sanitization can be safe for a product in contact with intact skin or for food utensils, but it is not considered safe for articles to be inserted in the human body. Effective sanitization is a requirement in the processing of reusable medical supplies before packaging and sterilization. It is also a requirement in the maintenance of utensils and containers used for food preparation.

Decontamination is a procedure to render safe for handling, disposal, or the subsequent processing of an article that may contain a large amount of potentially infectious organisms. Decontamination and sterilization are similar procedures, except that in the former case the bioburden is higher. In both cases, all organisms present are destroyed. However, decontamination is not expected to result reliably in the 10^{-6} probability of microbial survival, as in sterilization, because of the higher bioburden. Decontamination may include sanitization and disinfection steps, but it most frequently involves sterilization procedures at exposure times two to four times longer than usual. Incineration is a frequent choice for decontamination of single-use articles (see Incinerators). Germicides are agents capable of killing some specific forms of microorganisms.

10. Sterilization in the Food-Processing Industry

The concept of heat processing of foods in hermetically sealed containers was introduced in 1810 (23). The role of microorganisms was unknown at the time, and only the so-called agents of putrefaction were eliminated.

The problem of microbial contamination of foods is twofold: foods may act as nutrients for, and carriers of, pathogenic organisms; additionally, foods may be spoiled by the action of certain organisms (see Food processing). Generally, four specific types of organisms are considered to be food poisons, although it is possible for a number of different organisms present in foods to cause disease. Salmonella and *Clostridium perfringens* require the ingestion of the organisms in large numbers. *Staphylococcus aureus* and *Clostridium botulinum* produce toxins and the organisms need not be ingested for the symptoms to occur. Salmonella is relatively easy to destroy by boiling, and so is *Staphylococcus aureus*, although the toxin of the latter is more heat resistant. The *Clostridia* are spore-forming organisms. The germinated cells can easily be destroyed, but the spores are heat resistant. *Escherichia coli*, a universal contaminant of the colon, has been found to be a cause of food-related disease outbreaks, but usually as the result of direct contamination by employees in restaurants in combination with the serving of undercooked food.

Food spoilage can be caused by enzymatic action or microorganisms. Certain preservation techniques, such as freezing, may retard spoilage by preventing the multiplication of microorganisms or the catalytic action of enzymes. Freezing, however, does not kill the organisms and should not be regarded as a sterilization method.

The most widely used sterilization method in the food industry is moist heat. The heat is usually supplied by high pressure steam, but because most foods already contain moisture the role of steam is to heat the food to the required temperature. The cooking and sterilization processes can frequently be combined into one. The food may be sealed into impervious containers of glass, metal, or plastic film and undergo terminal sterilization, or it may be presterilized in batches or in a continuous operation and then filled into a presterilized container. The latter process is called sterile filling.

The sterilizers or retorts used to process canned or prepackaged foods must be designed in such a way as to assure uniform temperature distribution throughout. Adequate venting permits complete air removal. The air vent is located at the opposite end from the steam inlet (24). The retorts may be horizontal or vertical in design.

The destruction of microorganisms in foods follows the same kinetic relationship as for other materials. The process is strongly influenced by the nature of the food, size of the container, and temperature. Industry-wide standards have been established by the National Canners Association (24).

The effect of various pHs has been well known for some time. Acidic foods such as fruits tend to retard microbial growth and resist certain types of contamination. For this reason, the standards adopted industry-wide have been based on the processing of foods of high acidity (low pH). In the United States, the FDA has regulatory responsibility over the preparation, sterilization, and distribution of foods.

Because of the large volume of food production, continuous processing offers economic advantages. The continuous sterilization of prefilled cans may be accomplished with relative ease because some cans are capable of withstanding the high pressures generated in order to attain the required sterilization temperatures. Such a process is, in reality, not truly continuous, because the prefilled containers represent a discontinuous phase. Time delays in heating full large-volume cans to sterilization temperatures place a limitation on the can sizes; presterilization followed by aseptic filling solves this problem. The containers are presterilized by heat, steam, radiation, etc. The food may be processed in large discontinuous batches, or in modern continuous retorts. Sterilization conditions are checked by appropriate instrumentation. A truly continuous cooking-sterilization process employs back-pressure valves or water towers to allow the attainment of pressures that result in required temperatures.

There are four types of food sterilization processes: terminal sterilization in prefilled containers in a batchwise process; terminal sterilization in prefilled containers of appropriate design heated to the required temperatures in a continuous process; aseptic filling following batchwise cooking in an appropriate retort; and aseptic filling in a continuous cooking system equipped with appropriate valves to allow the necessary pressures for attainment of the required sterilization temperatures.

BIBLIOGRAPHY

"Sterilization" in *ECT* 1st ed., Vol. 12, pp. 896–916, by E. L. Gaden, Jr., and E. J. Henley, Columbia University; in *ECT* 2nd ed., Vol. 18, pp. 805–829, by J. E. Doyle, Castle Co.; "Sterilization Techniques" in *ECT* 3rd ed., Vol. 21, pp. 626–644, by T. A. Augurt, Propper Manufacturing Co., Inc.

Cited Publications

1. J. J. Perkins, *Principles and Methods of Sterilization in the Health Sciences*, 2nd ed., Charles C. Thomas, Springfield, Ill., 1969.
2. H. Chick, *J. Hyg.* **8**, 92 (1908).
3. L. L. Katzin, L. A. Sandholser, and M. E. Strong, *J. Bacteriol.* **45**, 265 (1943).
4. G. B. Phillips and W. Miller, *Industrial Sterilization*, Duke University Press, Durham, N.C., 1973, 239–282.
5. G. B. Phillips and W. Miller, *Validation of Steam Sterilization*, Technical Monograph #1, Parenteral Drug Association, 1978.
6. *The United States Pharmacopeia XXIII* (USP XX–NF XV), The United States Pharmacopeial Convention, Inc., Rockville, Md., 1980, 1037–1040.
7. Ref. 1, p. 163.
8. *Accreditation Manual for Hospitals*. Joint Commission on Accreditation of Hospitals, Chicago, Ill., 1996.
9. *Steam Sterilization and Sterility Assurance*, Association for Advancement of Medical Instrumentation, Arlington, Va., ANSI/AAMI ST46, 1993.
10. *Assoc. Oper. Room Nurses J.* **32**, 222 (Aug. 1980).
11. R. R. Ernst, *Industrial Sterilization: Ethylene Oxide Gaseous Sterilization for Industrial Applications*, Duke University Press, Durham, N.C., 1973.
12. Ref. 1, p. 165.
13. *Sterilization of Health Care Products—Chemical Indicators, Part 1: General Requirements*, AAMI 1114001-D, 1995-11-30, Association for the Advancement of Medical Instrumentation, Arlington, Va., 1995, proposed new American National Standard.
14. *Sterilization of Health Care Products—Chemical Indicators, Part 1: General Requirements*, ISO 11140-1:1995(E), International Standards Organization, Geneva, Switzerland, 1995.
15. U.S. Pat. 2,075,845 (1937), P. M. Gross and J. F. Dickson.
16. C. R. Phillips and S. Kaye, *Am. J. Hyg.* **50**, 270 (1949).
17. C. R. Phillips, *Am. J. Hyg.* **50**, 280 (1949).
18. S. Kaye and C. R. Phillips, *Am. J. Hyg.* **50**, 296 (1949).
19. R. R. Ernst and J. E. Doyle, *Biotechnol. Bioeng.* **10**, 1 (1963).
20. A. Heyl, *Surg. Bus.* **42** (Jan. 1972).
21. U.S. Pat. 3,016,328 (Jan. 9, 1962), R. E. Pepper and E. R. Lieberman (to Ethicon).
22. W. Horwitz, *Official Methods of Analysis of Association of Official Analytical Chemists*, AOAC, Washington, D.C.
23. *Chem. Week*, 40 (Mar. 10, 1982).
24. *Processes for Low Acid Foods*, National Canners Association, Washington, D.C., latest edition.

General References

25. S. S. Block, *Disinfection, Sterilization, and Preservation*, 2nd ed., Lea and Febinger, Philadelphia, Pa., 1977.
26. G. Sykes, *Disinfection and Sterilization*, 2nd ed., Spon, London, 1965.
27. S. Turco and R. E. King, *Sterile Dosage Forms, Their Preparation and Clinical Application*, Lea and Febinger, Philadelphia, Pa., 1974.
28. N. A. M. Eskin, H. M. Henderson, and R. S. Townsend, *Biochemistry of Foods*, Academic Press, Inc., New York, 1971.
29. *National Conference of Spacecraft Sterilization*, California Institute of Technology, U.S. National Aeronautics and Space Administration Technical Information Division, Calif., 1966.

30. *Canned Food, Principles of Thermal Processing*, Food Processing Institute, Washington, D.C.
31. *Validation and Routine Monitoring of Moist Heat Sterilization Processes*, ISO 11134, International Standards Organization, Geneva, Switzerland.

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Related Articles

Disinfectants and antiseptics; Industrial antimicrobial agents; Filtration; Food processing