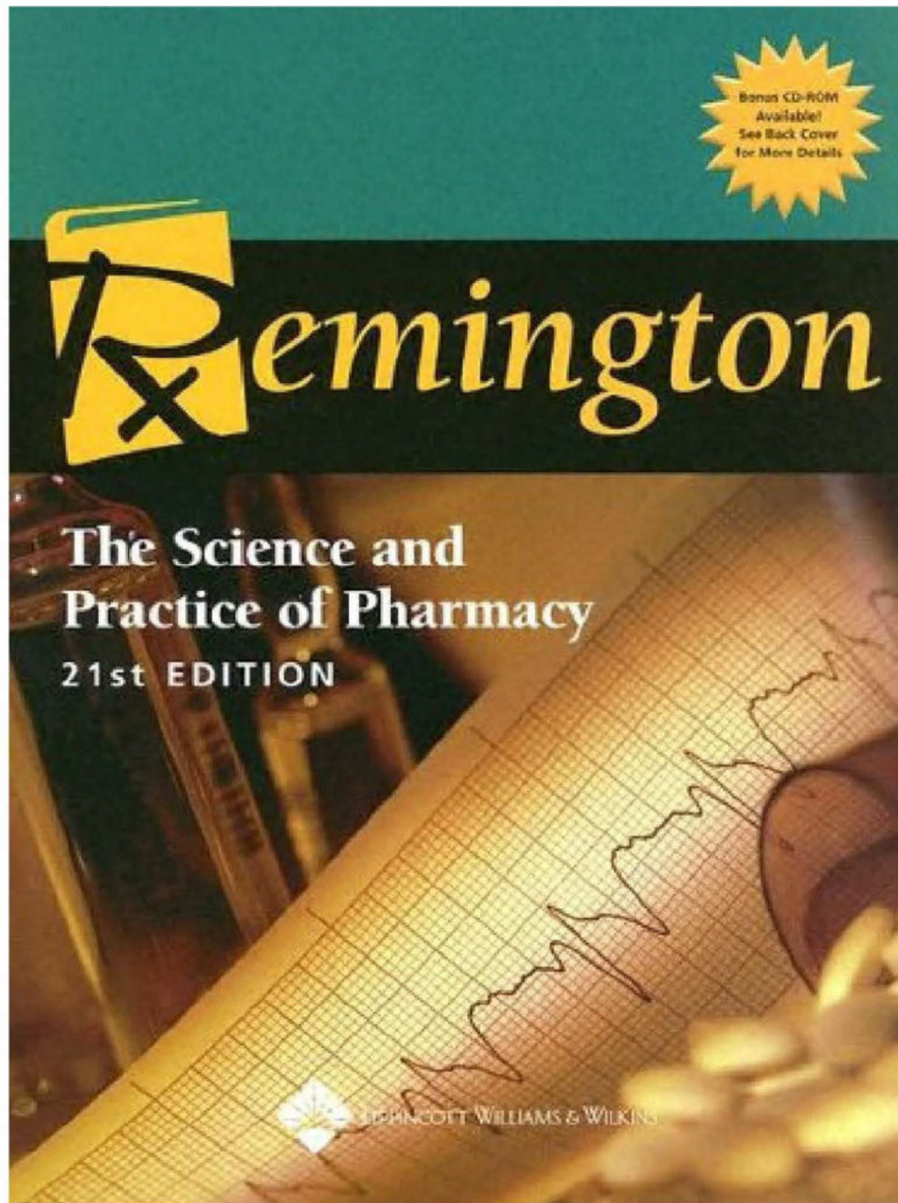

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ISBN 0-7817-4673-6

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CHAPTER 40

Sterilization

Donald E Hagman PhD

Sterilization is an essential concept in the preparation of sterile pharmaceutical products. Its aim is to provide a product that is safe and eliminates the possibility of introducing infection.

Mergers and acquisitions of pharmaceutical companies create multinational organizations faced with complying with all of the regulatory agencies of the involved countries. To date there is no global regulatory agency that oversees the production of sterile pharmaceutical products. Multinational companies must be familiar with the regulations of all countries in which they operate and meet those regulations. Although it is not the intent of this chapter to delineate the sterilization standards for all countries, it is to provide a detailed description of the techniques used throughout the world to sterilize pharmaceutical products. There are many attempts to standardize practices throughout the multinational industry. These include the efforts of the International Council on Harmonization (ICH) and the issuance of various technical ISO standards and compendial efforts of the various countries like United States Pharmacopoeias (USP) to set some basic standards. Additionally, organizations like International Society for Pharmaceutical Engineering (ISPE) and Parenteral Drug Association (PDA) have issued various documents, which include all facets of the international regulatory requirements.

Sterilization is a process used to destroy or eliminate viable microorganisms that may be present in or on a particular product or package. The process requires an overall understanding and control of all parts of the preparation for use of a particular product. Those areas include the selection and acceptance of all materials used for the product and package, environment in which the product is prepared and used and the ultimate disposition of the remaining materials after use. Sterilization may be required for several steps of the process using any one or a combination of the techniques listed in this chapter.

The aim of a sterilization process is to destroy or eliminate microorganisms that are present on or in an object or preparation, to make sure that this has been achieved with an extremely high level of probability and to ensure that the object or preparation is free from infection hazards. The currently accepted performance target for a sterilization process is that it provide for a probability of finding a nonsterile unit of less than 1 in 1 million. That is, the process (including production, storage, and shipment) will provide a *Sterility Assurance Level* (SAL) equal to or better than 10^{-6}. This is achieved through the processing of products in validated equipment and systems. Thorough validation and periodic requalification is essential to meeting these sterility requirements.

The purpose of this chapter is to provide a basic understanding of the following sterilization methods currently being used in pharmaceutical technology and the equipment employed to carry out these methods:

Method	Equipment
Moist heat sterilization	Saturated steam autoclaves
	Superheated water autoclaves
	Air over steam autoclaves
Dry heat sterilization	Batch sterilizers
	Continuous tunnel sterilizers
Chemical *cold* sterilization	Ethylene oxide
	Vaporized hydrogen peroxide
	Hydrogen peroxide/steam
	Other gases
Radiation sterilization	Electromagnetic
	Particulate
Filtration	Membranes

DEFINITIONS

The following terms, relating to sterilization, should be understood by those carrying out sterilization processes or handling sterile products:

Antiseptic—A substance that arrests or prevents the growth of microorganisms by inhibiting their activity without necessarily destroying them.

Aseptic—Refers to areas and practices where the intent is to be sterile.

Aseptic Processing—Those operations performed between the sterilization of an object or preparation and the final sealing of its package. These operations are, by definition, carried out in the complete absence of microorganisms.

Bactericide—Any agent that destroys microorganisms.

Bacteriostat—Any agent that arrests or retards the growth of microorganisms.

Bioburden—The number of viable microorganisms present prior to sterilization; Usually expressed in colony-forming units of volume.

Disinfection—A process that decreases the probability of infection by destroying vegetative microorganisms, but not ordinarily bacterial spores. The term usually is applied to the use of chemical agents on inanimate objects.

Germicide—An agent that destroys microorganisms, but not necessarily bacterial spores.

Sanitization—A process that reduces the level of bioburden in or on a product or object to a safe level.

Sterile—The absolute absence of viable microorganisms. There is no degree or partiality.

Sterility Assurance Level (SAL)—An estimate of the effectiveness of a sterilization process. It usually is expressed in terms of the negative power of 10 (ie, 1 in 1 million = 10^{-6}).

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Sterilization—A process by which all viable microorganisms are removed or destroyed, based on a probability function.

Terminal Sterilization—A process used to render products sterile to a preferred SAL.

Validation—The act of verifying that a procedure is capable of producing the intended result under prescribed circumstances and challenges to predefined specifications.

Viricide—An agent that will destroy viruses.

STERILITY AS A TOTAL SYSTEM

It is necessary to reiterate the concept already briefly addressed in the introduction. The task of the technology we are dealing with is to provide the product in sterile conditions to the end user. It is currently acknowledged that the quality of the product must be *built into* the process. This concept is particularly true when one of the essential qualities of the product is sterility.

Accordingly, the above-mentioned task is accomplished with a series of design, production, and distribution steps that can be summarized as activities for the selection and routine checking of the following items:

- Active constituents, additives, raw materials in general
- Water used both as solvent and as washing/rinsing agent
- Packaging suitable for the product and for the sterilization process that will be used
- Working environment and equipment
- Personnel

These procedures clearly have the purpose of providing the sterilization process with a product that has a minimum, definite, and consistent bioburden. There are also the following activities:

- Selection of the sterilization method that most suits the unit formed by the product and its packaging, and definition of the process variables for obtaining the intended SAL
- Selection of the machine that is most suitable for performing the selected method and of the utilities that this machine requires
- Qualification and validation of the machine and of the process
- Routine checking of the process
- Checking of the results of the sterilization process
- Proper storage of sterile goods and verification that their sterility is maintained with full reliability throughout the allowed storage period
- Delivering, opening, and using sterile goods without recontamination.

It also should be noted that, in December 2002, the US Food and Drug Administration (FDA) proposed new regulations for aseptic processing and terminal sterilization. The proposed rules as defined in their Concept Paper require that manufacturers of sterile products use validated and robust sterilization techniques wherever possible. The European Pharmacopeia and related pharmacopeias have modified their requirements in their rulings identified as Annex 1.

CONTAMINATION

Certain facts about microorganisms must be kept in mind when preparing sterile products. Some microbes (bacteria, molds, etc) multiply in the refrigerator, others at temperatures as high as 60°C. Microbes vary in their oxygen requirements from the strict anaerobes that cannot tolerate oxygen to aerobes that demand it. Slightly alkaline growth media will support the multiplication of many microorganisms while others flourish in acidic environments. Some microorganisms have the ability to use nitrogen and carbon dioxide from the air and thus can actually multiply in distilled water. In general, however, most pathogenic bacteria have rather selective cultural requirements, with optimum temperatures of 30° to 37°C and a pH of 7.0. Contaminating yeasts and molds can develop readily in glucose and other sugar solutions.

Actively growing microbes are, for the most part, vegetative forms with little resistance to heat and disinfectants. However, some forms of bacteria—among them the bacteria that cause anthrax, tetanus, and gas gangrene—have the ability to assume a spore state that is very resistant to heat as well as to many disinfectants. For this reason, an excellent measure of successful sterilization is whether the highly resistant spore forms of nonpathogenic bacteria have been killed.

The nature of expected contamination and the bioburden are important to pharmacists preparing materials to be sterilized. The raw materials they work with rarely will be sterile, and improper storage may increase the microbial content. Because the pharmacist seldom handles all raw materials in a sterile or protected environment, the environmental elements of the manufacturing area (air, surfaces, water, etc) can be expected to contribute to the contamination of a preparation. The container or packaging material may or may not be presterilized and thus may contribute to the total microbial load.

Understanding the nature of contaminants prior to sterilization and application of methods for minimizing such contamination is vital to preparing for successful pharmaceutical sterilization. Examples of such methods include:

- Maintenance of a hygienic laboratory
- Frequent disinfection of floors and surfaces
- Minimization of traffic in and out of the area
- Refrigerated storage of raw materials and preparations that support microbial growth
- Use of laminar airflow devices for certain critical operations
- Use of water that is of appropriate USP quality and is free of microbial contamination (It is preferable to use presterilized water to avoid any possible contamination.)

METHODS

General

The procedure to be used for sterilizing a drug, a pharmaceutical preparation, or a medical device is determined to a large extent by the nature of the product. It is important to remember that the same sterilization technique cannot be applied universally because the unique properties of some materials may result in their destruction or modification. Methods of inactivating microorganisms may be classified as either physical or chemical. Physical methods include moist heat, dry heat, and irradiation. Sterile filtration is another process, but it only removes, not inactivates, microorganisms. Chemical methods include the use of either gaseous or liquid sterilants. Guidelines for the use of many types of industrial and hospital sterilization are available.[1–10]

Each sterilization method can be evaluated using experimentally derived values representing the general inactivation rates of the process. For example, a death rate or survival curve for a standardized species can be diagramed for different sterilization conditions. This is done by plotting the logarithm of surviving organisms against time of exposure to the sterilization method. In most instances, these data show a linear relationship, typical of first-order kinetics, and suggest that a constant proportion of a contaminant population is inactivated in any given time interval. Based on such inactivation curves, it is possible to derive values that represent the general inactivation rates of the process. For example, based on such data, it has become common to derive a decimal reduction time or D value, which represents the time under a stated set of sterilization exposure conditions required to reduce a surviving microbial population by a factor of 90%.

D values, or other expressions of sterilization process rates, provide a means of establishing dependable sterilization cycles. Obviously, the initial microbial load on a product to be sterilized becomes an important consideration. Beyond this, however, kinetic data also can be used to provide a statistical basis for the success of sterilization cycles. A simple example will

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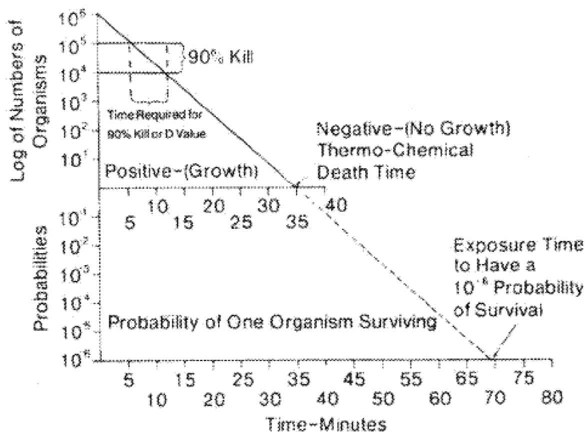

Figure 40-1. Sterilization model using D values.

suffice (Fig 40-1). When the initial microbial contamination level is assumed to be 10^6, and if the D value of the sterilization process is 7 minutes, complete kill is approached by application of 6 D values (42 minutes). However, at this point reliable sterilization would not be assured because a few abnormally resistant members of the population may remain. In this example, by extending the process to include an additional 6 D values, most of the remaining population is inactivated, reducing the probability of one organism surviving to one in 1 million.

Moist Heat

ESSENTIALS OF STEAM STERILIZATION KINETICS

Let us suppose a system contaminated by microorganisms (which we assume, for the sake of simplicity, to be pure and homogeneous) is immersed in pressurized saturated steam, at constant temperature; for example, it could be a vial containing an aqueous suspension of a certain spore-forming microorganism.

It has been shown experimentally that, under the above conditions, the reaction of thermal degradation of the microorganism obeys the laws of chemical reactions: the rate of reduction of the number of microorganisms present in the system in each moment is proportional to the actual number itself. The proportionality coefficient is typical of the species and conditions of the chosen microorganism.

Thus, the degradation reaction (the sterilization process) develops like a first-order chemical reaction in which the reaction rate is proportional, in each moment, only to the amount of microorganisms still to be inactivated. This seems to be obvious for dry sterilization, but less rigorous for steam sterilization, in which the water vapor molecules also seem to take part in the reaction. Actually, this bimolecular reaction is of the first order, as the steam is present in high excess during the entire reaction and its concentration may be regarded as constant.

The most frequently used mathematical expression of the above facts is

$$N = N_0\, 10^{-t/D} \tag{1}$$

where N_0 is the initial number of microorganisms, t is the elapsed exposure (equal to sterilization time), N is the number of microorganisms after the exposure time t, and D is the *decimal decay time*, defined as the time interval required, at a specified *constant* temperature, to reduce the microbial population being considered by 1/10 (ie, by one logarithmic value; eg, from 100% to 10% or from 10% to 1% of the initial value).

The D value is inversely proportional to the first-order reaction coefficient and is therefore typical of the species and conditions of the chosen microorganism. Depending on the initial hypothesis of exposure at constant temperature, each D value always refers to a specified temperature.

Equation 1 allows one to draw a first very important conclusion: the time required to reduce the microorganism concentration to any preset value is the function of the initial concentration. The sterilization reaction is therefore neither an *all-or-nothing* process nor a *potential barrier* process as was once thought.

It also is evident immediately that the effect of sterilization at the same constant temperature will be very different depending on the D value of the contaminating microbial species (or on the largest D value in the usual case of mixed contamination). Figure 40-2 shows that the same reduction ratio for different species is achieved after exposure time proportional to the D value of each species. The graph derives only from Equation 1 and from the definition of D value. The basic hypothesis of the temperature being constant is thoroughly valid.

Sterility Is a Probable Effect of Exposure Time—Let us now consider what happens within a batch of units (vials, bottles, or others) with an initial constant unit contamination of 100 microorganisms equal to 10^2. If the D value at 121°C is assumed to be 1, after 1 min at 121°C, a reduction equal to $10^1 = 10$ microorganisms is achieved; after another minute, only $10^0 = 1$ microorganism is still surviving. After another minute, the surviving microbial population would be $10^{-1} = 1/10$ microorganism. A contamination of 1/10 must not be understood to mean that each unit contains 1/10 of a microorganism, which is biologically meaningless (in this case the unit probably would be sterile) but that there is a probability of having 1/10 of the units still contaminated within the batch of sterilized units.

In fact, 3 min would be the necessary time to reduce the microbial population to a single surviving microorganism if the initial population were 10 times larger than the one at issue. This higher initial contamination could be regarded either as a 10 times larger number of microorganisms in the same unit, or as the initial contamination of a 10 times larger unit.

If the unit is not considered any longer as the single vial or bottle, but as the whole of all the items produced over a period of time, the initial number of microorganisms present in each item has to be multiplied times the number of items produced, and the exposure time to achieve the reduction to the same number of viable microorganisms left in the whole of the items produced, has to be increased correspondingly. The following example will be helpful to focus the matter.

A new sterile product in ampules has to be manufactured; the number of ampules to be produced over all the life period of the product is expected to be 10^{10}. The maximum number of contaminated ampules deemed to be acceptable is $10 = 1$; this obviously means that the probability of having nonsterile ampules after sterilization must not exceed 10^{-10}. Let us also suppose that the microbial population within each ampule after the filling and the sealing does not exceed 10^3 microorganisms. These must be destroyed by means of moist heat-terminal sterilization at 121°C. The applicable D value is 1 min. The total number of microorganisms to be destroyed during the life of the product will be

$$10^{10+3} = 10^{13}$$

If this whole microbial population were exposed to moist heat at 121°C over a period of 13 min, it would be reduced to 10^{-13} times its initial number (ie, to $10^{13-13} = 10^0 = 1$. The exposure time of 13 min thus would be sufficient (under all the other above hypotheses) to prevent the total number of contaminated ampules from exceeding the value of 1.

From the point of view of each single ampule, 13 min of exposure would reduce the microbial population to the theoretical value of

$$10^{3-13} = 10^{-10}$$

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To interpret this numeric value as the probability of still having one contaminated ampule in 10 billion sterilized ampules means that a single ampule will still be contaminated out of a whole lot of 10^{10}. This probability value is defined as PNSU (probability of nonsterile unit).

In recent times the PNSU as a sterility evaluation criterion is being replaced by the SAL. The name itself could generate some misunderstanding, because a level of assurance commonly is deemed to be good if high, but SAL seems to have been defined in such a way that its numerical value is the same as PNSU. This notwithstanding, it is sometimes calculated as the reciprocal value of PNSU. The SAP (sterility assurance probability) criterion has been proposed as well and SAP seems for the moment to have been granted the same definition of PNSU, even if it would be better understandable if its value approached unity after a satisfactory sterilization.

The above discussion and example lead to the conclusion that the optimum exposure time for a sterilization process must take into account not only the initial microbial population within the single item to be sterilized and the species and conditions of the contaminating microorganism, but also the total number of items expected to be sterilized over the life of the product.

Effect of Temperature Changes—All the above considerations have been developed under the basic assumption that the temperature is kept constant during the entire exposure time. It seems rather obvious that the D value will change as the temperature changes. If the D values experimentally obtained for a given microbial species are plotted on a semilogarithmic chart as the function of the temperature T, a path similar to Figure 40-3 is obtained.

In this case, it can be seen that D value is 1 min at 121°C (ie, the average value which very often is assumed to be acceptable in the absence of more exact experimental data). It also can be seen that D value varies by a factor of 10 if the temperature varies by 10°C.

The z value is defined as the temperature coefficient of microbial destruction, the number of degrees of temperature that causes a 10-fold variation of D (or, more generally, of the sterilization rate). The z values generally oscillate between 6 and 13 for steam sterilization in the range 100° to 130°C, and z value often is assumed to be equal to 10 in the absence of more precise experimental data.

The fact that D value varies by 10 times for a variation of 10°C when $z = 10$ must not lead to the false assumption that D varies by one time (ie, doubles) for an increase of 1°C. Obviously, this is not true. It is actually a matter of finding the number which yields 10 when raised to the tenth power. This number is 1.24. Therefore, a variation of 1°C entails a variation of D value of 24%. This is quite a significant number, which illustrates the dramatic effects that are generated when the sterilization temperature is also only a few degrees lower than the expected value, perhaps only in some areas of the sterilizer load.

It is also useful to remember that the effect of temperature variation decreases considerably as the temperature rises and drops to approximately 1/2 (or even less) for dry sterilization at approximately 200°C. Under these conditions the z value is about 20 instead of about 10. Therefore, the small temperature differences that can be so dramatic in steam sterilization have much less effect in dry sterilization.

The foregoing refers to average values because the actual D values and z values depend to a large extent on the medium that contains the microorganisms and on their history. At 121°C no microorganism has exactly $D = 1$ and $z = 10$. However, the combined use of these two parameters in calculating F_0 and PNSU provides ample margins of safety with regard to the microorganisms with which we deal commonly.

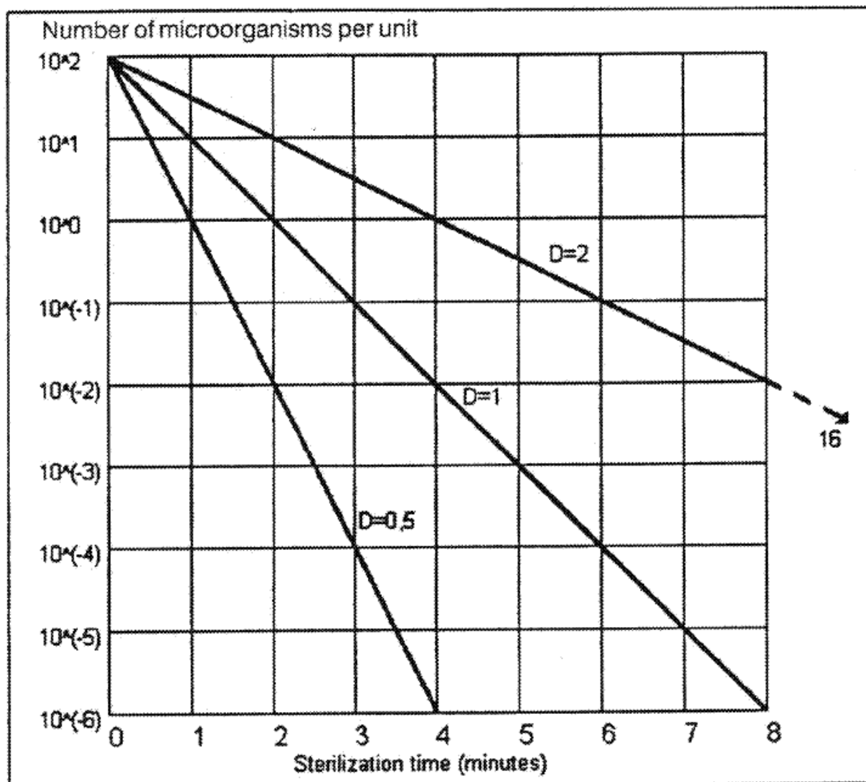

Figure 40-2. Effect of varying D values on sterilization rate (courtesy, Fedegari Autoclavi).

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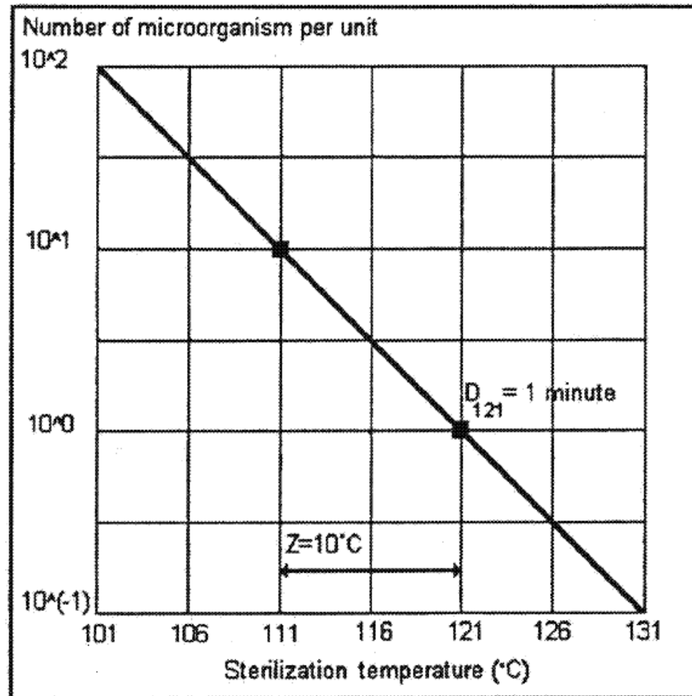

Figure 40-3. Effect of temperature on microbial destruction (courtesy, Fedegari Autoclavi).

F_0 or Equivalent Sterilization Time at 121°C—It is of the utmost interest to calculate the lethal effect of the exposure of a microbial population to a variable temperature, T, by relating it to an hypothetical sterilization performed at a constant temperature, T_0, for the time, t_0. If the constant reference temperature is assumed equal to 121.1°C (originally 250°F) and the z value equal to 10, the equivalent time is termed F_0. Thus, F_0 is the equivalent exposure time at 121.1°C of the actual exposure time at a variable temperature, calculated for an ideal microorganism with a temperature coefficient of destruction equal to 10.

First introduced in the *Laboratory Manual for Food Canners and Processors* by the National Canners Association in 1968, F_0 has become a common term in pharmaceutical production since the FDA used it extensively in the "Proposed Rules" of June 1, 1976 (21 CFR 212.3) with the following meaning:

> F_0 means the equivalent amount of time, in minutes at 121.1°C (250°F), which has been delivered to a product by the sterilization process. For the calculation of it, a z value of 10°C or 18°F is assumed; the term z value means the slope of the thermal death time curve and may be expressed as the number of degrees required to bring about a 10-fold change in the death rate.

In practice, the knowledge of the temperature values as the continuous function of elapsing time is not available, and F_0 is calculated as

$$F_0 = \Delta t \, \Sigma \, 10^{\frac{T - 121.1}{z}} \tag{2}$$

where Δt is the time interval between two consecutive measurements of T, T is the temperature of the sterilized product at time t, and z is the temperature coefficient, assumed to be equal to 10.

Saturated Steam

PRINCIPLES

Sterilization with saturated steam is the method that provides the best combination of flexibility in operation, safe results and low plant and running costs. The sterilizing medium obviously is pressurized saturated steam and the typical operating temperature is 121°C (250°F), but higher or lower temperatures often are used.

The term *dry* saturated steam sometimes is used: it should be made clear that this is an *ideal* condition of steam, and that moist saturated steam is used in practice for sterilization. However, the steam must entrain the smallest possible amount of condensate. The *water vapor ratio* of the steam defines the amount of condensate entrained by 100 parts by weight of moist steam; a water vapor ratio of 0.95 means that 100 g of steam consist of 95 g of dry saturated steam plus 5 g of condensate which is, or should be, *at the same temperature as the steam.*

The reliability of sterilization performed with saturated steam is based on several particular characteristics of this medium. When steam condenses, it releases calories at a constant temperature and in a *considerable amount:* 1 kg of pure saturated steam condensing at 121°C (turning into water at 121°C, thus without cooling) releases as much as 525 kcal. The temperatures and pressures of saturated steam have a two-way correlation. Once the temperature of the steam is determined, so is its pressure, and *vice versa.* Saturated steam at 121°C inevitably has a pressure of 2.05 abs bar. This entails two very interesting practical possibilities:

1. A pure saturated steam autoclave can be controlled indifferently according to the temperature parameter or according to the pressure parameter.
2. Regardless of the parameter used for control, the second parameter can be used easily to cross-monitor the first one.

A 1 gram molecule of water (18 g, or 18 mL in the liquid state) as steam at 121°C and 2.05 abs bar occupies a volume of approximately 15 L. This means that when steam condenses at 121°C it shrinks in volume by almost 1000 times. Accordingly, additional available steam *spontaneously* reaches the object to be sterilized. The condensate that forms can be removed easily from the autoclave chamber by means of a condensate discharge or, with a more modern technique, by continuous and

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forced bleeding (as occurs for example in so-called *dynamic steam* sterilizers).

However, several other phenomena must be considered. To perform its microorganism inactivating action (coagulation of cellular proteins), the steam, or more generally the moist heat, must make contact with the microorganisms. This can occur directly or indirectly. For example, it occurs directly when the steam that is present in the autoclave chamber is in direct contact with a surgical instrument. It instead occurs indirectly when moist steam is generated (by heat exchange with the steam present in the chamber) inside a sealed ampule that contains an aqueous solution. However, it is evident that it is not possible to steam-sterilize the inside of an empty closed ampule or the contents of an ampule if they are constituted by an anhydrous oil-based solution.

The air that is initially present in the autoclave chamber and the *incondensables* that possibly are entrained by the steam (generally CO_2) have molecular weights, and thus densities, 1.5 to 2.0 times higher than steam (under equal temperature/pressure conditions). Therefore, the air must be eliminated initially from the chamber and the steam must not introduce incondensables in the chamber; otherwise, these tend to stratify in the lower portions of the chamber, creating intolerable temperature gradients.

When closed nondeformable containers that contain aqueous solutions are sterilized, the pressures inside them can reach values far above those of the chamber. All air has been removed from the chamber, which in fact only contains steam: accordingly, at 121°C the pressure is 2.05 abs bar. The container instead almost always has a head space that contains air (or other gases). During sterilization, the aqueous solution of the container produces a vapor pressure that is approximately equal to 2.05 abs bar, but this value is increased by the partial pressure of the air of the head space; assuming that its initial value is 1.0 bar, it will increase to approximately 1.3 bar due to heating.

Pressure increases also will occur due to the thermal expansion of the solution (which is not entirely compensated by the expansion of the glass of the container) and because any gases dissolved in the solution may leave it.

Generally, in the conditions described above the total pressure inside the container exceeds by approximately 1.4 bar the pressure in the chamber if the initial head space is, as usually occurs, 10 to 20% of the total volume of the container. This overpressure generally is well tolerated by glass ampules, even those of considerable capacity (20 to 30 mL). However, it becomes hazardous for glass containers fitted with rubber stoppers held in place by a seal (due to the risk of stopper lifting) and intolerable for deformable containers, such as rigid (and even flexible) plastic containers, prefilled syringes, or cans. In all these cases, it is necessary or convenient to use the *counterpressure* sterilization methods (described later).

SATURATED STEAM AUTOCLAVES

Materials—All autoclaves intended for the pharmaceutical industry are made of Class AISI 316 stainless steel, including valves and piping (Fig 40-4). Only the service elements arranged *downstream* of the autoclave (for example the vacuum pump or the condensate discharge) are accepted if they are made of other materials. The service elements *upstream* of the autoclave (eg, heat exchangers or water pumps) also must be made of stainless steel.

Silicone rubber or Teflon and derivatives thereof generally are used for the gaskets (of doors, valves, etc).

Structure—Saturated steam autoclaves generally have a quadrangular, or rarely cylindrical, chamber. The doors are generally quadrangular even if the structure is cylindrical; in this case, the doors are inscribed in the circumference. There may be one or two doors: when the autoclave leads to a sterile room, there are always two doors.

Two-door autoclaves often are used when this requirement does not occur but the need is nonetheless felt to separate the loading area, where products to be sterilized are placed, from the unloading area, where already sterilized products are placed. *This concept applies to all types of sterilizers.*

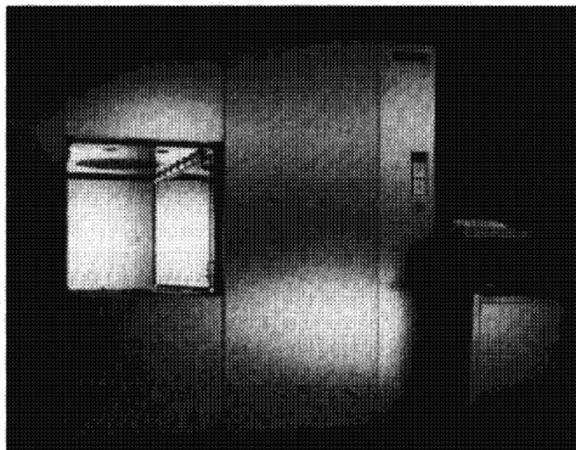

Figure 40-4. A modern computerized steam autoclave with horizontal sliding door (courtesy, Fedegari Autoclavi).

Doors may be of various kinds. The most common types are

- Hinged, manually operated, retained by radial locking bars, with a solid and fixed gasket
- Hinged, semiautomatically operated, retained by means of abutments in which the door engages automatically and with a movable gasket activated by compressed air
- Vertically or laterally sliding, with retention and gaskets as mentioned immediately above

Saturated steam autoclaves generally are jacketed. There is no room here to discuss the various kinds of jacket and their purposes. However, there are two ways to feed steam into the jacket and into the chamber:

Single Feed—the steam circulates first in the jacket and passes from the jacket into the chamber.

Separate Feed—usually the chamber is fed pure steam and the jacket is fed industrial steam.

Single-feed steam has some advantages in terms of control, but separate-feed steam is preferred because it provides better assurances of lack of microbiological and particle contamination.

MANAGEMENT SYSTEMS

The management systems used on currently manufactured autoclaves are programmable logic controllers (PLCs) or personal computers (PCs), or sometimes combinations of PLCs and PCs. This is also true for other kinds of autoclaves and sterilizers, which will be discussed later. However, a very large number of autoclaves controlled by electropneumatic systems are still in operation and still perform acceptable work. Naturally, the current control systems offer a kind of performance that was undreamed of earlier.

Pressure or temperature control (as mentioned previously, these parameters are interchangeable for a saturated steam autoclave) generally is performed with a proportional-integral-derivative method. Control by temperature is the generally accepted scheme because it is not influenced by trapped air. Sterilization can be time-managed or F_0-managed (with the F_0 being accumulated by heat probes enabled for this function), or time-managed with simultaneous calculation of F_0 for monitoring purposes.

Some management systems offer exceptional flexibility in composing programs and in setting parameters even to operators who have no knowledge of electronic programming. The information provided in real time (on same display device) is ex-

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tremely detailed, as is the permanent information, which can be produced on paper or stored on various kinds of electronic medium.

PROCESS

Initial Removal of the Air from the Chamber—The main reason the air must be removed from the autoclave chamber has been pointed out above.

Loads often are made up of porous materials or materials packaged in sterilization paper or in plastic/paper bags, or contained in filter boxes. All these situations require reliable and rapid removal of the air from the load. The so-called *gravity* removal method is considered obsolete. Modern autoclaves have a water-ring vacuum pump that can produce a vacuum of approximately 70 residual mbar in the chamber. Accordingly, only about 10% of the air remains in the chamber. There are essentially two methods for completing air removal:

Pulsed Vacuum—Once the initial vacuum has been reached, the pump is stopped and steam is introduced in the chamber (up to approximately atmospheric pressure), then vacuum is produced again. Three or more of these vacuum/steam pulses are performed.

Dynamic Vacuum—Once the initial vacuum has been reached, the pump continues to run, but at the same time a 5- to 10-min injection of steam is performed (from the side of the chamber that lies opposite the vacuum drain).

Modern autoclaves are capable of performing either of these methods, chosen according to the load to be processed.

Heating-Sterilization—During heating phases, and much less during the sterilization phase, considerable amounts of condensate form in the chamber. Except for particular instances, this condensate must be removed from the chamber. There are basically two extraction methods:

- *A condensate trap located at the bottom of the chamber.* This is the simplest and cheapest method, but it causes significant pressure drops, and therefore temperature drops, inside the chamber due to the inertia of the condensate trap. Essentially, it discharges not only the condensate but also significant amounts of steam, which cause instantaneous expansion, and thus cooling, of the steam that remains in the chamber.
- *Dynamic steam.* This is the most reliable and elegant system, but is also more expensive. During the heating and sterilization phases, the vacuum pump is kept running and draws from the chamber all the condensate that forms in it through a low-capacity valve. A certain amount of steam is naturally aspirated continuously, and a dynamic condition of the steam is thus produced, hence the name of the method.

Autoclaves also are required to have a continuous steam bleed past the controlling sensor in the drain line.

Post-Sterilization Phases—These may be different according to the material to be sterilized and depending on the results to be obtained on the material itself. The most common solutions are listed below.

1. **Vacuum and Time-Controlled Vacuum Maintenance**—This method is used to dry and simultaneously cool loads of solid materials, both porous and nonporous. It is performed by restarting the vacuum pump until a preset value (eg, 100 mbar) is reached; the pump then is kept running for a preset time (eg, 20 min).
2. **Cooling by Circulating Cold Water in the Jacket**—This method is used to cool containers that are partially filled with solution (eg, culture media) and closed with sleeve (Bellco-type) stoppers. Naturally, with these loads Item 1 is not applicable, because the solution would boil, and Item 3 is dangerous due to possible contaminations. This method is performed by removing the steam present in the chamber through the introduction of compressed sterile air at a pressure that is equal to, or greater than, the sterilization pressure. Then, cold water is circulated in the jacket. The pressurized compressed air in the chamber has two purposes: (1) to prevent the solution from boiling and (2) to improve heat exchange between the load and the jacket.
3. **Cooling by Spraying Water on the Load**—This method generally is used for loads of filled and closed ampules and plastic intravenous containers. It is performed with deionized water (to avoid salt residues on the ampules) which is nebulized onto the load by means of a sparger provided in the ceiling of the chamber. Naturally the ampules, which preferably are arranged in an orderly fashion, must be contained in trays with a perforated bottom. Nebulization of the water causes a rapid condensation of the steam that produces a sudden pressure drop in the chamber, whereas the pressure inside the ampules still remains rather high because the solution cools rather slowly. Ampules of good quality (even large ones up to approximately 20 mL) tolerate this method adequately. Cooling stops when the solution inside the ampules has reached the temperature of 70° to 80°C. In this manner, the load, removed from the autoclave, still contains enough heat energy to dry spontaneously.
4. **Ampule Tightness with Fast Vacuum**—The pressure stress described in Item 3, above, is produced deliberately and increased by activating the vacuum pump as soon as the sterilization phase ends. The pressure in the chamber quickly drops to values that can reach 150 to 200 mbar (obviously this value can be controlled easily), whereas the pressure inside the closed ampules initially remains above 3.0 bar. The ΔP thus produced breaks ampules with *closed defects*, such as thinner regions, tensions in the glass, and closed cracks.

   Obviously, if the ampules have *open defects* (ie, holes at the tip or open cracks), the ΔP does not arise or is very small and thus the ampules rarely break. What happens instead is that the solution in the ampule boils and thus evaporates, reducing the volume of the solution. Unfortunately, this evaporation is very limited. Because it requires a considerable amount of energy, the solution cools very quickly and the boiling ends. One cannot rely on the transmission of heat from the adjacent ampules or from the jacket, because the chamber is evacuated. It is evident that in such conditions, solution in the liquid state leaks from the ampules; at least from the *open defects* that lie below the level of the solution. Accordingly, it may be convenient to load the ampules upside down (ie, with their tip pointing downward) if it is known that most defects occur at the tip or shoulder of the ampules. Naturally, the breakage of the ampules or the leakage of solution soils the load, which must therefore be washed and dried. With appropriate methods it usually is possible to achieve all this in the autoclave itself.
5. **Cooling as in Item 3, but with Air Counterpressure**—In many cases it is not possible or reasonable to subject the load, during cooling, to the pressure stress that arises with the method described in Item 3. In such cases, it is possible to remove the steam present in the chamber by replacing it with sterile compressed air at a pressure that is equal to, or higher than, the sterilization pressure. Only after this has occurred does the cooling water spray described in Item 3 begin. This method only prevents the load from suffering the pressure stress of the cooling phase, whereas the stress of the sterilization phase is unavoidable. Reference is made to the section on *Counterpressure Methods* below for an explanation of this phenomenon and for the autoclaves that allow to avoid it.
6. **Spontaneous Cooling**—In some particular cases it may be necessary to resort to this cooling method, which is the simplest but also obviously requires a very long time. Clearly, at the end of this cooling the autoclave will be in vacuum, and the longer the cooling the deeper the vacuum.
7. **Ampule Tightness Test with Dye Solution Penetration**—This test generally is performed with an aqueous solution of methylene blue. However, it is also possible to use other dyes. This test is effective only on *open defects* of ampules and is performed as follows:
   a. Vacuum in the chamber to approximately 100 to 150 mbar.
   b. The chamber is filled with the colored solution until the load is completely covered; the ampules must of course be contained in appropriate trays that do not allow them to escape, because they tend to float.
   c. During this filling operation, the chamber vacuum reached in Item 1 is maintained continuously by connecting the vacuum pump to the ceiling of the chamber.
   d. The colored solution is pressurized at 2 to 3 bar and is maintained in this condition for 30 to 60 min or more.
   e. The colored solution is discharged and recovered.
   f. The load is washed several times with spray water.
   g. The load is washed by flooding the chamber.
   h. The washing water is discharged.

There are alternatives to this method, such as electronic spark discharge inspection which detects leakage of liquid from the ampule by a decrease in resistance across electrodes placed across the ampule.

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- The vacuum is not maintained continuously while the chamber is being filled with the colored solution.
- The vacuum is produced only after filling the chamber with the colored solution.
- The vacuum is not produced at all.

This test has in any case the following problems:

- It has been demonstrated extensively that with usual values for dye concentration, differential test pressure, and test time, tip holes with a diameter of less than 5 to 10 μm allow very small amounts of colored solution to enter. This prevents detection of the coloring of the ampules during subsequent checking.
- The preparation of sterile colored solution for each test entails very high costs.
- Recovery and reuse of the colored solution entails keeping it in conditions that prevent microbial proliferation (80°C) and subjecting it to sterilizing filtration prior to each test. All these procedures are expensive and complicated. In any case, the solution recovered from each test is contaminated chemically by the broken or defective tested ampules.
- Decolorization/destruction of the solution is very difficult, because methylene blue is very stable; however, good decolorization results have been achieved by using ozone. The use of amber glass ampules makes detection of the dye difficult.

STERILIZING THE AIR INTRODUCED IN THE CHAMBER

In the previous paragraphs we noted that it is often necessary to introduce air in the chamber, especially in post-sterilization phases. This air must be sterile, otherwise it may recontaminate the sterilized load and can, in any case, contaminate the sterile environment if the autoclave is of the two-door type connected to the sterile area.

The air generally is sterilized by filtration using a system that is part of the autoclave. It is thus necessary to

- Provide a filtration cartridge with sterilizing porosity
- Allow *in situ* sterilization of the assembled filtration system with an appropriate sterilization program of the autoclave itself
- Ensure that the filtration system and the line for connecting it to the autoclave maintain their sterility between one production sterilization program of the autoclave and the next
- Allow validation of all of the above described procedures

If one wishes to operate in perfect safety, the filtration system also should be subjected to an integrity test each time it is operated.

Counterpressure Methods

Autoclaves operating with counterpressure are defined as devices able to control, during sterilization, the pressure of the moist sterilizing medium independently of its temperature. Conventional pure saturated steam autoclaves do not belong to this category. The temperature of the pure saturated steam present in the chamber in fact automatically generates a specific pressure that cannot be modified without modifying the temperature as well. If the temperature of the steam is 121°C, its pressure is unavoidably 2.05 bar abs and *vice versa,* assuming no trapped air.

For many kinds of load it is necessary or convenient to use an autoclave operating with counterpressure. To understand this need, let us see what happens in a conventional autoclave during the sterilization of a rigid container partially filled with an aqueous solution and closed tight. For the sake of simplicity, let us assume that the container is filled with pure water.

A glass bottle is filled partially in standard conditions: 20°C and 1.013 bar; the bottle is closed with a rubber stopper and aluminum seal. In the head space there is a total pressure of 1.013 bar, which is actually the sum of two factors: a partial water-vapor pressure which corresponds to the vapor pressure of water at 20°C, ie, 0.025 bar, and a partial air pressure of 0.988 bar.

When the bottle is subjected to the sterilization phase at 121°C, these two factors change as follows:

	Initial Condition		Sterilization Condition
Partial water-vapor pressure	0.025	→	2.050 bar (1)
Partial air pressure	0.988	→	1.330 bar (2)
Total pressure in head space	1.013	→	3.380 bar abs

Value 1, 2.050 bar, is obviously the pressure of water vapor at 121°C and *corresponds to the pressure that occurs in the autoclave chamber.* Value 2, 1.330 bar, is a theoretical value that is calculated by applying the law of perfect gases to air:

$$0.988 \times \frac{121 + 273}{20 + 273} = 1.330$$

Therefore, the total pressure of 3.380 bar abs is also a theoretical value.

There are some reports that demonstrate that the *practical* value is slightly higher than the theoretical one and largely depends on the ratio between the head space and the volume of the filling solution. The practical pressure of the head volume is, on average, higher at 121°C by approximately 1.40 bar, with respect to the pressure in the chamber. This is caused by two mechanisms:

The thermal expansion of water is significantly greater than that of glass and increases very rapidly as the temperature rises. The specific volumes of water at the temperatures we are interested in are in fact

Temperature °C	Specific Volume mL/g
0	1.0002
4	1.0000 (maximum density)
20	1.0017
120	1.0606

In passing from 20° to 121°C, water increases its volume by approximately 6% according to the following ratio:

$$\frac{1.0606}{1.0017} = 1.058$$

This fact must be considered carefully by those who tend to reduce or eliminate the head space in containers and then are surprised to find that such containers explode or warp during sterilization. Solutions (especially if filtered under gas pressure) contain considerable amounts of dissolved gases that leave the liquid phase as the temperature rises.

The overpressure of approximately 1.40 bar that occurs in the bottle naturally generates a force of approximately 1.4 kg per cm^2 of internal surface of the bottle. A rubber stopper with a diameter of 24 mm is subjected to an expulsion force of approximately 6.3 kg.

These conditions therefore prevent or advise against the use of a pure saturated steam autoclave to sterilize solutions contained in a wide variety of containers. For example,

- Large-Volume Parenterals (LVP) in glass containers
- Small-Volume Parenterals (SVP) in glass vials with rubber stopper
- LVP or SVP in plastic containers (flexible, semirigid, or rigid plastic)
- Prefilled syringes
- Jars or similar containers with press-on or screw on closures
- Blisters containing various materials, such as disposable contact lenses

Two counterpressure methods currently in use are

- Superheated water spray method (water cascade process)
- Air overstream method (steam plus air method)

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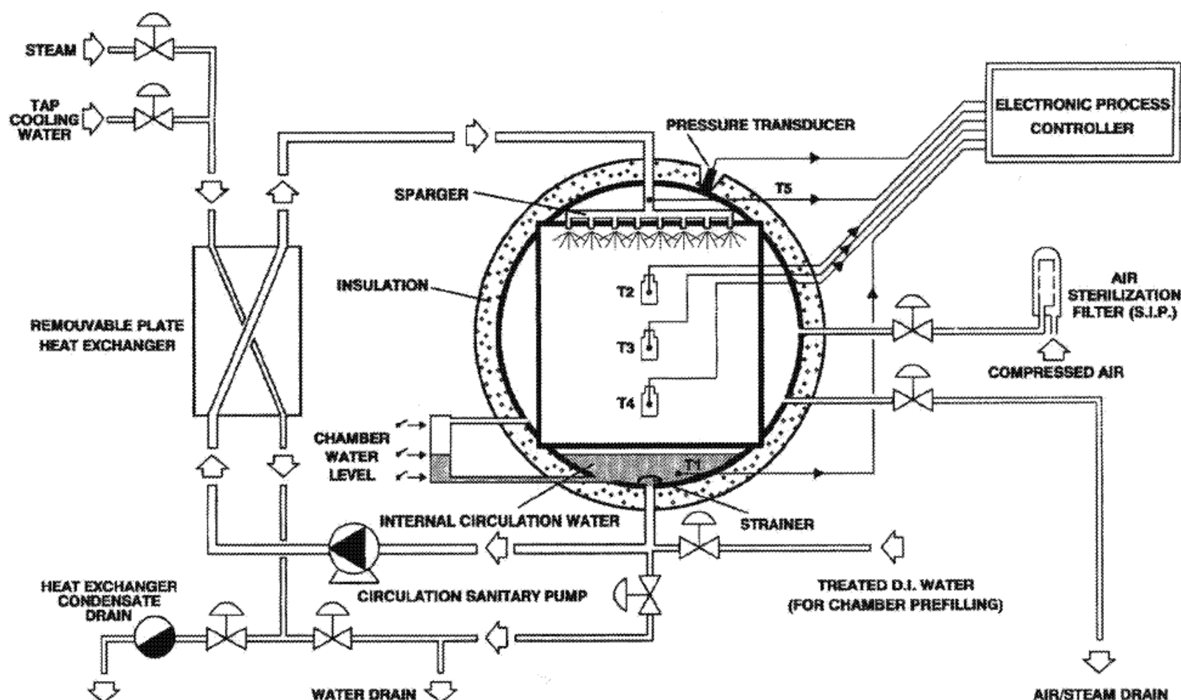

Figure 40-5. Superheated water-spray autoclave: simplified piping and instrumentation diagram (courtesy, Fedegari Autoclavi).

SUPERHEATED WATER SPRAY AUTOCLAVES

A typical functional diagram of this autoclave is shown in the Figure 40-5. Obviously, different solutions are also possible which, however, do not change the essence of the method. The chamber generally has a circular cross-section (with quadrangular door(s) inscribed in the circumference) and has a single wall.

At the beginning of the program, after the goods have been loaded, the lower circular sector is filled with purified water. The air contained in the chamber is *not* removed. The water, drawn by a sanitary-type pump, circulates in a heat exchanger (plate or other sanitary type), which is *indirectly* heated in countercurrent with industrial steam. The water returns then into the upper part of the chamber and is distributed to the load by a system of solid-cone spray nozzles. The uniform redistribution of the water on the lower layers of the load is ensured by appropriate perforated racks that support the load. Side spray bars sometimes are used, even if their actual usefulness is not demonstrated.

The heating of the circulation water, and therefore of the load, is gradual but quite fast; for example, the temperature of 121°C is reached in approximately 20 to 30 min *inside* 500-mL containers, mainly dependent on the solution and the material and shape of the containers.

The sterilization phase lasts 15 to 20 min, and temperature uniformity (in time and space) is excellent: it is well within the quite narrow limits required by FDA for LVP sterilization, ±0.5°C. This allows very small F_0 dispersions, and therefore minimum sterilization times.

The cooling phase is performed while the circulation water, now sterile, continues to circulate. However, cold tap water now flows in the plates of the exchanger, where steam was flowing earlier. In less than 15 min, the temperature *inside* the 500-mL containers drops to approximately 70°C, which is also the ideal temperature for obtaining a rapid and spontaneous drying of the load removed from the autoclave.

During all the phases of the process, an appropriate sterile air counterpressure is maintained inside the chamber to counterbalance the overpressure in the bottles. There are various methods for controlling this counterpressure in each phase. With computerized management, it is even possible to generate a total pressure (steam plus air) inside the chamber that is correlated, in each phase, to the average of the internal temperatures of two or more *witness* containers.

The load suffers no thermal or pressure shock and the differential pressure between containers and chamber can be eliminated or maintained in a direction convenient, in each phase, for the particular type of load. Even highly deformable products (semirigid plastic containers or plastic–aluminum blisters) or products that are particularly sensitive to differential pressures (eg, prefilled syringes) can be treated (from 60° to 127°C) without problems.

The autoclaves are obviously highly specialized machines, and as such they have some limitations in application:

- It is illogical to attempt to dry the load inside the autoclave by putting the chamber in vacuum or by circulating warm air.
- In the case of materials with concavities directed upward, these concavities will be filled with water at the end of the program: the most obvious solution is to load these materials upside down.
- When PVC bags are sterilized, the phenomenon of *blushing*—the whitening of the PVC due to water absorption—usually occurs. The intensity of this phenomenon and the time required for its disappearance depend on the type of PVC and of plasticizer employed. Blushing does not occur with rigid or semirigid plastic or with polylaminate plastics; it also is reduced considerably with PVC containing special plasticizers.

AIR OVER STEAM AUTOCLAVES

A typical functional diagram of this type of autoclave is shown in Figure 40-6. Alternatives are also possible in this case. The most important one is the use of horizontal fans placed on a side of the chamber. As in the previous case, the chamber has a cir-

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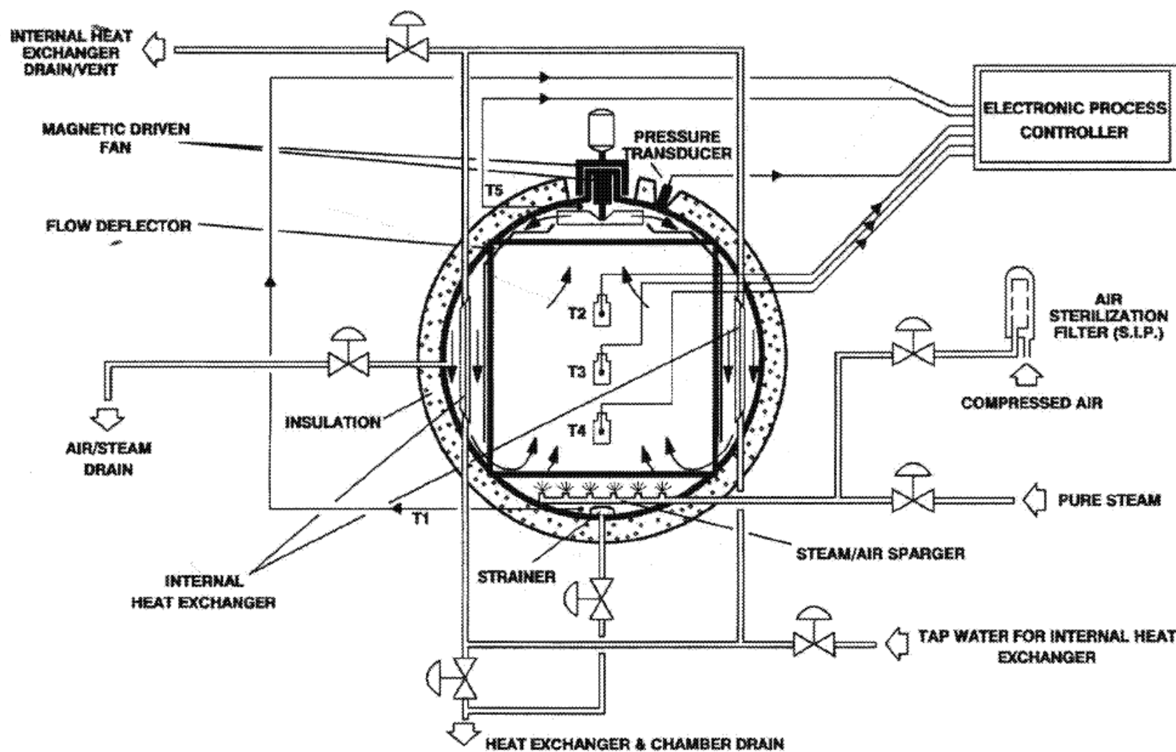

Figure 40-6. Air over steam autoclave: simplified piping and instrumentation diagram (courtesy, Fedegari Autoclavi).

cular cross-section (with a quadrangular door or doors inscribed in the circumference) and has a single wall.

There are two reasons for choosing a circular cross-section for autoclaves operating in counterpressure.

1. These autoclaves operate at significantly higher pressures than conventional pure saturated steam autoclaves, and generally are not put in vacuum. It is well known that a cylindrical structure withstands internal pressure much better than a quadrangular one.
2. The circular sectors of the chamber that are not occupied by the load are used to place elements required for the operation of these autoclaves.

The air is *not* removed initially from the chamber. The steam enters directly into the chamber through a sparger located in the chamber's lower portion. The partial air pressure of the mixture can be adjusted during the entire process, similarly to what occurs for the previously described superheated water spray autoclaves.

The fan(s) placed against the ceiling of the chamber and the flow deflectors have the purpose of homogenizing the steam plus air mixture that forms inside the chamber. The task of these fans is very important and demanding. In fact, for equal pressure and temperature conditions, the air is approximately 1.6 times denser than the steam (one only has to consider their respective molecular weights) and would tend to stratify on the bottom, producing intolerable temperature gradients.

The cooling phase consists of feeding air into the chamber (to condense and replace all the steam that is present) while maintaining the same sterilization pressure or possibly increasing it. Cold tap water then is fed into the heat exchangers, which are constituted by batteries of hollow plates located in the two circular sectors of the sides of the chamber (only one plate is shown in the diagram for the sake of simplicity). A tube heat exchanger can be used as an alternative. The load is thus cooled while constantly maintaining a controlled pressure inside the chamber.

However, this cooling comprises two solid-gas heat exchanges (plates → air; air → load) that, as is known, have a very poor efficiency. An attempt is made to improve this exchange by increasing the pressure of the air in the chamber (within the limits allowed by the product and the autoclave) so as to increase its density and therefore its heat-exchange capacity. The fans obviously continue to run during the cooling phase. Despite these refinements, the cooling phase is definitely longer than the same phase in superheated water spray autoclaves.

A critical mechanical aspect of these autoclaves is the tightness of the fan shaft. This aspect can be solved completely by using magnetic-drive fans.

With steam plus air mixture autoclaves, the blushing of PVC bags is less intense than with water spray autoclaves and generally affects essentially the regions where the bag rests on the supporting racks.

Table 40-1 compares the characteristics of the two kinds of counter pressure autoclaves.

Dry Heat Treatments

STERILIZATION AND DEPYROGENATION

Dry heat treatments have two targets: microorganisms and their by-products. The aim of sterilization is to destroy the ability of microorganisms to survive and multiply. Depyrogenation seeks to destroy the chemical activity of the by-products: pyrogens or endotoxins (these terms do not mean exactly the same thing, but we will consider them to be synonymous for the sake of simplicity).

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Table 40-1. Counter Pressure Autoclave Comparison

CRITICAL COMPARISON	WATER SPRAY (WS) AUTOCLAVES	AIR OVER STEAM (AS) AUTOCLAVES
Temperature uniformity in time	Very good easily in ±0.5° limits	Very good easily in ±0.5° limits
Temperature uniformity in space	Very good requested by FDA for LVP	Very good requested by FDA for LVP
Total pressure uniformity in time	Very good	Very good
Counterpressure management flexibility	Excellent	Excellent
Consumption of high microbiological quality water	Yes, modest, for initial filling	No
Consumption of tap water for cooling	Yes, acceptable	Yes, approx. 3 times higher than WAS
Consumption of compressed air	Yes, acceptable	Yes, acceptablke
Consumption of industrial steam	Yes, acceptable	No
Consumption of ultraclean steam	No	Yes, acceptable
Condensate recovery	Possible and easy	Not possible
Cooling water recovery	Possible, recovered water is initially very hot	Possible, recovered water is initially very hot
Autoclave price	Acceptable	Approx. 1.1 times higher than WS
Total process duration	Short	Approx. 1.3 times higher than WS
Autoclave productivity/price	High	Approx. 70% of WS
Operation principle	Very simple and straightforward	More complicated than WS
Mechanical construction	Simple	More complicated than WS
Qualification/validation	Normal	Normal
Operating flexibility according to type of load	Suitable for any kind of container with the following remarks: • Upward concavities collect water • Product is unloaded wet • PVC bags can produce blushing phenomena	Suitable for any kind of container: • Upward concavities collect condensate only • Other kinds of container can be unloaded slightly damp • Blushing phenomena of PVC bags are limited
Possibility of combination with pure saturated steam processes	Strongly discouraged: It is complex and expensive and complicates validation	Very frequent, but moderately expensive

Both processes consist of an oxidation that is almost a combustion. However, the temperatures required to achieve depyrogenation are distinctly higher than those needed to obtain sterilization. We can summarize the situation as follows:

- If an effective dry heat depyrogenation is performed, sterilization generally is achieved *as well*.
- Effective dry heat sterilization can be performed even *without* achieving depyrogenation.
- If moist heat sterilization is performed, in normal operating conditions depyrogenation is *not* achieved.

The kinetics of dry heat treatments are not substantially different from those of moist heat sterilization. The values of the algorithms F_T and F_H (analogous to F_0) and those of the parameters D and z, however, are different not only from those of moist heat sterilization but also from each other. Furthermore, the two dry heat treatments are verified biologically with different biochallenges. Accordingly, the two dry heat treatments require different validation approaches.

The materials subjected to dry heat treatments naturally must be heat-stable: the most common are glass containers for parenterals. Elastomeric compounds generally are unable to tolerate these treatments.

The literature generally mentions the following operating conditions:

Sterilization:	160°C—120 to 180 min
	170°C—90 to 120 min
	180°C—45 to 60 min
Depyrogenation:	230°C—60 to 90 min
	250°C—30 to 60 min

However, the current trend is toward using treatments at higher temperatures than those listed.

The sections that follow describe the most common types of equipment used to perform the above processes. If the load (bottles/vials/ampules made of glass or other materials) is wet when it is introduced, a large part of the energy required by the process is used initially to evaporate the water that wets the load, and the process accordingly takes more time. The equipment uses large amounts of air, which generally is recirculated partially and must be filtered in HEPA filters to have, in the critical regions of the equipment, the Class 100 environment. This is relatively easy to achieve in the sterilization phases (or regions) in which the *thermal situation* of the filters is stable. It is much less easy to achieve in the heating/cooling phases (or regions), because the changes in temperature entail expansions/contractions of the filters, with consequent release of particles.

DRY HEAT BATCH STERILIZERS

The forced-convection batch sterilizer is a type of dry heat unit widely used in the industry. It uses the principle of convective heat transfer to heat the load. Figure 40-7 is a schematic diagram of a modern unit. It shows a two-door sterilizer in which the unloading door leads to the sterile area. The two doors are, of course, parallel to the plane of the drawing and are hinged vertically.

The pressure inside the chamber must be controlled continuously so that it is slightly higher than the pressure in the loading area (nonsterile) and slightly lower than the pressure in the unloading area (sterile).

The unit is made entirely of stainless steel; particular care must be taken in selecting the insulating materials and in the methods for applying them. It is important also to avoid the forming of so-called *thermal bridges;* these allow dissipation, and thus excessive external temperatures of the sterilizer and *cold spots* in the chamber.

The main features shown in the sketch are:

1. Air-circulation fan
2. Water-cooled battery (for the cooling phase)
3. Circulation HEPA filters
4. Launch/recovery bulkheads
5. Trolley and load
6. Discharge duct
7. HEPA filter on the discharge duct to prevent back-flow contamination
8. Variable-speed fan for chamber pressurization (proportionally controlled)
9. Prefilter and HEPA filter on the chamber pressurization loop
10. Electric heater (proportionally controlled)

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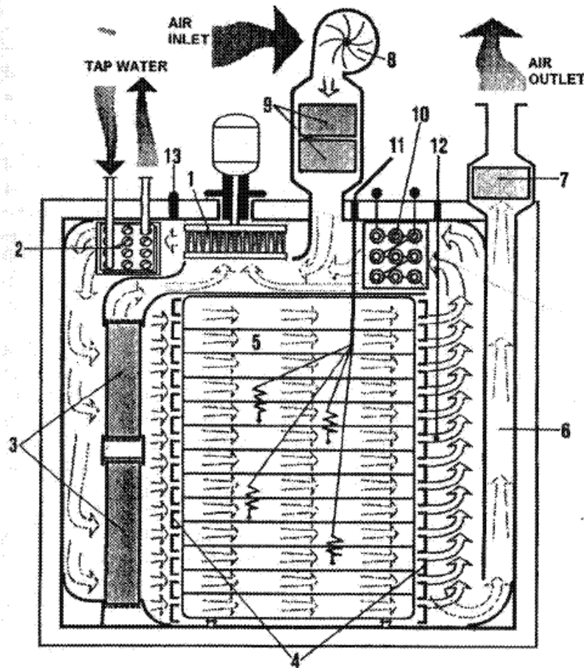

Figure 40-7. Dry heat batch sterilizer: simplified diagram (courtesy, Fedegari Autoclavi).

11. Four flexible Pt100 4-wire RTDs
12. Main control Pt100 4-wire RTD
13. Pressure transducer

DRY HEAT TUNNELS

The drying, sterilizing/depyrogenation, and cooling tunnel is the only continuous sterilizing apparatus widely used in the pharmaceutical industry (apart from filters). It basically consists of a horizontally rotating transport belt made of a stainless-steel mesh (some devices must be provided to confine the product on the transport belt without particulate generating friction), installed in a thermally insulated *tunnel* that directly connects an upstream cleaning machine to the downstream sterile area or to *isolated* devices.

Inside the tunnel, the product (most frequently glass vials) is dried; heat-treated either by radiant heat or, as more usual today, by hot air; and finally cooled. In both cases the internal part of the tunnel must be pressurized dynamically by ventilation at an intermediate pressure level between the downstream system and the loading room. From a process point of view, higher temperature and shorter exposure time are used than in batch sterilizers. During the last 10 years the practice has changed from 20 min at 280°C to 3 or 4 min at 300°C or more. Because a minimum safety margin is required for the duration of exposure, and glass of most types becomes more difficult to handle above 320°C and more fragile after such a treatment, it is likely that the trend toward higher temperature values has reached its practical limit.

In infrared (IR) radiant heat tunnels, heat is supplied by resistance-in-glass heaters located above and below the transport belt; prefiltered and HEPA-filtered air is fed into the cooling zone mainly for pressurizing and cooling. This air, a countercurrent slowly flowing through the entire tunnel, has also an important drying and preheating effect of the load in the infeed zone. Figure 40-8 schematically represents an IR

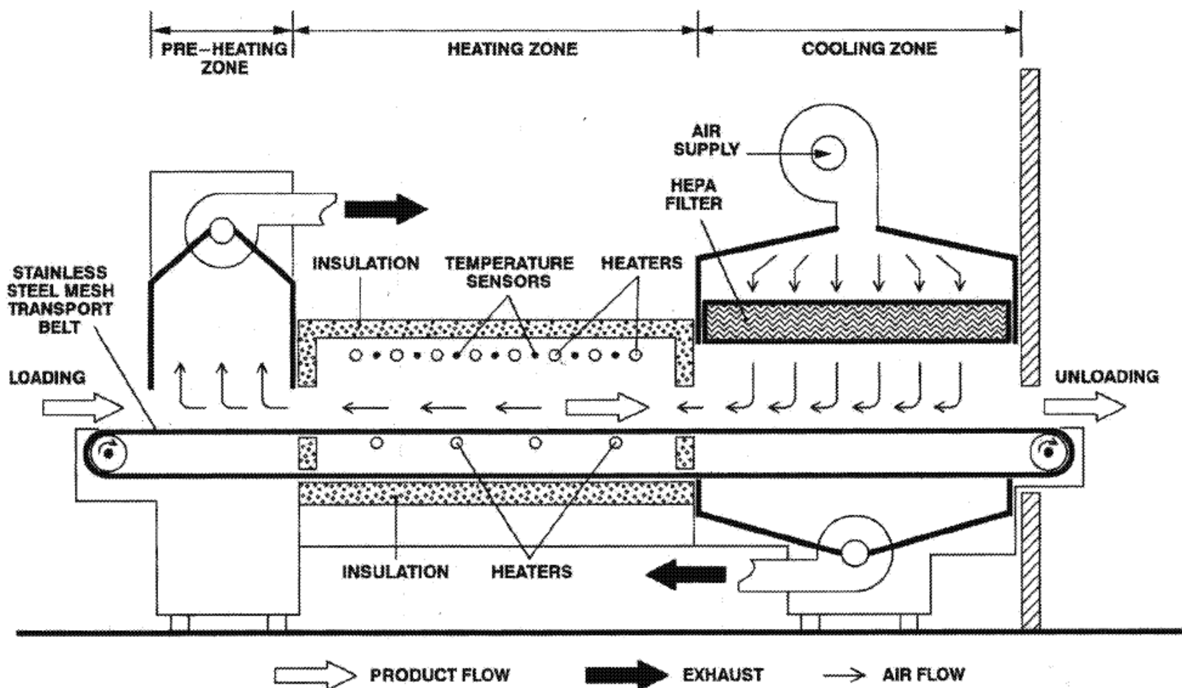

Figure 40-8. Dry heat tunnel: simplified diagram (courtesy, Fedegari Autoclavi).

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tunnel: even if this type of apparatus is no longer widely used, the basic concepts have not been modified in the hot-air laminar flow tunnel, but airflow patterns are a little more complex.

Hot-air laminar flow (LF) tunnels do not radiate heat directly to the product, but rather heating is provided by circulation of hot filtered air forced onto the product. A circulation fan withdraws the air; it leaves the product through heating bars below the transport belt and is fed again to the inside of the tunnel through HEPA filters suitable for operating at high temperature. Airtightness of the coupling of HEPA filters with tunnel framework is of utmost importance from the point of view of particulate contamination. It must cope with the strong thermal expansion of different materials. Some makeup air is required in the heating zone, and the total number of installed fans may be as high as five or even six if an additional extraction below tunnel outfeed is required in case of high pressure in the sterile room.

Despite the complexity of its airflow, the LF tunnel has the main advantage of quicker heating and consequent shorter process time. This results in reduced size compared with the IR tunnel, because the belt speed cannot be reduced below a certain value. As the name itself declares, the air speed in the LF tunnel is kept around 0.5 m/sec (1.5 ft/sec), aiming to avoid particulate contamination.

The comparison between continuous tunnel and batch oven is favorable to the continuous tunnel from the point of view of handling the product. No batch work is needed after the unpacking of the components and loading of them into the cleaning machine until the final removal of the packaged product from the line after the filling and the following operations. This can be very important in the case of large-scale production.

The batch oven provides a much easier isolation of the sterile area. In the case of continuous tunnel, there must be a steady flow of air through the open connection from the sterile area to the tunnel. The pressure difference between the two systems must be such that the sterile area always is kept at a higher pressure level than the tunnel. Too big a difference would result in an excessive escape of air to the tunnel, both reducing the pressure in the sterile area and disturbing the laminar airflow and the temperature profile inside the tunnel. Experience has proved that these problems can be solved satisfactorily only if the design of the air-conditioning system of the sterile area is developed from the very beginning, keeping in mind the foreseen installation of a specified tunnel. Baffle systems also aid in maintaining pressure differentials between the aseptic-processing area and the sterilizing tunnel.

Chemical "Cold" Sterilization

Many products do not tolerate the sterilization conditions of moist-heat or dry heat processes. In such cases it is possible to resort to cold or at least low-temperature sterilization methods performed with chemical means, by gases or vapors. The continuously increasing use of plastic disposable products or components for medical treatments has been made possible by the development of reliable cold sterilization processes.

A variety of gases and vapors have shown germicidal properties: chlorine dioxide, ethylene oxide, propylene oxide, formaldehyde, betapropiolactone, ozone, hydrogen peroxide, peracetic acid, etc. Ethylene oxide (EtO) is currently in widespread use for medical product sterilization. However, EtO has been shown to have detrimental effects on the environment; thus, other agents are being developed on a commercial scale, with the intent to reduce the use of EtO. Vaporized hydrogen peroxide and hydrogen peroxide/steam mixtures are being used to sterilize a variety of materials and work surfaces. Chlorine dioxide recently has become available for these applications.

ETHYLENE OXIDE

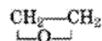
The sterilizing action of EtO is based on an alkylation reaction: it is, accordingly, a truly chemical action rather than a physical one. This chemical reaction must be activated by the presence of water vapor (approximately 60% of RH or relative humidity) and is increased by temperature and EtO concentration.

The process temperature is limited by the characteristics of the product. Generally, it is between 40° and 60°C, but it must be remembered that the reaction rate increases by approximately 2.5 times for each 10°C increase in temperature. The normally used EtO concentrations range between 400 and 1200 mg/L. It has in fact been demonstrated that beyond 1200 mg/L the consequent increase in the reaction rate is no longer economically convenient.

The EtO must make *direct* contact with the microorganism for the microbe to be inactivated. Any packagings that contain the object to be sterilized must therefore be permeable to air, EtO, and any dilution gases (as discussed later). Generally, it is not possible to use EtO to sterilize liquids, solutions, or emulsions. Powders, too, are difficult to treat unless microbial contamination is only on the outside of the granules.

Fortunately, EtO, air, and dilution gases easily penetrate most of the plastic and paper barriers used for the packaging of medical products. However, the good penetrating properties of EtO are also a disadvantage, because large amounts of it are absorbed by plastic or rubber materials. Products sterilized on an industrial scale using EtO normally require about 14 days of quarantine to spontaneously eliminate absorbed EtO residuals. This time can be reduced by using forced desorption methods. Sterilized goods must be monitored for toxic EtO residual, ethylene glycol, and ethylene chlorhydrin breakdown products of EtO.

EtO in standard room conditions is a vapor (indeed, its boiling point is about 11°C at atmospheric pressure). It is colorless, heavier than air, and has an ether-like odor. Its formula is



The presence of the oxygen bridge, which can be opened easily, explains its reactivity and its sterilizing action, as well as its tendency to polymerize.

Unfortunately, EtO has several drawbacks: it is toxic, carcinogenic, teratogenic, inflammable, and explosive when mixed with more than 3% air by volume. These characteristics make the use of EtO highly controversial, and many countries have issued regulations or requirements for its use as a sterilizing agent.

EtO often is used in a mixture with dilution gases, with weight ratios of 85 to 90% of diluent. The diluent gas most often used is CO_2; use of Freon is shrinking, due to the well-known international restrictions to its use; N_2 is beginning to be used quite often. These mixtures are considered nonflammable and nonexplosive, and many countries consider them mandatory for use in industrial autoclaves. These countries allow the use of pure EtO in small, individual, single-use cylinders only for small autoclaves (100 to 200 L). If a 10 to 12% mixture of EtO in CO_2 is used to obtain an acceptable EtO concentration (at least 500 mg/L), it is necessary to work at 3 to 4 absolute bar. Accordingly, one must use an autoclave that can withstand relatively high pressures; these autoclaves are expensive, and the duration of the sterilization process is rather long because of EtO concentration is rather low.

Other countries accept the use (including industrial use) of pure EtO or of inflammable/explosive mixtures with a low percentage of dilution gas (the presence of the dilution gas generally is ascribed to a reduction in the tendency of pure EtO to polymerize). In such circumstances one can operate at less than atmospheric pressures and still reach high EtO concentrations that shorten the sterilization time. Thus, it is not necessary to use true autoclaves, but merely sterilizers capable of tolerating the very hard vacuum required for the initial elimination of the air from the chamber and from the load and for the final ex-

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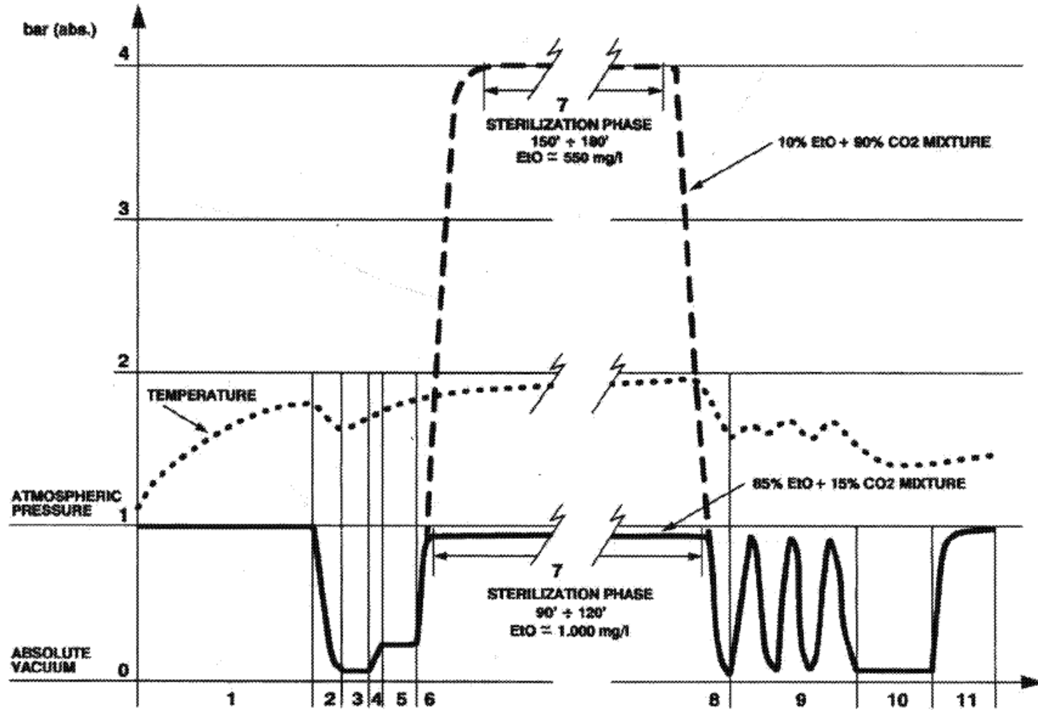

Figure 40-9. EtO sterilization pressure–time diagram: overpressure and subatmospheric pressure (courtesy, Fedegari Autoclavi).

traction of the EtO. Obviously, in these circumstances the use of plants constructed with explosion-proof criteria cannot be avoided.

The P/T/t diagrams of EtO sterilization are therefore different, depending on whether one or the other of the above described principles is used. A typical diagram of an overpressure sterilization with a mixture using 10% EtO and 90% CO_2 is shown in Figure 40-9. These are the steps:

1. Load and/or chamber heating
2. Vacuum
3. Vacuum hold for leak test
4. Humidification by steam injection
5. Penetration of humidity in the load
6. Loading of EtO mixture
7. Sterilization
8. EtO mixture evacuation
9. Air/vacuum pulses
10. Vacuum hold
11. Vacuum breaking

A typical diagram of a subatmospheric sterilization with a mixture using 85% EtO and 15% CO_2 is shown equally in Figure 40-9. One can see clearly that the phases are substantially the same as in Figure 40-8; the changes are the sterilization pressure, the EtO concentration, and therefore the duration of the sterilization phase.

In performing industrial sterilizations, which accordingly involve large loads, the load is heated and humidified before placing it in the sterilizer, in adequately conditioned rooms. Thus, the heating/humidification phases described above in the diagrams of Figures 40-8 and 40-9 are reduced drastically.

The layout of an industrial EtO sterilization plant is shown in Figure 40-10. This unit contains:

- The EtO or EtO-mixture cylinders
- The automatic devices that connect/disconnect the various cylinders to and from the sterilizer; disconnection of a cylinder (especially for mixture cylinders) often is controlled by its weight reduction, which must accordingly be checked individually

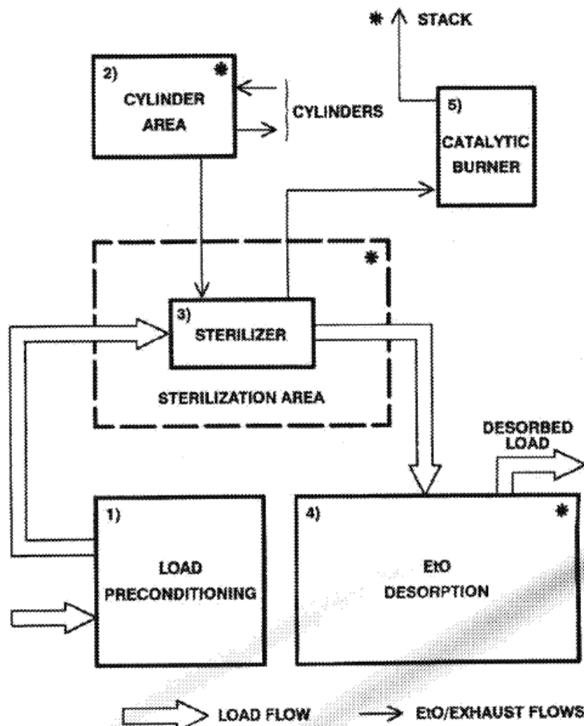

Figure 40-10. Flow diagram of an EtO sterilization industrial plant (courtesy, Fedegari Autoclavi).

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- The heat exchanger that must provide the vaporization calories to the liquid EtO mixture
- The pressure reduction unit that brings the liquid EtO mixture to the vapor state
- Any cylinders of N_2, which is used in the most advanced plants to *wash*, after each process, the pipes that have carried EtO

The EtO that is produced in the desorption chamber is at a very low concentration and it is generally too expensive to eliminate it with a catalytic burner. It is preferred to absorb it on activated-charcoal columns through which the air of the desorption chamber is recirculated.

Obviously, the EtO discharged by the sterilizer (and possibly the EtO arriving from the desorption chamber) must not be discharged into the atmosphere. Catalytic burners generally are used today: they convert the EtO into $CO_2 + H_2O$. These burners must be highly efficient, and their efficiency must be checked systematically, because the laws enforced in the various countries are generally very strict as to the limits of residual EtO. The asterisks (*) in Figure 40-10 indicate points where continuous monitoring of EtO concentration must be provided.

EtO sterilizers generally are made of stainless steel, although there also are machines made of carbon steel coated with epoxy paints. The chamber generally is jacketed in order to circulate thermostat-controlled warm water to maintain the sterilization temperature. Use of water vapor for the same purpose is fading because of the difficulties in using this method when the temperature must be kept below 100°C.

Process sensors are more numerous than in heat sterilizers, because there are essentially four sterilization parameters:

- EtO concentration
- Temperature
- Humidity or relative humidity
- Time

The EtO concentration generally is monitored by the pressure rise that occurs in the chamber when the EtO mixture is introduced; a pressure transducer is therefore used as a sensor.

Many guidelines require, in addition to the pressure rise, a second monitoring method that can be chosen among the following:

1. Weight difference of gas cylinder
2. Volume of gas delivered
3. Sampling from the sterilizer chamber and analysis

When EtO mixtures are used, Methods 1 and 2, like the pressure rise method, assume confidence in the concentration of EtO that is present in the mixture and that reaches the sterilizer. Method 3 is certainly more reliable, but it also is more difficult to apply. Infrared spectrometry or gas chromatography methods generally are used for analysis; these methods can be continuous and allow the simultaneous determination of the relative humidity.

Temperature generally is monitored by temperature sensors located in the chamber; these may be placed inside the load as well. The relative humidity generally is monitored on the basis of the temperature and of the pressure rise of the steam injection of the humidification phase. This method obviously is not very reliable, and many guidelines recommend also using a sensor that can determine the relative humidity. Unfortunately, sensors of this kind are generally "poisoned" by the EtO and become unreliable after a few cycles. The solution often used is to keep the sensor inside the chamber during the humidification phases, removing it before introducing the EtO in the chamber.

Finally, it is evident that if the load is preconditioned, the preconditioning temperature/humidity/time also must be monitored.

HYDROGEN PEROXIDE

Hydrogen peroxide (HP), chemically H_2O_2, is normally a liquid at room temperature. However, it can be vaporized and the resultant gas is an effective sterilant for certain packaged materials and for equipment and enclosures used in processing sterile materials. The most frequent and successful use of HP as a sterilant is for *isolators* (also known as barriers, locally controlled environments, etc). These units are very sophisticated versions of their ancestors, the *glove boxes* used to isolate processes in the past.

Isolators now are used widely for sterility testing, transporting sterilized goods from moist and dry heat units to sterile areas or processing isolators, and processing of supplies. HP also is being used to sterilize more sophisticated processing equipment, such as freeze dryers and filling lines, and even may be used to sterilized small clean rooms. High humidity can inhibit the effectiveness of vaporized HP and must therefore be controlled during the exposure of the gas. Figure 40-11 represents a typical vaporized HP cycle.

Although HP is broken down readily to water and oxygen, the effluent gas can represent a safety hazard at higher levels. Just as with EtO, catalytic converters are used to ensure that all materials are rendered safe before they are released to the atmosphere. Figure 40-12 represents a typical installation using vaporized HP to sterilize a freeze dryer and condenser system. VHP DV1000 is a model manufactured by Am Sterilizer/Finn Aqua, which holds many of the patents on the use of this technology.

Systems for larger applications may require fans to aid in uniformly distributing the vaporized HP. In addition, auxiliary air systems may be added to reduce the time required to dehumidify at the beginning of the cycle and to aerate the load at the end of the cycle. Figure 40-13 shows a transfer isolator connected to a sterilizer and a vaporized HP generator. This particular unit also has a protective half-suit to allow full access to the large internal area. These units allow the unloading of the sterilizer directly into a sterilized isolator. The isolator excludes direct human intervention, which greatly reduces the potential for microbial contamination.

A typical freeze-dryer sterilization involves several vacuum *pulses* during which the temperature is brought to 40° to 60°C and the humidity is reduced (dry phase). A vacuum hold cycle is run to check for leaks and the temperature is reduced to about 25°C for the sterilization cycle. The sterilant is introduced and is monitored and controlled by weight using an electronic balance. Filtered air is pulsed with sterilant to push the vapors into any deadlegs and to compress the vapors, thus increasing the concentration. Finally, the vacuum is pulsed again to aerate the chamber, and the residual vapor is verified to be below acceptable levels before proceeding to the processing cycle.[11]

HYDROGEN PEROXIDE PLUS STEAM

For certain applications, one can combine moist heat and hydrogen peroxide methods. The combination can produce some effects that may be more desirable than either of the techniques run separately. Cycles can be as effective in shorter times and may improve the removal of residual peroxide. The system must be able to withstand exposure to steam at atmospheric pressure. The air-handling equipment can be moved outside the processing area, which simplifies the system and minimizes any mechanically generated particles, because the air, steam, and peroxide are introduced through the same type of HEPA filters used for laminar-flow hoods.[12,13]

The process area is raised to about 80°C by introducing dry heated air through the HEPA filters. The steam is introduced and surfaces are raised to about 100°C. During the steam cycle, hydrogen peroxide is introduced and is carried with the steam. When the cycle has been completed, the steam and peroxide are stopped and the dry heated air is started again. This aids in removal of residual condensate and helps break down the peroxide to water and oxygen. After sufficient heat has been introduced to dry and remove residuals, cool air is introduced to bring the unit to the desired operating temperature.

Because the hydrogen peroxide is mixed intimately with the steam, temperature can be used to monitor the progression of the cycle. However, the heated portions of the cycle must be

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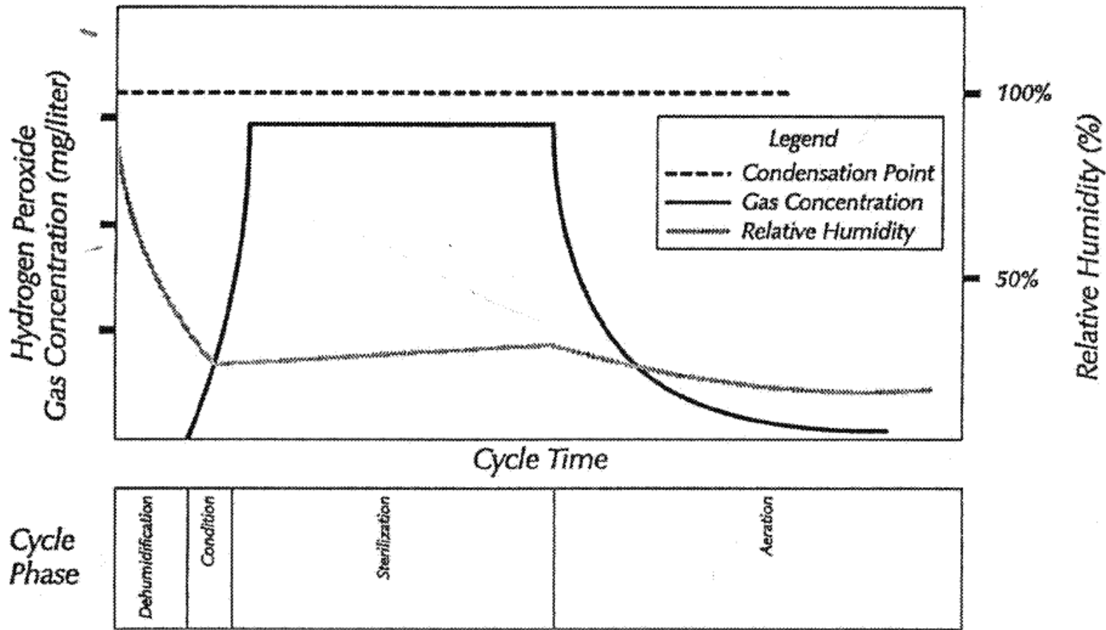

Figure 40-11. A typical vaporized HP cycle (courtesy, Am Sterilizer/Finn Aqua).

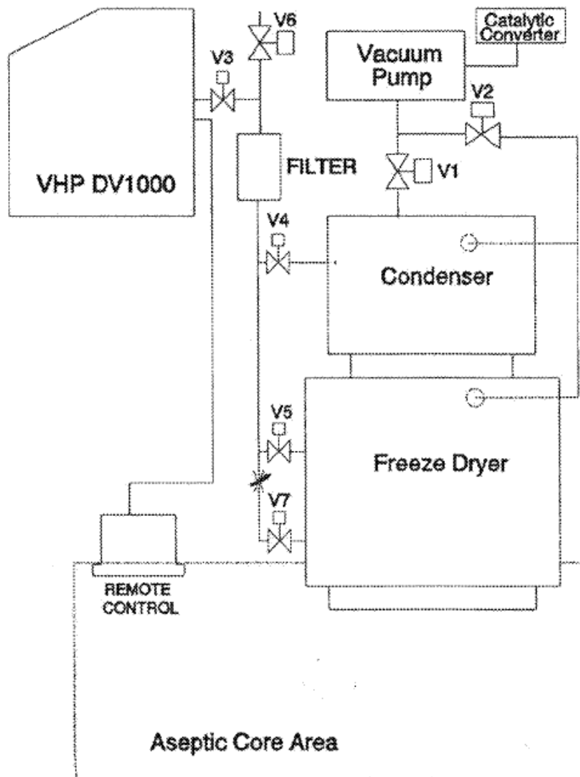

Figure 40-12. A typical installation using vaporized HP to sterilize a freeze dryer and condenser (courtesy, Am Sterilizer/Finn Aqua).

validated using biological indicators and residual peroxide measurements, to ensure their effectiveness in sterilizing and removing residuals to a safe level. Figure 40-14 diagrams a cycle using steam and hydrogen peroxide to sterilize as a filler in an isolator.

Figure 40-15 is included to show the synergistic effects of steam and hydrogen peroxide in some sterilization cycles. The challenge organism was *Bacillus stearothermophilus*, which typically is used to validate steam cycles. It should be noted that the kill rate was not only considerably faster, but was accomplished using atmospheric steam. This means that instead of 121°C the equipment was only subjected to 100°C and was exposed for 15 min less to achieve the same reduction in microorganism count.

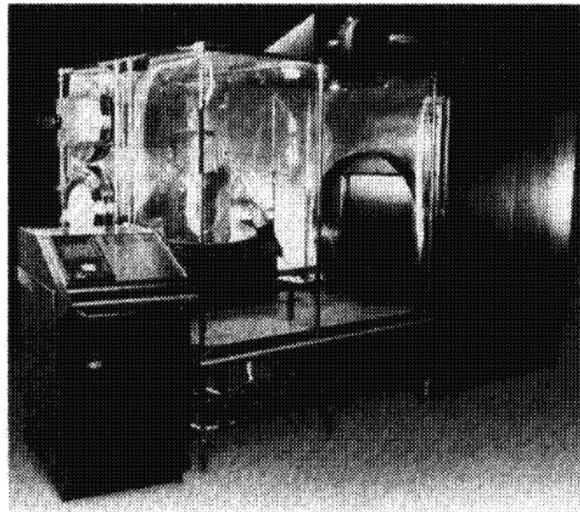

Figure 40-13. A transfer isolator connected to a sterilizer and a vaporized HP generator (courtesy, Am Sterilizer/Finn Aqua).

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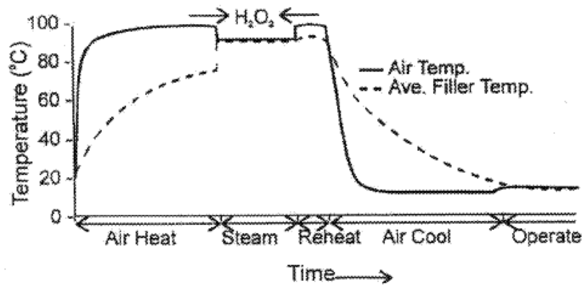


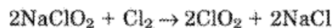
Figure 40-14. A steam/hydrogen peroxide cycle (courtesy, TL Systems and Despatch Industries).

CHLORINE DIOXIDE (CD)

The compound chlorine dioxide (CD) was discovered in 1811. It is a greenish-yellow gas with the common name euchlorine. It is a single electron transfer–oxidizing agent that has a chlorine-like odor. CD has been recognized since the beginning of the 20th century for its disinfecting properties. CD possesses the bactericidal, virucidal, and sporicidal properties of chlorine, but unlike chlorine, does not lead to the formation of trihalomethanes or react with ammonia to form chlorinated organic products (chloramines). These properties have led to the widespread use of CD in the treatment of drinking water. Despite numerous applications for CD in aqueous systems, only recently have the sterilizing properties of gaseous CD been demonstrated.

CD has been shown to have low toxicity in humans and is nonmutagenic and noncarcinogenic; it is not an ozone-depleting chemical. Used at comparatively low concentrations and at subatmospheric pressure, gaseous CD sterilization lacks many of the hazards associated with EtO, and it has been suggested as an attractive potential replacement.[14,15] Gaseous CD does not require expensive damage-limiting construction and is cost-competitive with EtO. Capability for spectrophotometric in-chamber measurement of gas concentration makes the process amenable for the validation of parametric release.

CD gas cannot be compressed and stored in high-pressure cylinders, but is generated upon demand using a column-based solid phase generation system. The chemical reaction used for CD generation is based upon the reaction of solid flaked sodium chlorite with dilute chlorine gas:



$$2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl$$

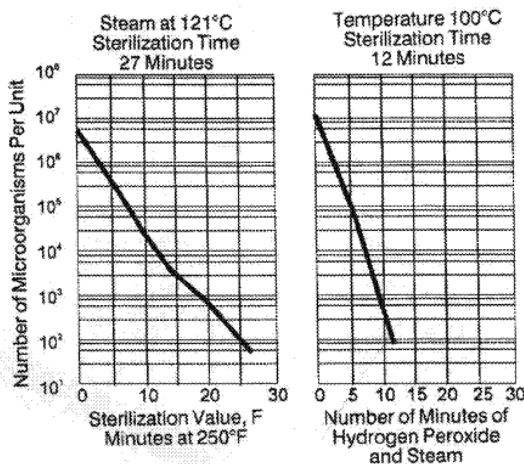


Figure 40-15. Comparison of steam under pressure with hydrogen peroxide/atmospheric steam mixture (courtesy, TL Systems and Despatch Industries).

Table 40-2. Effect of CD Gas Concentration on the Rate of Inactivation of 10^6 *B subtilis* Spores on Paper Strips Within a Load of Overwrapped Foil Suture Packages[a]

	FRACTION NONSTERILE[b]		
EXPOSURE PHASE TIME (min)	10 mg/L	20 mg/L	40 mg/L
0	NT	20/20	19/20
15	NT	19/20	1/20
30	20/20	4/20	0/20
60	9/60	0/60	0/20
90	3/20	NT	NT
180	0/20	NT	NT
240	0/20	0/20	NT

[a] The paper spore strips were placed next to the foil suture package and then overwrapped with Tyvek/Mylar. Sterilization exposures were performed at 30 to 32°.
[b] NT = not tested.

A block diagram for a CD gas sterilization system is shown in Figure 40-16. The output of the primary generation column is monitored spectrophotometrically, as is the gas concentration within the chamber. The scrubber system uses a sodium thiosulfate solution to chemically convert the CD to sodium sulfate. The scrubber system is highly efficient; therefore, the effluent released into the atmosphere is mainly process N_2 and air with the CD component reduced to low ppm levels. A typical gaseous CD sterilization process is quite similar to that used with EtO and has these steps:

1. Initial vacuum to remove air from the chamber and load.
2. Moisture conditioning at 70 to 85% relative humidity for 30 to 60 min.
3. CD gas injection: 10 to 30 mg/L.
4. Air or N_2 injection to attain a constant subatmospheric pressure, generally 80 kP_a.
5. CD gas exposure, generally 60 min.
6. Chamber and load aeration by evacuation and air replacement, tailored to load materials and density.

The temperature of the process in a sterilizer application is 30 to 32°C; for isolation systems, it is at ambient temperature.

Feasibility studies on the application of gaseous CD for medical sterilization were performed with over-wrapped foil suture packages.[16] The studies focused on the effect of gas concentration on the rate of inactivation of paper-strip biological indicators (BIs). The results of these studies are shown in Table 40-2. As with other gaseous sterilants, as the CD concentration increases, the time it takes to attain all sterile BIs becomes progressively shorter.

More detailed CD sterilization process development and validation studies were performed using polymethylmethacrylate (PMMA) intraocular lenses as the test system. A diagram of the sterilization process used for these studies is shown in Figure 40-17. The following results were obtained after 30 minutes of gas exposure at 30 mg/L (half cycle):

Packages/Load	Fraction Nonsterile
800	0/8, 0/8
1600	0/16, 0/16
25	0/25, 0/25

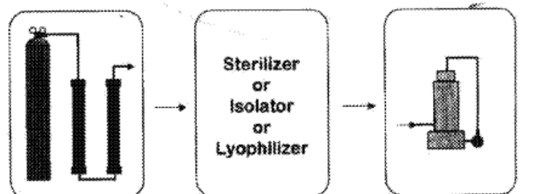


Figure 40-16. Block diagram of a gaseous CD sterilization unit.

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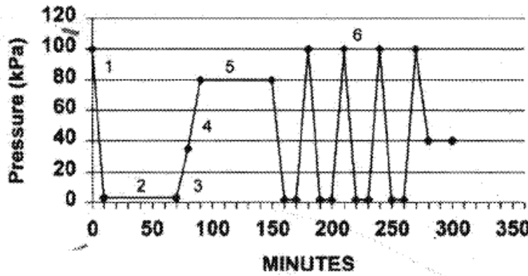

Figure 40-17. Pressure excursion diagram of a typical gaseous CD sterilization process. (1) Initial vacuum; (2) Moisture conditioning; (3) CD gas injection; (4) N_2 or air injection; (5) CD gas exposure phase; (6) aeration by evacuation and air replacement.

As can be seen, all of the *B. subtilis* BTs were sterilized and varying the load size had no discernible effect upon process lethality. CD also has been evaluated for the sterilization of blood oxygenators.[17]

CD also has great potential for the decontamination/sterilization of barrier-isolation systems. Initial studies on the efficacy of gaseous CD for the decontamination/sterilization of a sterility testing isolator used a gas concentration of 10 mg/L. This concentration yielded a relatively rapid process with a complete kill of 10^6 spores in approximately 15 min. The effect of gas concentration upon the observed D_{10} value with *B. subtilis* spores was determined at 10, 20, and 30 mg/L of CD:

mg/L CD	D Value in Seconds
10	45
20	16
30	7

As expected, the D_{10} value decreases with increasing CD concentration. These low D_{10} values yield very rapid decontamination/sterilization processes for barrier-isolation applications.

Very low residuals of CD are observed when examining product and packaging materials from medical devices or isolation technology systems. CD does not appear to have the *solvent-like* quality of EtO. Residual CD is generally less than 10 ppm following a 15-min exposure at 10 mg/L. Rapid aeration also is observed with levels often less than 1 ppm following 15 min of aeration. A typical aeration curve of CD from flexible-wall isolator PVC material is shown in Figure 40-18.

The impact of CD exposure on a number of polymeric materials and metals has been evaluated. Commonly used polymers such as ABS, nylon, PMMA, polyethylene, polypropylene, polystyrene, Teflon, and Viton appear highly compatible. Polycarbonates and polyurethanes, depending upon the particular formulation, may exhibit a loss in tensile properties and/or discoloration. Stainless steel is compatible with CD; uncoated copper and aluminum are affected.

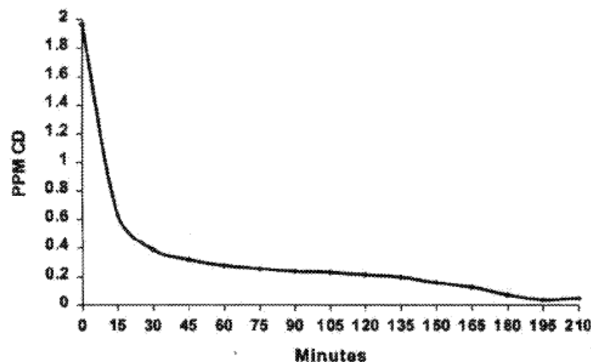

Figure 40-18. Aeration of CD from flexible-wall isolator PVC material; aqueous extraction from treated samples (10 mg/L, 15 min) followed by polarographic measurement of dissolved CD.

OTHER GASES

Formaldehyde (HCHO) sometimes is used for sterilizing certain medical products. It is not in widespread use in the United States but as a gas or in combination with low-pressure steam, it is used in some European hospitals instead of ethylene oxide. Formaldehyde, a toxic chemical and a human carcinogen, is an alkylating agent and destroys microorganisms by alkylation of susceptible cell components.

Filtration

Filtration is the removal of particulate matter from a fluid stream. Sterilizing filtration is a process that removes, but does not destroy, microorganisms. Filtration, one of the oldest methods of sterilization, is the method of choice for solutions that are unstable to other types of sterilizing processes.

Pasteur, Chamberland, Seitz and Berkfeld filters have been used in the past to sterilize pharmaceutical products. These types of filters were composed of various materials such as sintered glass, porcelain, or fibrous materials (ie, asbestos or cellulose). The filtration mechanism of these depth filters is random adsorption or entrapment in the filter matrix. The disadvantages of these filters are low flow rates, difficulty in cleaning, and media migration into the filtrate. Fiber-releasing and asbestos filters now are prohibited by the FDA for the filtration of parenteral products.[18,19]

Over the past 35 years, membrane filters have become the method of choice for the sterilization of heat-labile sterile products. Membrane filters are thin, strong, and homogenous polymeric structures. Microorganisms, present in fluids, are removed by a process of physical sieving and are retained on or near the membrane surface. Membrane filters of 0.1 and 0.22-µm pore size are employed commonly as sterilizing filters.

When solutions are sterilized by filtration, the filters must be validated to ensure that all microorganisms will be removed under known conditions. Filter manufacturers normally validate sterilizing membrane filters using a protocol similar to the one developed by the Health Industry Manufacturers Association (HIMA).[20] In this procedure, *Pseudomonas diminuta* (ATTC 19146) is cultivated in saline lactose broth. Leahy and Sullivan[21] have shown that when *P. diminuta* is cultivated in this medium the cells are discrete and small (approximately 0.3 µm in diameter)—a range recommended for sterilizing filtration with 0.22-µm filters. Each cm^2 of the filter to be validated is challenged with 10^7 microorganisms at a differential pressure of 30 psig. The entire filtrate is collected and tested for viable microorganisms. The retention efficiency (log reduction value) of the membrane filter may be calculated using the procedure described in the HIMA protocol. Dawson and co-workers[22] have demonstrated that the probability of a nonsterile filtration with a properly validated membrane filter is approximately 10^{-6}. Another aspect in filter validation is adsorption of the product by the filter and extractables from the filter and housing.

Once the performance of the membrane filter has been validated, a nondestructive integrity test that has been correlated to the bacterial challenge test (the bubble point or diffusion test) can be used routinely prior to and after a sterilizing filtration to ensure that the membrane filter is integral.[23,24] Unique to membrane filtration is the condition that beyond a certain challenge level of microorganisms, the filter will clog. For a typical sterilizing filter this level is 10^9 organisms per cm^2. Initially, membrane filters were available only in disc configuration. Advances in membrane technology have provided filters in stacked-disc, pleated-cartridge and hollow fiber configurations. These advances have provided larger surface areas and higher

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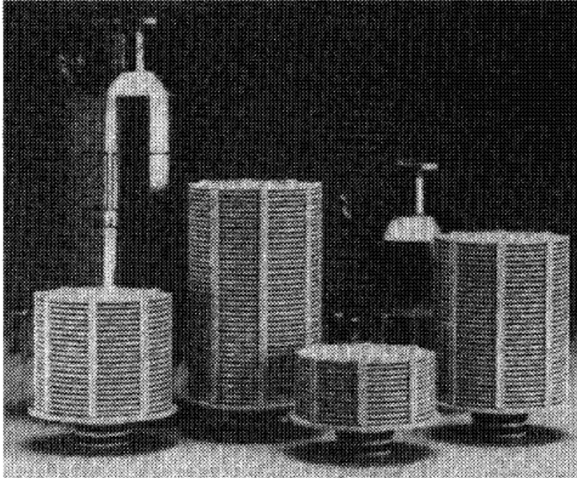

Figure 40-19. Stacked-disk membrane filters. This new technology allows filter manufacturers to supply filters with large surface area in relatively small packages (courtesy, Millipore); vaporized HP generator (courtesy, Am Sterilizer/Finn Aqua).

flow-rate capabilities. Figure 40-19 is an example of these larger surface area filters.

Membrane filters are manufactured from a variety of polymers, such as cellulosic esters (MCE), polyvinylidiene fluoride (PVF), and polytetrafluoroethylene (PTFE). The type of fluid to be sterilized will dictate the polymer to be used. The listing below is intended to serve only as a guide for the selection of membrane filters for a particular application. The filter manufacturer should be consulted before making a final choice.

Fluid	Polymer
Aqueous	PVF, MCE
Oil	PVF, MCE
Organic solvents	PVF, PTFE
Aqueous, extreme pH	PVF
Gases	PVF, PTFE

Figure 40-20 is an example of a sterilizing filtration system commonly used in the pharmaceutical industry.

Positive pressure commonly is used in sterilizing filtrations. It has the following advantages over vacuum: it provides higher flow rates, integrity testing is easier, and it avoids a negative pressure on the downstream (sterile) side of the filtrate, thus precluding contamination. Membrane filters are sterilized readily by autoclaving, by *in-situ* steaming, or by using ethylene oxide.

In addition to their use in the pharmaceutical industry, membrane filters are used in many applications in the hospital pharmacy. The membrane filters commonly used in these applications are small disposable units. Examples of these are shown in Figures 40-21 and 40-22. Typical applications for membrane filters in hospital pharmacies include sterilization of intravenous (IV) admixtures and hyperalimentation solutions, sterilization of extemporaneously compounded preparations, sterility testing of admixtures, as well as in direct patient care (see Chapter 42).

Radiation Sterilization

The retail or hospital pharmacist probably has little opportunity to use radiation sterilization. However, they should be aware that many of the products sold in stores and used daily in hospitals are sterilized by this technology. Products such as contact lens solutions, bandages, baby bottle nipples, and teething rings (the kind containing water/gel) are a few exam-

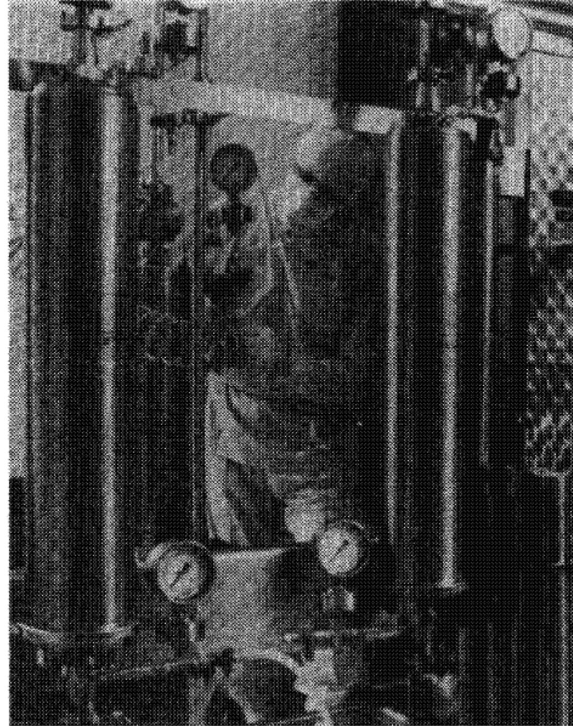

Figure 40-20. An example of a process filtration system in a pharmaceutical plant (courtesy, Millipore).

ples of the everyday type of product encountered in a pharmacy. Several drugs, including some anticancer drugs, also are terminally sterilized using gamma radiation.

The hospital pharmacist is likely to encounter the use of gamma or X-ray treatment of blood to eliminate white blood cells in host-versus-graft reactions following transplant surgery. The serum used for tissue cultures is frequently ster-

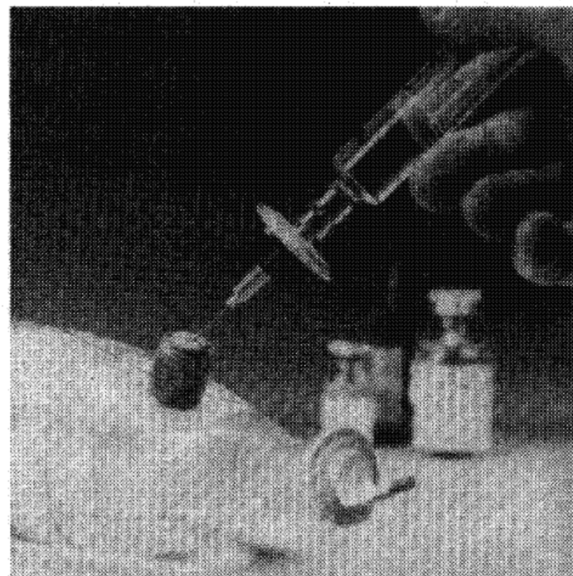

Figure 40-21. Intravenous additive filtration using a small disposable membrane filter (courtesy, Millipore).

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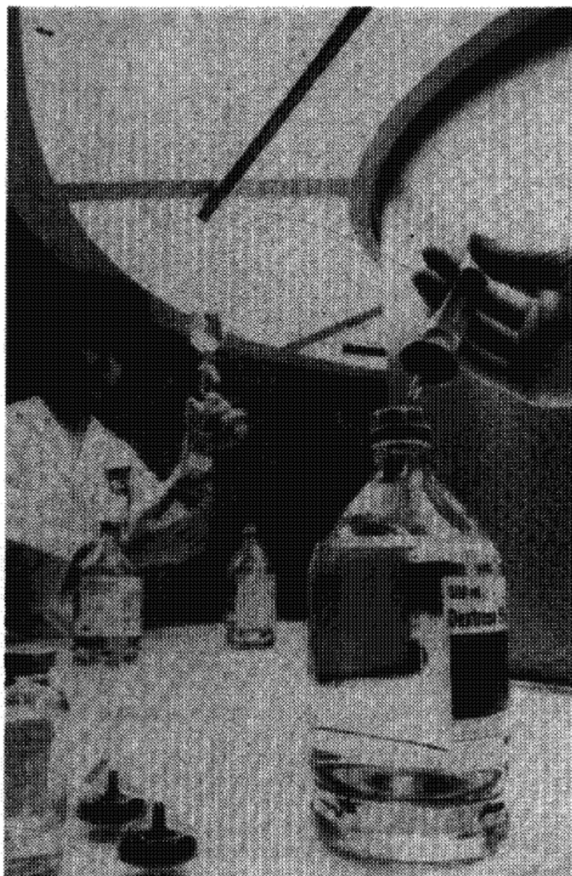

Figure 40-22. Intravenous additive filtration and sterility testing. Both procedures employ membrane filtration (courtesy, Millipore).

ilized with gamma radiation to eliminate viruses, virus-like particles, and mycoplasms.

The pharmaceutical industry historically has relied on steam, dry heat, ethylene oxide gas, filtration, and chemical processes to meet sanitization or microbial load reduction requirements. Sterilization by radiation may employ either electromagnetic radiation or particle radiation.

Electromagnetic radiation, composed of photons of energy, includes ultraviolet, gamma, X-, and cosmic radiation. Gamma radiation, emitted from radioactive materials such as Cobalt-60 or Cesium-137, is the most frequently used source of electromagnetic radiation. Of these two, only Cobalt-60 is used in the large industrial irradiator (Fig 40-23). Cesium-137 is used in blood irradiators.

Particulate or corpuscular radiation includes a formidable list of particles. The only one that currently is being employed for sterilization is the electron. These electrons are machine generated using the technique illustrated in Figure 40-24; Figures 40-25 and 40-26 illustrate two methods of presenting products to a commercial electron-beam sterilizer.

Radiation-processing technology, and its application in the manufacture of pharmaceuticals, is being investigated more actively now than at any other time. This renewed interest is in part due to the development of aseptic and barrier technology, as well as an overall improvement in the environment in which pharmaceuticals are manufactured.

In the past the use of a radiation dose of 25 kGy was required to ensure that all viable microbes had been inactivated, and that a SAL of 10^{-6} was achieved. This level of radiation proved detrimental to many pharmaceuticals. With the advent of clean rooms, and aseptic and barrier technologies, the microbial environment has improved dramatically. No longer are spores or even the number of organisms as daunting. It is more appropriate now to determine the resistance of the bioburden to radioaction and to tailor the minimal sterilization dose to meet the most resistant strain of the bioburden. In this way many more drugs and other products are capable of being sterilized terminally. This provides an SAL of 10^{-6} or greater, depending upon the microorganism.

The increased use of radiation processing to sterilize medical devices has led to the development of more efficient and economical irradiation equipment and processes. It also has generated new scientific data. The positive experience of the medical-device industry should be a *signpost* for the pharmaceuticals industry.

Several pharmaceutical raw materials and finished products are being sanitized/sterilized successfully with gamma radiation. Although it is possible to use electron beam radiation, we are presently unaware of any pharmaceuticals being treated using this technology. This should not preclude others from investigating its potential. The superior penetrating ability of gamma radiation provides the edge for this technology in this application.

HOW RADIATION KILLS MICROORGANISMS

The principles of sterilization by irradiation have been known since the early 1940s. Basically, charged particles or electromagnetic radiation interact with matter to cause both ionization and excitation. Ionization results in the formation of ion pairs, comprised of ejected orbital electrons (negatively charged) and their counterparts (positively charged). Charged particles such as electrons interact directly with matter causing ionization, whereas electromagnetic radiation causes ionization through various mechanisms that result in the ejection of an orbital electron with a specific amount of energy transferred from the incident gamma ray. These ejected electrons then behave similarly to machine-generated electrons in ionization reactions. Thus, both particle and electromagnetic radiation are considered as ionizing radiation and differ from ultraviolet radiation in this respect.

Ionizing radiation kills or inactivates microorganisms through the interaction of the ion pairs or excitations altering the molecular structure or spatial configuration of *biologically active* macromolecules. In particular, those involved in cell replication are most critical. It can do this in two ways. The first is to deposit energy directly in a bond of the macromolecule. This can cause a rearrangement of its structure, altering or destroying its normal function. The second is to generate free radicals, primarily from the water contained within the cytoplasm. The free radicals thus generated react with the macromolecules to subvert their normal function. In either case the result is the loss of reproductive capability of the microorganism.

The number of microorganisms inactivated by a given radiation dose is a statistical phenomenon. It depends upon the sensitivity of the biologically active macromolecule(s) to alteration (denaturation), the number of alterations elicited within the cell and the ability of the cell to repair these alterations. Different microorganisms have different capabilities to withstand or repair such alterations. This sensitivity is referred to as the D_{10} value. The size of the microorganism, its state of hydration, and the presence or absence of radical scavengers affect the outcome of exposure to ionizing radiation.

The ability of gamma radiation to inactivate microorganisms has been well documented. New documentation relating to viruses or new strains/reclassifications of microorganisms is being added continually. The major benefit of using radiation sterilization as the terminal step in the manufacturing process, as opposed to autoclaving or dry heat methods, is the minimal product degradation usually observed with this technology.

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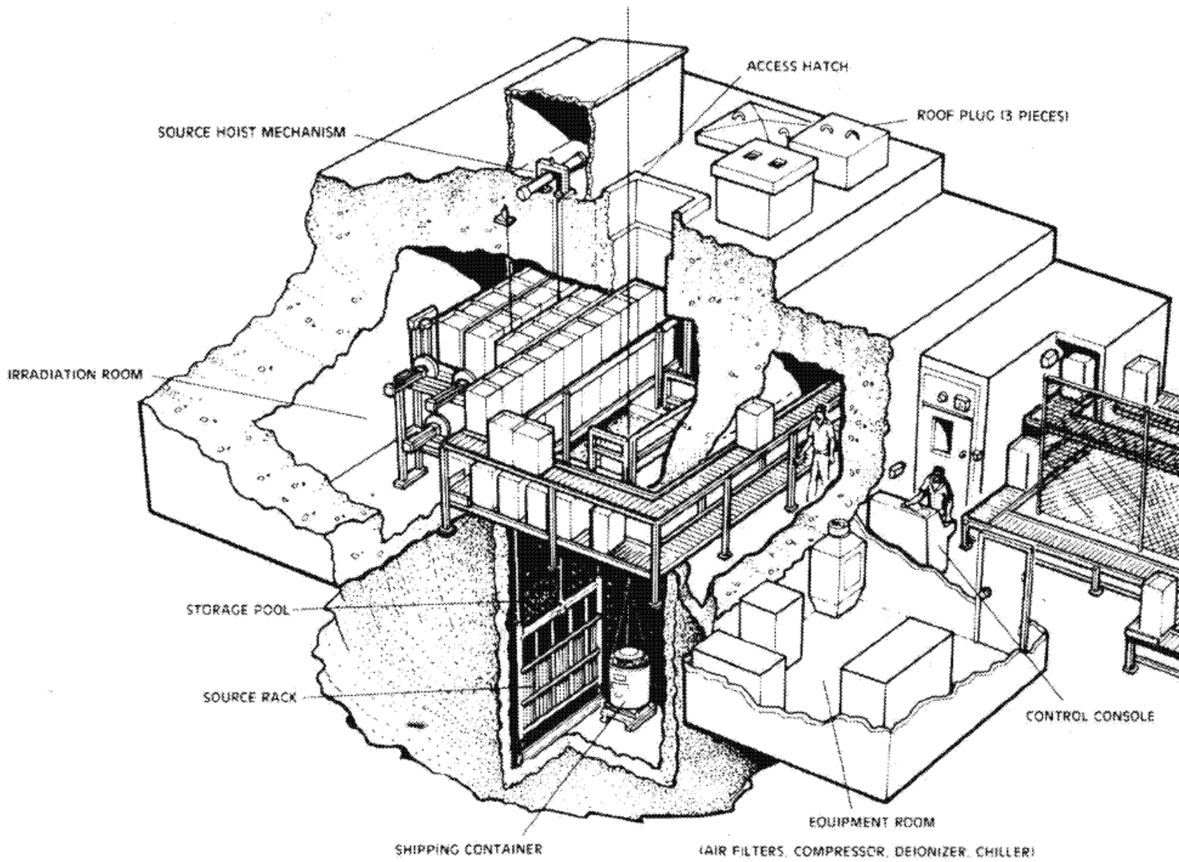

Figure 40-23. Tote box irradiator: automatic (courtesy, Nordion Intl).

The process has been in use in the medical device industry for over 25 years. Ample evidence as to its efficacy exists in scientific literature. Materials and processes have been developed to reduce the impact of radiation on the product. Some materials, such as Teflon and polypropylene, are severely degraded by radiation and must be avoided. It is the intent of this update to present some of the process developments that will facilitate the use of this technology for the terminal sterilization of pharmaceutical products. It also will assist those wishing to improve the microbial quality of raw materials entering the manufacturing process. Clean materials reduce the bioburden levels present in a clean room facility.

Sterilization by ionizing radiation requires consideration of the minimum and maximum doses (or the amount of radiation that is absorbed by the material), the energy level available (which along with the bulk density of the material will determine the depth of penetration), and the power output available (which determines the rate at which the dose can be applied).

The unit of absorbed dose is the Gray (Gy), where 1 Gy = 1 joule/kg, independent of the nature of the irradiated substance.

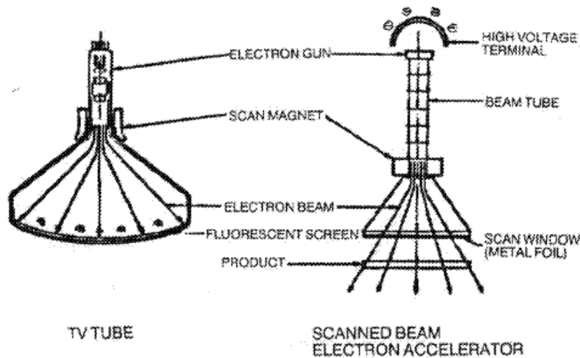

Figure 40-24. To produce an electron (courtesy, RDI).

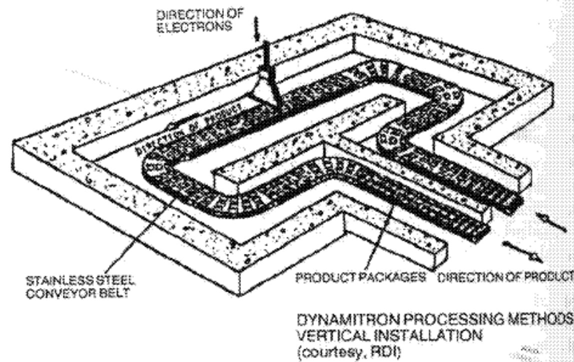

Figure 40-25. Dynamitron processing methods: vertical installation (courtesy, RDI).

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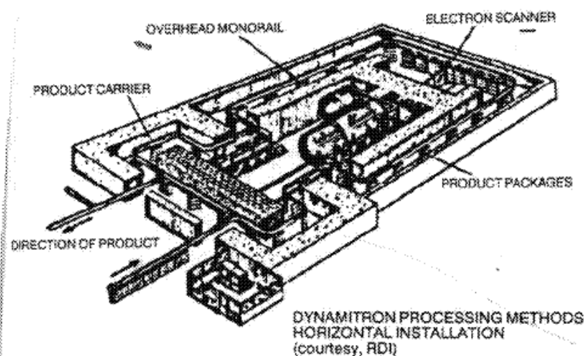

Figure 40-26. Dynamitron processing methods: horizontal installation (courtesy, RDI).

Sterilization doses, for convenience, are predominantly expressed in kilogray (kGy).

Many investigators have studied the relative resistance of microorganisms to sterilization by radiation. The consensus is that vegetative forms are most sensitive, followed by molds, yeasts, viruses and spore-formers. While historical practice has been to use 15 to 25 kGy, today the minimal sterilization dose is more closely tailored to the resistance of the bioburden. It is not unusual to use doses as low as 2 to 8 kGy. The use of the AAMI/ISO or EN standards is highly recommended.

Modern gamma sterilization facilities used by pharmaceutical and medical device companies generally hold up to 4 MCi of Cobalt-60. The largest facility holds 12 MCi. Figure 40-23 shows a schematic of a modern Cobalt-60 radiationsterilization facility.

Two types of electron accelerators are used in sterilization: alternating-current machines with ranges up to 50 kW of power and 5 to 12 meV of energy, and direct-current machines with ranges of 30 to 200 kW and 0.5 to 5 meV. These machines generate electrons at high voltage, accelerate the electrons, and project them into the product to be sterilized. The greater the machine power (kW), the more electrons can be generated per unit time. The higher the energy (meV), the greater the penetration capability of the electrons into the material to be sterilized.

Ultraviolet (UV) Radiation

Artificially produced UV radiation in the region of 253.7 nm has been used as a germicide for many years. Although UV radiation often is used in the pharmaceutical industry for the maintenance of aseptic areas and rooms, it is of limited value as a sterilizing agent.

Inactivation of microorganisms by UV radiation is principally a function of the radiant energy dose, which varies widely for different microorganisms. The primary mechanism of microbial inactivation is the creation of the thymidine dimers in DNA, which prevents replication. Vegetative bacteria are most susceptible, while bacterial spores appear to be 3 to 10 times as resistant to inactivation and fungal spores may be 100 to 1000 times more resistant. Bacterial spores on stainless-steel surfaces require approximately 800 $\mu W\ min/cm^2$ for inactivation. By comparison, the black spores of *Aspergillus niger* require an exposure of over 5000 $\mu W\ min/cm^2$. Even with an adequate dose, however, the requirements for proper application of germicidal UV radiation in most pharmaceutical situations are such as to discourage its use for *sterilization* purposes. On the other hand, as an ancillary germicidal agent, UV radiation can be useful.

When using UV radiation, it is very important that lamps be cleaned periodically with alcohol and tested for output: also its use requires that personnel be properly protected; eye protection is particularly important.

The principal disadvantage to the use of germicidal UV radiation is its limited penetration—its 253.7 nm wavelength is screened out by most materials, allowing clumps of organisms, and those protected by dust or debris, to escape the lethal action. The use of UV radiation as a sterilizing agent is not recommended unless the material to be irradiated is very clean and free of crevices that can protect microorganisms. Many organisms are capable of repairing the UV-induced DNA damage using photoreactivation (light repair) and dark repair.

PULSED LIGHT

Recently, high-intensity visible light has been developed to a level that allows it to be used for certain sterilization applications. The advantages include extremely short exposure times (eg, 2 to 3 pulses of a few seconds) and relative ease in shielding the operations to provide operator safety. It can be used for surface sterilization and certain terminal sterilization applications. This is limited to packaging materials that are transparent to the wavelengths used. It is applicable for certain plastic materials, but not for Type I glass. This technique requires additional study, but has been shown to be effective against all organisms studied thus far.

Aseptic Processing

Although not actually a sterilization process, aseptic processing is a technique frequently used in the compounding of prescriptions or commercial products that will not withstand sterilization but in which all of the ingredients are sterile. In such cases, sterility must be maintained by using sterile materials and a controlled working environment. All containers and apparatus used should be sterilized by one of the previously mentioned processes and such work should be conducted only by an operator fully versed in the control of contamination. The use of laminar-airflow devices or barrier technology for aseptic processing is essential.

With the availability of sterile bulk drugs and sterilized syringe parts from manufacturers, the purchase of several pieces of equipment permits pharmacies to produce filled sterile unit-dose syringes with minimum effort. The equipment needs have been described in a paper by Patel and associates.[25] Figure 40-27[25] illustrates this system.

PACKAGING

Following exposure of a product to a well-controlled sterilization treatment, the packaging material of the product is expected to maintain sterility until the time of use. Packaging must be durable, provide for permanent-seal integrity, and have pore sizes small enough to prevent entry of contaminants. Obviously, the packaging must be compatible with the method of sterilization.

The package design is important if the contents are to be removed without recontamination. Tearing of plastics or paper can be tempered by coatings, and sealed containers should be tested carefully to ensure retention of sterility at the time of use.

If sterile material passes through many hands, it is important to provide a tamperproof closure to indicate if the container has been opened inadvertently. These four features—compatibility with sterilization, proven storage protection, ease of opening, tamper-proofing—are highly desirable characteristics of medical packaging.

For hospitals and pharmacies, there are a wide variety of woven reusable materials or nonwoven disposable materials that provide acceptable sterile barriers and are offered by major packaging suppliers. These suppliers normally conduct extensive programs to ensure the ability of the material to maintain sterility. Both hospitals and industry have guidelines and accepted practices for sterile-product packaging.[5]

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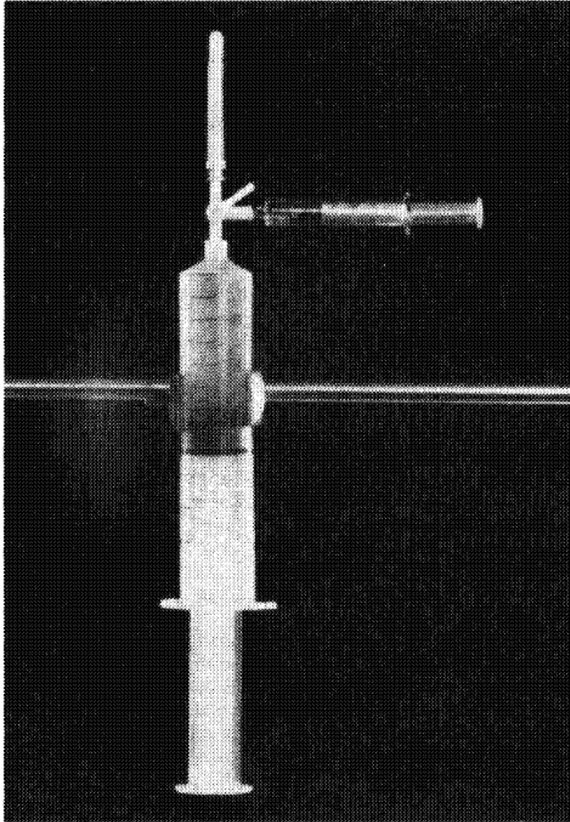

Figure 40-27. Unit-of-use system for sterile injectable medication.[19]

A review of the principles of sterile-material packaging by Powell[26] discusses the suitability of packaging materials for various sterilization methods, including resistance to bacteria, types of openings, strength of packaging, testing of packaging, and types of packaging. These topics also are discussed in Chapter 54.

UNIDIRECTIONAL AIRFLOW

Unidirectional airflow equipment is essential for proper performance of sterility tests and aseptic filling or assembling operations. These procedures require exact control over the working environment, but while many techniques and different types of equipment for performing these operations have been used over the years, unidirectional airflow devices are superior to all other environmental controls.

The unidirectional airflow procedure for producing very clean and dust-free areas was developed in 1961. In a unidirectional airflow device the entire body of air within a confined area moves with in one direction with uniform velocity along parallel flow lines. By employing prefilters and high-efficiency bacterial filters, the air delivered to the area essentially is sterile and sweeps all dust and airborne particles from the chamber through an open side. The velocity of the air used in such devices is generally 90 fpm ± 20%. Unidirectional airflow devices that deliver the clean air in a vertical, horizontal, or curvilinear fashion are available. The devices can be in the form of rooms, cabinets, or benches. For a comprehensive discussion of the biomedical application of unidirectional airflow the reader is referred to Runkle and Phillips.[27]

Each unidirectional airflow cabinet or bench should be located in a separate, small, clean room having a filtered air supply. The selection of the type of cabinet will depend on the operation itself. For most sterility-testing operations, horizontal unidirectional airflow units appear to be superior to vertical-flow hoods because the air movement is less likely to wash organisms from the operator's hands or equipment into the sterility test media. Figure 40-28 shows the sterility testing of syringes in a horizontal unidirectional airflow hood. Figure 40-29 shows the design of a typical horizontal, unidirectional airflow hood.

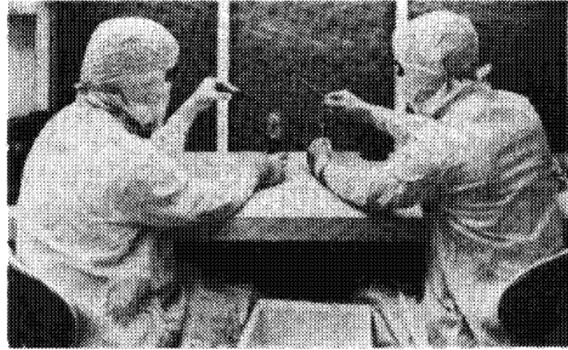

Figure 40-28. Sterility testing of plastic disposable syringes in a horizontal laminar-airflow bench (courtesy, Becton Dickinson & Co).

The major disadvantage of the horizontal unidirectional airflow units is that any airborne particulate matter generated in the units is blown directly into the room and against the working personnel. In situations where infectious material is involved, or where one must prevent contamination of the environment with a powder or drug, the use of specifically designed vertical, recirculating unidirectional airflow units is recommended. Units are available that do an excellent job of providing both product and personnel protection. Such a unit is shown in Figure 40-30.

To achieve maximum benefit from unidirectional airflow, it is important first to realize that the filtered airflow does not itself remove microbial contamination from the surface of objects. Thus, to avoid product or test contamination, it is necessary to reduce the microbial load on the outside of materials used in sterility testing. Unidirectional flow will do an excellent job of maintaining the sterility of an article bathed in the airflow; however, to be accurate, the sterility-testing, or product-assembly procedure must create the least possible turbulence within the unit. Moreover, an awareness of the turbulent air patterns created by the operation is necessary to avoid performing critical operations in turbulent zones. To illustrate how effectively airborne particles are washed from an environment by laminar airflow, Figure 40-31 shows the distance that particles of various sizes will travel horizontally before falling 5 ft in a crossflow of air moving at 50 fpm.

Unidirectional airflow clean benches should supply Class 100 air as defined in Federal Standard 209B.[28] They should be certified to this standard when installed and then tested periodically. An air velocimeter should be used at regular intervals to check the airflow rates across the face of the filter. Smoke tests are use-

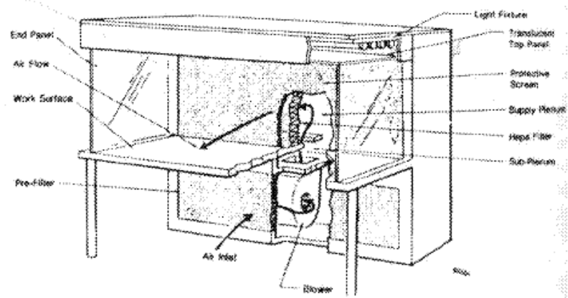

Figure 40-29. Horizontal laminar-airflow hood.

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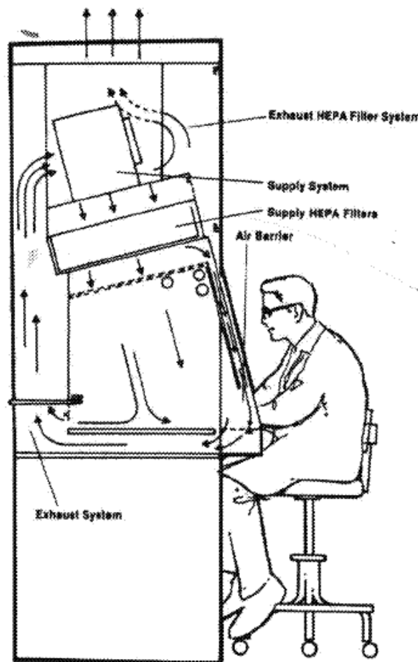

Figure 40-30. Sketch of a biological cabinet with vertical, recirculating laminar-airflow and HEPA-filtered exhaust. HEPA-filtered air is supplied to the work area at 90 fpm 20%. Airflow patterns in combination with a high-velocity curtain of air form a barrier at the front access opening that protects both the work and the worker from airborne contamination (courtesy, Bioquest).

ful in visualizing airflow patterns and a particle analyzer can be used to check the quality of the air. Filter efficiency testing determines the validity of the filter and its seal using a smoke (mean particulate diameter of 0.3 μm) and a light-scattering aerosol photometer. The smoke, at a concentration of 80 to 100 mg/L, is introduced to the plenum of the unit and the entire perimeter of the filter face is scanned with the photometer probe at a sampling rate of 1 ft^3/min. A reading of 0.01% of the upstream smoke concentration is considered a leak.

In addition to the routine airflow measurements and filter-efficiency testing, biological testing should be done to monitor the effectiveness of laminar-airflow systems. Microbial air sampling and agar-settling plates are useful in monitoring these environments. Phillips evaluated horizontal laminar-flow hoods by tabulating the number of *false positives* appearing in sterility-test media over a period of time. These results (Table 40-3) showed very low numbers of *false positives*.

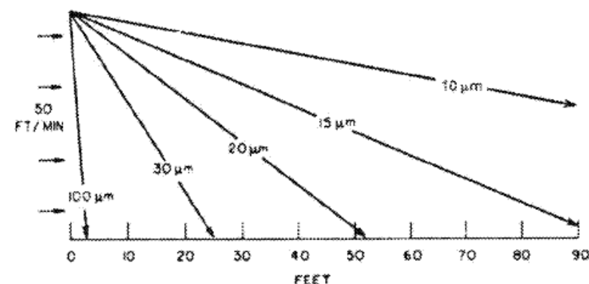

Figure 40-31. Distance traveled by particles settling from a height of 5 ft.

Table 40-3. False Positives Occuring in a Laminiar-Flow Hood[26]

PRODUCT	NO. OF UNITS STERILITY TESTED	NO. OF FALSE POSITIVES	% FALSE POSITIVES
Syringes	9793	2	0.02
Needles	4676	2	0.04
Misc	306	0	0

See Figure 40-29 for laminar-flow hood.

TESTING

After sterilization, there are several techniques for determining whether the particular lot of material is sterile. The only method for determining sterility with 100% assurance would be to run a total sterility test, that is, to test every item in the lot.

Representative probabilities are shown in Tables 40-4 and 40-5 to illustrate more specifically how low levels of contamination in treated lots of medical articles may escape detection by the usual sterility-test procedures. The data are calculated by binomial expansion, employing certain assumed values of percent contamination with large lot sizes (greater than 5000) and including standard assumptions with regard to the efficiency of recovery media and so on.

In Table 40-4 the probability data are calculated for lots with various degrees of assumed contamination when 10 random samples per lot are tested. For example, a lot that has one in each 1000 items contaminated (0.1% contamination) could be passed as satisfactory (by showing no positive samples from 10 tested) in 99 tests out of 100. Even at the 10% contamination level, contamination would be detected only two out of three times.

Table 40-5 shows the difficulty in attempting to improve the reliability of sterility tests by increasing sample size. For contamination levels as low as 0.1%, increasing the sample size from 10 to 100 has a relatively small effect in improving the probability of accepting lots. Even a sample size of 500 would result in erroneously accepting a lot 6 times out of 10. On the other hand, with a lot contaminated to the extent of 10%, by testing 100 samples the probability of acceptance of the lot would be reduced to a theoretical zero.

The information in Table 40-5 may be viewed in another way. If, for the probability values shown for each different sample size, the value that approximates the 95% confidence level ($P = 0.05$) is selected, it is clear that using 20 samples only will discriminate contamination levels of 15% or more. If the 20 tubes show no growth, the lot could, of course, be sterile but there would be no way of knowing this from the test. From such a test it could be stated only that it is unlikely that the lot would be contaminated at a level higher than 15%. It is clear from these data that product sterility testing is a poor method of validating sterilization procedures.

The USP provides two basic methods for sterility testing. One involves the direct introduction of product test samples into culture media; the second involves filtering test samples through membrane filters, washing the filters with fluids to remove inhibitory properties, and transferring the membrane

Table 40-4. Probabilities for Sterility Testing of Articles With Assumed Levels of Contamination

"TRUE" % CONTAMINATION	PROBABILITY OF DESIGNATED POSITIVES OUT OF 10 SAMPLES TESTED			
	0	2	5	10
0.1	0.990	(Total = 0.010)		
1.0	0.904	0.091		
5.0	0.599	0.315		
10.0	0.349	0.387	0.001	
30.0	0.028	0.121	0.103	
50.0	0.001	0.010	0.246	0.001

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Table 40-5. Relationship of Probabilities of Acceptance of Lots of Varying Assumed Degrees of Contamination to Sample Size

NUMBER OF SAMPLES TESTED (*n*)	PROBABILITY OF NO POSITIVE GROWTH "TRUE" % CONTAMINATION OF LOT					
	0.1	1	5	10	15	20
10	0.99	0.91	0.60	0.35	0.20	0.11
20	0.98	0.82	0.36	0.12	0.04	0.01
50	0.95	0.61	0.08	0.007		
100	0.91	0.37	0.01	0.00		
300	0.74	0.05				
500	0.61	0.01				

aseptically to appropriate culture media. Test samples may be sterilized devices that simply are immersed aseptically into the appropriate culture-broth washings of the sterile object with sterile diluent, or dilutions of sterile materials. The USP recommends three aqueous diluting fluids for sterility tests while the Antibiotic Regulations list four; all are nontoxic to microorganisms. In the case of petrolatum-based drugs, a nonaqueous diluting fluid is required.

Many studies have been conducted to find the minimum number of culture media that will provide the greatest sensitivity in detecting contamination. Internationally recognized experts and bodies now recommend the use of two culture media: Soybean-Casein Digest Medium, incubated at 20° to 25°C, and Fluid Thioglycollate Medium, incubated at 30° to 35°C. The time of incubation specified usually is 7 days for the membrane filtration method and 7 to 14 days for the direct-inoculation method, depending on the method of sterilization. The requirements are described in detail in the USP.

The preferred method of verifying sterility is not by testing sterilized materials but by the use of biological indicators. This is not possible, however, when products are sterilized by filtration and filled aseptically into their final containers, as is the case with such important drugs as antibiotics, insulin, or hormones. The indicators generally are highly resistant bacterial spores present in greater numbers than the normal contamination of the product and with equal or greater resistance than normal microbial flora in the products being sterilized. Various properties of commercially available bacterial spores have been recommended for specific methods of sterilization based on unique resistance characteristics.

Commonly accepted species of bacteria used for biological indicators are shown in Table 40-6. Other species can be employed, probably without serious impact on the validity of sterility interpretation, so long as the prime requirements of greater numbers and higher resistance, compared to material contamination characteristics, are maintained.

Included with the materials being sterilized, biological indicators are imbedded on either paper or plastic strips or are inoculated directly onto the material being sterilized. Obviously, the indicator has greater validity in verifying sterility if it is located within product spaces that are the most difficult to sterilize. For example, in the case of a syringe, the location of a paper strip or inoculation of spores between the ribs of the plunger stopper is recommended.

The use of isolators (barrier technology) for processing materials is discussed in the section on advanced aseptic processing.

Table 40-6. Species of Bacteria Used as Biological Indicators

METHOD OF STERILIZATION	BACTERIAL SPECIES
Moist heat	*B stearothermophilus*
Dry heat	*B subtilis*
Ethylene oxide	*B stearothermophilus*
Radiation	*B pumilus, B stearothermophilus, B subtilis*

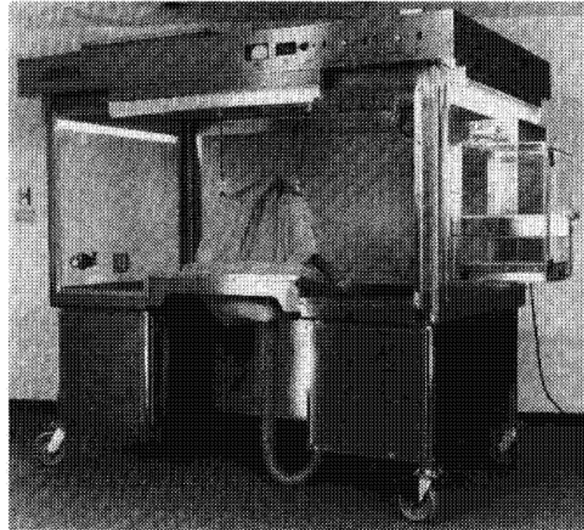

Figure 40-32. Stainless steel half-suit isolator (courtesy, Laminar Flow).

The first widespread use of these modern *glove boxes* in the pharmaceutical industry was in sterility testing. As filling speeds became faster, batches became larger. This, coupled with more expensive drug substances, created the need to avoid false-positive sterility tests. Even with laminar-flow hoods becoming widely used, the large number of manipulations carried out by people, created a significant chance for contamination through the testing procedure.

Government standards for SAL basically eliminated the possibility to repeat sterility tests. This means that batches that fail for any reason cannot be released. They are only useful for investigation of potential contamination hazards. Industry in turn needed more assurance that the product was indeed not sterile and the test was valid. This led to the development of more sophisticated isolation units. Figure 40-32 shows a stainless-steel half-suit system that is typical of those used in sterility testing. The units can be *docked* to a sterilizer, which eliminates the possibility of contamination during transfer of materials to the test area. The units can be sterilized using vaporized hydrogen peroxide. The exterior of any test materials required to be transferred into the units also can be sterilized in this manner. Validation of these steps allows one to virtually eliminate false-positive test results. Most manufacturers have adopted this technique and have agreed to a policy of essentially no sterility retests. Only where obvious system breakdowns can be shown to have led to failures will a retest be considered.

ADVANCED ASEPTIC PROCESSING

Isolator technology also is being used with increasing frequency in the processing of sterile products and associated packaging materials. This is driven by the same need to minimize human intervention and thus increase dramatically the assurance of sterility (SAL). The minimization of people was expanded throughout the 1990s with the advent of more widespread use of form, fill, and seal technology. This involved the on-line molding, filling, and sealing of plastic bottles containing sterile products. The technology was housed in rigid walled areas and product was supplied through filters and sterilized in place, at the last possible area before filling.

While form, fill, and seal is a technology on its own, it did lead to the recognition that by updating significantly the older concepts of *glove boxes,* one could dramatically affect the sterility assurance of an aseptic process. People contribute the

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largest percentage of the contamination risk. By minimizing their interaction, the probability of nonsterility is greatly reduced.

Glove boxes were not designed to support modern (and especially more automated) operations. This may explain why they did not become popular as aseptic processing units. Usage began to expand only when the need for increased assurance drove designers to develop ergonomically designed isolators.

More recently, the manufacturers of high-speed processing equipment have begun to redesign their machinery in line with the principles of isolator units. Because the mechanics of these machines have been proved to be very reliable and require very little human intervention, the timing seems to be correct for such modifications. Isolator units are relatively inexpensive also. They have allowed for aseptic processing without the construction of large processing areas, sterile suites or gowning areas. The development of relatively safe sterilization methods, such as vaporized hydrogen peroxide (with or without atmospheric steam) also has allowed the technology to become more viable for widespread use. Prior to this, the use of toxic (and sometimes corrosive) materials was required. This limited their use to more sophisticated operators, who were able to afford the resources required to build and maintain facilities for their use.

TRAINING

It is desirable that personnel involved with sterilization or aseptic processing be instructed in the basic behavior of microorganisms. This would include the differentiation of vegetative, spore-forming, and slow-growing life forms such as molds and yeasts. This would allow those being trained in the operations to understand the reasons for many of the restrictions necessary to carry out these processes. It is imperative that each person involved in these operations be instructed in two main areas.

Safety is the first and foremost area of concentration for a training program. Each of the pieces of equipment and processes described above have unique hazards associated with them. The operators must be made to understand the dangers of steam under pressure and exposure to gaseous sterilants prior to their neutralization.

The equipment design and installation should undergo safety reviews prior to its general operation. This review for potential hazards must be done by highly trained individuals and should include computer control and piping systems. It is important that the equipment fail (should a failure occur) in a manner that is safe to the operators. Valves should fail in a way to vent pressure to some safe area and/or gases to a relatively safe, unoccupied site.

The second major area of training involves gowning for entry into the sterile areas and subsequent performance of aseptic operations. Personnel must be instructed in proper gowning techniques so that they do not contaminate the exterior of garments and gloves during the process. Gowning areas should be supplied with full-length mirrors so that personnel can verify that all areas of their body have been covered fully and properly prior to entering a sterile work area. Recent trends indicate that gowning training should be followed by personnel monitoring with contact plates containing growth media. This allows one to verify the effectiveness of the training and, should growth occur, one can use this growth as a training tool to emphasize the importance of careful attention to detail during the gowning process. Because these plates require incubation, one does not allow operators to enter the sterile area until the results of these tests have been collected and reviewed with the candidate.

Continuing with the above approach, those performing aseptic operations require additional training and subsequent verification. This principle of competency-based training (ie, verifying the capabilities of those being trained) is necessary to ensure that the operators have developed the skills to carry out these vital operations while minimizing the risk of contamination. Again, it allows for constructive feedback to those who have not yet become fully accomplished in the techniques. It is prudent to reinforce these skills periodically through refresher sessions, and reverification of the skills. It has become standard practice to do unannounced spot-checks of the gloves and gowns of aseptic operators. This practice helps to maintain a level of vigilance, with regard to proper gowning and operating technique.

ACKNOWLEDGMENTS—Special thanks to the previous authors Barry Garfinkle and Martin Henley for writing such a good treatise on this subject. A thank you to the Cardinal-Health ElPaso, Texas facility for their review and comments on the radiation section.

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