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CHAPTER 40

Sterilization

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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}.

The variety and amounts of sterile products and their packages required for health care have increased continuously and been modified in recent years. Accordingly, sterilization technologies have adapted to the changing need. Some of these also are brought about by changing requirements and guidelines issued by regulatory or advisory bodies.

Not many years ago, sterility testing of the finished product was the basic means of monitoring the success of a sterilization process. Today, qualification and validation of the equipment and the process carried out in that equipment is considered essential. This stems from the general principles of Total Quality systems. National and international standards that define this system (such as ISO-9000 and EN-29000) indeed state that "sterilization is a special process because its efficacy cannot be verified by simple inspection and testing on the final product . . . For this reason, sterilization processes have to be validated before use, the performance monitored routinely and the equipment regularly maintained . . ."

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 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 in or on an object or preparation entering a sterilization step (usually expressed in colony forming units per unit 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.
Sterility—The absence of viable microorganisms.
Sterility Assurance Level (SAL)—A term related to the probability of finding a nonsterile unit following a sterilization step. It usually is expressed in terms of the negative power of 10 (ie, 1 in 1 million = 10^{-6}).
Sterilization—A process by which all viable microorganisms are removed or destroyed, based on a probability function.
Terminal Sterilization—A process that destroys all viable microorganisms within the final, sealed package.
Validation—The act of verifying that a procedure is capable of producing the intended result under all expected circumstances. This usually is accomplished through appropriate challenge(s).
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, def-

inite, 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, on October 11, 1991, the US Food and Drug Administration (FDA) proposed new regulations for aseptic processing and terminal sterilization. The proposed rules require that manufacturers of sterile products use terminal sterilization wherever possible. The proposal will affect 21 CFR 211, 314, and 514. Aseptic processing may be used only in those cases where terminal sterilization has significant detrimental effects on the product. This ruling is based on the ability to prove higher SAL's with current terminal sterilization processes, thus reducing the risk of a nonsterile unit reaching the patient.

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°. 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° 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 suffice (Fig 40-1). When the initial microbial contamination level is assumed to be 10^6, and if the D value of the sterilization

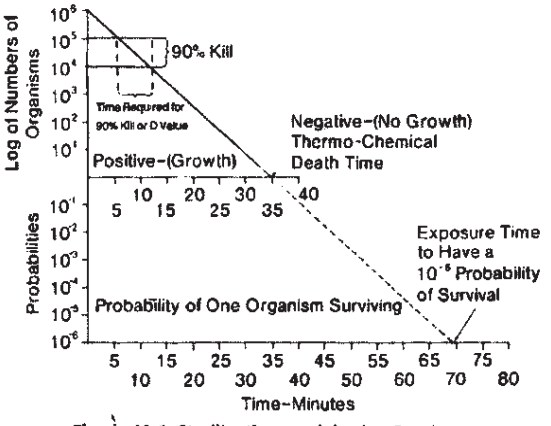

Figure 40-1. Sterilization model using D values.

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

$$N = N_0\, 10^{-t/D} \qquad (1)$$

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 $\frac{1}{10}$ (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.

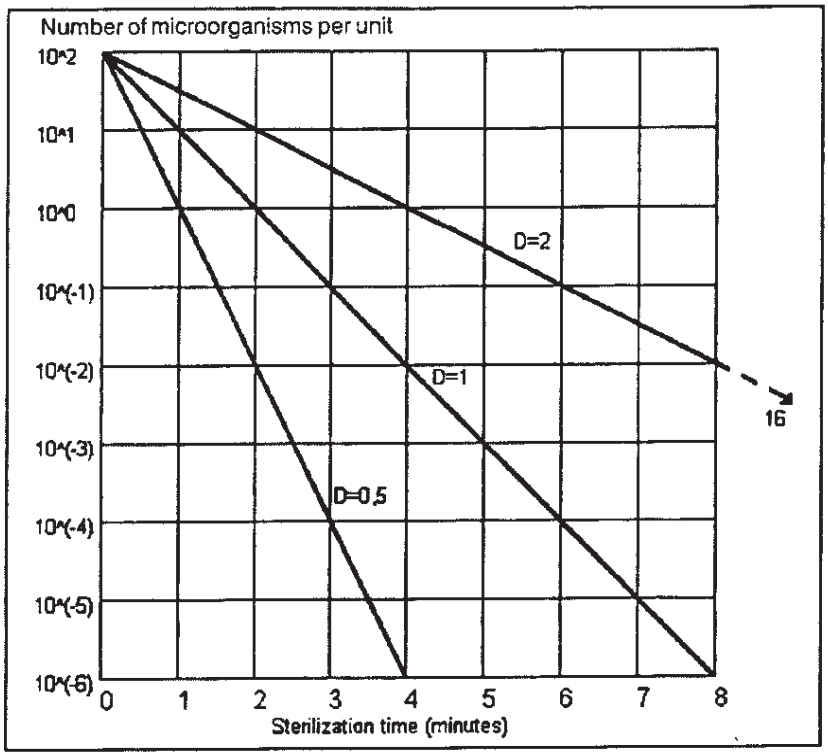

Figure 40-2. Effect of varying D values on sterilization rate (courtesy, Fedegari Autoclavi).

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