

An Overview of the Validation Approach for Moist Heat Sterilization, Part I

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This article illustrates a **qualification-validation strategy for moist heat sterilization** and briefly discusses the sterility concept and common sterilization principles. In Part I, the authors present examples for cycle types, parameter requirements for a standard cycle as defined by pharmacopeias, methods used to design sterilization cycles, and various approaches used to measure the efficiency of the sterilization process.

This article provides an update of the validation of moist heat sterilization. It brings together practical information one needs when validating an autoclave, from procurement through routine use. In Part I of this article, the sterility concept, sterilization principles, development of sterilization cycles, and the measurement of sterilization efficiency are discussed. Part II will be published in *Pharmaceutical Technology's* October issue and will review the qualification-validation procedure and the probability of nonsterility of a load during the validation of the steam sterilization process.

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The sterility concept and sterilization principles

Sterility is defined as the absence of any microorganism capable of reproduction (1). Sterility can be achieved through the destruction of all viable life forms by applying a lethal agent to the item that must be sterilized. Several sterilization principles exist according to the lethal agent used (2). Each treatment destroys microorganisms in a unique manner and with a different degree of effectiveness. A few examples of these treatments are

- heat sterilization with dry or moist heat
- radiation sterilization with gamma and X-rays, which produce positively charged ions in their passage through the matter, thereby further generating free radicals with lethal effects in the cells
- chemical sterilization with glutaraldehyde, chlorine, iodine, hydrogen peroxide, quaternary ammonium salts, ozone, peracetic acid, and phenolic compounds
- gaseous sterilization with chemical agents such as ethylene oxide, propylene oxide, and formaldehyde, all of which have a lethal effect that consists of the alkylation of proteins, DNA, and RNA
- filtration sterilization, which physically removes microorganisms from the product by means of retentive filters.

New technologies also have been developed, including sterilization with free radicals generated by the combination of UV irradiation with hydrogen peroxide and sterilization with chlorine dioxide.

The sterility criterion is a mandatory requirement for parenteral products, ophthalmic preparations, and products applied to injured skin or used to irrigate the body cavities. All products that must be manufactured as sterile should pass the sterility test described by the pharmacopeias.

When exposing homogenous populations of microorganisms to a sterilizing agent, the microbial inactivation follows an exponential decrease curve (see Equation 1). Mathematically, the inactivation of microorganisms can be expressed as a first-order kinetic process. A finite probability of surviving organisms, independent from the magnitude of the delivered sterilizing agent, can be expressed as:

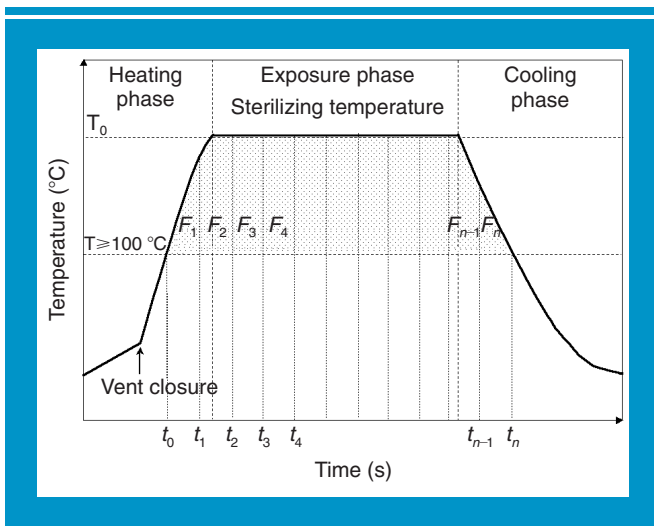


Figure 1: The temperature profile for a saturated steam-vented cycle. t_0 to t_n is the time interval in the computation of the F value. F_1 to F_n areas represent the lethal rates in the product calculated for each discrete time interval. The shaded area under the curve obtained through graphical summation of F_1 to F_n values represents the total calculated F value per cycle. In the heating phase, saturated steam is admitted into the chamber, displacing the cold air until the exposure temperature and corresponding saturated steam pressure are attained. In the exposure phase, the sterilizing temperature is maintained in the chamber by saturated steam for the prescribed exposure time. The cooling phase can be achieved by slow exhaust (for containers filled with liquids) or fast exhaust (for goods required to be dry after sterilization). This phase is completed when the pressure in the chamber reaches atmospheric pressure.

$$N_t = N_0 e^{-kt} \quad [1]$$

in which N_t is the number of surviving organisms after time t , N_0 is the number of microorganisms at time zero (i.e., the bioburden), t is the exposure time, and k is a microbial inactivation rate constant.

For a given process, the probability of survival is determined by the number, type, and resistance of the microorganisms present and by the environment in which the organisms exist during the treatment (e.g., moisture content, thermal energy, and time for steam sterilization) (3). For pharmaceutical products, the term *sterile* is applied to products that have been treated in such a manner that, on completion of the process, individual items have a probability of being nonsterile or have a sterility assurance level (SAL) equal to 1×10^{-6} or more (4). Such a level of sterility assurance is required for terminally sterilized injectable products. This definition of sterility as a probability function does not assume that one in a million products is allowed to be nonsterile but admits a finite statistical probability that a microorganism may survive the sterilizing process (3).

Sterilization by saturated steam in the autoclave

When heat is used as a sterilizing agent, the vibratory motion of every molecule of a microorganism is increased to levels that induce the cleavage of intramolecular hydrogen bonds between proteins. Death is therefore caused by an accumulation of irreversible damage to all metabolic functions of the organism (5).

Heat can be used either alone or mixed with steam. In the autoclave chamber, terminal sterilization is based on highly efficient heat transfer from the saturated steam to the autoclave load (6). Heat transfer occurs by the release of the latent heat from saturated steam under pressure as it condenses.

Heat transfer has maximum efficiency if the steam is kept along the phase separation line of the water-steam phase diagram. Heat transfer from saturated steam to the chamber environment is much more effective and timely for the coagulation and denaturation of nucleic acids and proteins than from dry heat or superheated heat (7). Moist heat in the form of saturated steam under pressure is therefore the most reliable sterilizing agent and is the method of choice whenever it can be used, especially for aqueous preparations (2,8).

Types of cycles used in moist heat sterilization

For saturated steam sterilizers, the physical parameters governing the efficiency of the sterilization process are exposure time, temperature, and pressure. The last two parameters vary in a direct proportional relationship to each other.

Generally, a cycle comprises heating, sterilizing, and cooling phases (9). The choice of a cycle for a particular load depends on the heat sensitivity of the load material and on the knowledge of the heat penetration in the articles. Figures 1 and 2 illustrate the cycles typically used in the moist heat sterilization process.

For a saturated steam-vented cycle (see Figure 1) the steam is injected at the top of the chamber and displaces the cold air, which exits through a trap. This cycle is recommended for containers filled with aqueous preparations (i.e., parenterals, ophthalmics, media, and buffers). The prevacuum cycle (see Figure 2) ensures a more effective penetration of the load by saturated steam and is generally used for porous loads (e.g., surgical dressings and wrapped materials).

For moist heat sterilization, the accepted range of sterilizing temperatures is 118–134 °C. The *US Pharmacopeia (USP)* explains in a footnote that “an autoclave cycle, where specified in the compendia for media or reagents, is a period of 15 min at 121 °C, unless otherwise indicated” (10). The *European Pharmacopoeia (EP)* and the *British Pharmacopoeia (BP)* recommend a heating process at a minimum of 121 °C for 15 min as a reference condition for aqueous preparations (3–4). The text of both books states that other conditions of time and temperature may be used provided that the process chosen has been satisfactorily demonstrated to deliver an adequate and reproducible level of lethality when operating routinely within the established tolerances (3–4).

Development of sterilization cycles

Theoretically, the timing of the exposure phase begins when the temperature sensor placed in the chamber drain (i.e., the coldest spot, theoretically) reaches the set sterilizing temperature. Experiments using thermocouples have shown that the temperature profile of the chamber under loaded conditions is different from the one obtained for the empty chamber. The rate of heating and cooling a product in a container is a function of the container type and size, the viscosity of liquids, and the size and arrangement of the load (2). Therefore, to ensure sterili-

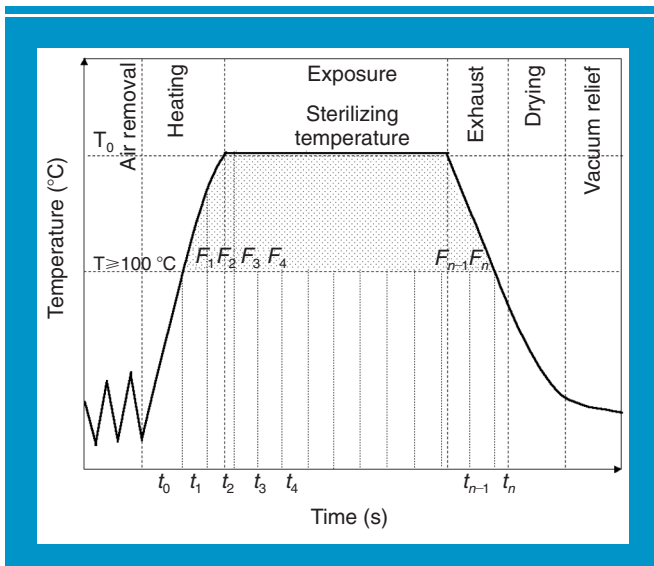


Figure 2: The temperature profile for a saturated-steam with forced air-removal cycle. t_0 to t_n is the time interval in the computation of the F value. F_1 to F_n areas represent the lethal rates in the product calculated for each discrete time interval. The shaded area under the curve obtained through graphical summation of F_1 to F_n values represents the total calculated F value per cycle. In the air-removal phase, air is removed from the chamber and load by several vacuum pulses. In the exhaust phase, steam is exhausted from the chamber until the atmospheric pressure is reached. In the drying phase, the temperature in the jacket and the vacuum in the chamber are maintained for a predetermined time period for products required to be dry. In the vacuum-relief phase, air is admitted to the chamber through a microbiologically retentive filter until atmospheric pressure is reached.

zation efficiency, sterilization must begin after a certain equilibration time when the temperature of the material being sterilized has reached the set sterilizing temperature. This means that a lag time must be added to the cycle exposure time that represents the time necessary for the coldest spot inside the load to reach the sterilizing temperature. The lag time must be determined experimentally and validated with each loading configuration that will be used in the autoclave for any further routine sterilization. The larger the containers and the volumes to be sterilized are, the longer the lag time is.

The modern design of a sterilization cycle can be based on either the initial population of microorganisms in the product that is examined during a suitable time period or on the measurement of the physical parameters attained in the load during the sterilization process. *BP* indicates that “sterilization methods must be validated with respect to both the assurance of sterility and integrity of the product and to ensure that the final product complies with the requirements of the monograph” (4). *USP* states that “the design or choice of a cycle for given products or components depends on a number of factors, including the heat lability of the material, knowledge of heat penetration into the articles, and other factors described under the validation program” (10). Three approaches are used in designing sterilization cycles: the bioburden approach, the overkill approach, and a combination of the two approaches.

The bioburden approach is based on determination of the

number, type, and resistance characteristics of the organisms contained in the bioburden (11). For heat-sensitive products, the heat exposure must be minimized in such a way to reach an optimum balance between an acceptable degree of sterilization and an acceptable stability of the product after its sterilization (12). In the bioburden approach, the process will provide less than 1×10^{-6} probability of microorganism survival. The sterilizing conditions used for the bioburden approach are less extreme than the conditions used for an overkill approach.

The design of a sterilization cycle also can be based on the heating characteristics of the containers located in the slowest heating zone of the load. The overkill approach stems from the concept that the sterilization process will inactivate a high microbiological challenge with an additional safety factor. The microbiological challenge will consist of a biological indicator with a specific number of microorganisms (10^3 – 10^6), and a worst-case assumption is made that the heat resistance of the bioburden is equivalent to that of the biological indicator. Therefore, the cycle conditions established are more severe than those required to inactivate the real product bioburden, and a theoretical spore reduction of 10^{-12} is expected to prove the overkill assurance. Considerably exceeding the sterilization time is recommended for heat-resistant products (10). Therefore, F_0 values (see definition in the “Mathematical approach” section) in the range of 30–60 are expected.

The bioburden approach emphasizes the stability of the final product while the optimum sterility is achieved by knowing the characteristics of the bioburden; however, the overkill approach places the emphasis on sterility. A combination of the two approaches is sometimes useful to reach a balance between the maximum acceptable SAL and the maximum allowable concomitant adverse effect upon the material to be sterilized.

Measuring the efficiency of the sterilization process

Assessment of sterility is based on the demonstration of the absence of growth following the sterility test. Performance of the sterility test is not always a guarantee of sterility for a product (13). Therefore, two methods that incorporate microbial inactivation data have been developed to measure, control, and demonstrate the efficiency of a sterilization process.

Biological approach. In the biological approach, known also as the *Latin approach*, one determines the lowest probability to detect a nonsterile unit in a sterile load and uses biological indicators (BIs) (14). BIs are standardized preparations of microorganisms specific to the type of sterilization process being studied. These BIs are used to show a reproducible logarithmic inactivation. Many types of BIs consist of a given population of bacterial spores considered to be the most resistant forms against a particular sterilization principle.

In sterilization microbiology the D value is frequently used instead of k as a measure of the rate of microbial death. Equation 1 can be formulated in a logarithmic manner as follows:

$$\log \frac{N_0}{N_t} = \frac{kt}{2.303} = \frac{t}{D} \quad [2]$$

The D value represents the temperature coefficient for the lethal process and is the exposure time in minutes required to

cause a 1-logarithm or 90% reduction in the population of a particular microorganism (9,10). The resistance of an organism changes with alterations in temperature. The smaller the D value, the more sensitive the organism is to the lethal agent.

The Z value is defined as the number of degrees of temperature required for a 1-logarithm change in the D value. The Z and D values are significant only under precisely defined experimental conditions and are generally assumed for aqueous solutions. For saturated-steam sterilization, a BI containing spores of *Bacillus stearothermophilus* strain ATTC 7953 with a D value greater than 1.5 min, a Z value of 10 °C, and a population greater than 5×10^5 is recommended (15). *B. stearothermophilus* is considered the reference organism for saturated-steam sterilization because it is known for its great resistance to the lethal agent when compared with the organisms contained in the bioburden (4). For a standard cycle, as recommended by *BP*, no growth should be recorded for the BI after an exposure time of 15 min at 121 ± 1 °C and a poststudy incubation period. *USP* states that for *B. stearothermophilus* spores with a D value of 1.5 min under total process parameters (i.e., 121 °C), if they are treated for 12 minutes, the lethality input is $8D$ (10). However, it is not specified that, after 15 min, no growth of the reference microorganism should exist.

For meaningful results, at least 20 BIs per cycle should be used to ensure that statistical distribution is differentiated from true deviations to demonstrate homogenous conditions within the chamber autoclave. Although the biological approach presents difficulties specific to statistical methods, it allows the determination of the lethality effect within the loads in which it is difficult or impossible to place temperature probes (e.g., inside ampuls).

Mathematical approach. The F value is a unit of lethality and is a measure of the microbial inactivation capability of a heat sterilization process. It is defined as “the equivalent in minutes at 250 °F of all heat considered with respect to its capacity to destroy spores or vegetative cells of a particular organism” (2) and allows the comparison of lethal effects at various temperatures. Sterilization cycle parameters can be found by using other temperatures than 121 °C with the help of the F_0 concept. F_0 , as defined by *USP* (10) at a particular temperature other than 121 °C, is the time (in minutes) required to provide the lethality equivalent to that which is provided at 121 °C for a stated time. The temperature of 250 °F or 121.1 °C represents the temperature of saturated water vapor at ~100 kPa. The F value allows the comparison of lethal effects at various temperatures.

The total F value of a process can be calculated by the integration of lethal rates with respect to time at discrete temperature intervals (16). Integrating the lethality constant $10^{(T-T_0)/Z}$ between two time points, t_0 and t_n , will yield the shaded area under the curves indicated in Figures 1 and 2.

The mathematical approach, also known as the *Anglo-Saxon approach*, uses the F_0 value as the reference unit of lethality. The F_0 value can be calculated using Equation 3. Product-

Table I: Combinations of temperature and time calculated to provide F_0 values of 8- and 12-min equivalents based on reference spores of *Bacillus stearothermophilus* with a D_{121} of 1.5 min and a Z value of 10 °C.

Temperature (°C)	Sterilization Exposure	Sterilization Exposure
	Time (min) for $F_0 = 8\text{-min Equivalents}$	Time (min) for $F_0 = 12\text{-min Equivalents}$
110	103.05	154.58
115	32.59	48.88
118	16.34	24.50
121	8.18	12.27
124	4.10	6.15
126	2.58	3.88
130	1.03	1.54

temperature data accumulated during the entire process are converted to the equivalent lethality at 121.1 °C. For practical purposes, temperatures higher than 100 °C are considered in calculations. It should be emphasized that the F_0 value must be calculated at 121.1 °C, not at 121 °C, because the difference of 0.1 °C introduces an error of 2.4% in the computation of the F_0 value.

$$F_0 = \int_{t_0}^{t_n} 10^{\left(\frac{T-121.1}{z}\right)} dt \quad [3]$$

In a nonmandatory section of the *EP*, General Text 5.1.5 offers guidance concerning the validation of the steam sterilization process by means of the F_0 concept, whose value can be determined from Equation 4 (3). D_{121} is the exposure time at the indicated temperature required to cause a 90% reduction in the spore population. The text of *BP* states that the application of the F_0 concept requires microbiological validation.

$$F_0 = D_{121} (\log N_0 - \log N_t) \quad [4]$$

The lethality of the sterilization process is influenced by the effectiveness of heat penetration into actual articles and the time of sterilization. Pfeiffer, quoting *BP* 1983, indicates that the steam sterilization process must be sufficient to produce an F_0 value of at least 8-min equivalents (17). This means that the coolest location in the autoclave loading configuration must be exposed to an equivalent time of at least 8 min of exposure to a temperature of 121.1 °C. This represents the most conservative estimate of the lethality and therefore the safest conditions for determining cycle times.

An F_0 of 8-min equivalents is considered a realistic minimum value because most mesophilic spore-forming microorganisms have D values between 30 s and 1 min at 121.1 °C. When designing a cycle, one should introduce an additional safety factor that takes into account the extra time that may be required for steam to penetrate certain containers in the middle or cool locations of the chamber. For practical purposes one should design sterilization cycles with an F_0 value of 12-min equivalents.

Depending on the nature of the load, one may use nonstandard conditions and try to achieve an equivalent level of kill from new conditions (18). A high activation energy needed to

kill spores can lead to chemical changes, especially in aqueous preparations (2). To avoid product degradation, a longer cycle at lower temperatures is recommended. For other products such as vitamins media that may suffer decomposition processes from a prolonged treatment at a lower temperature, it would be more advantageous to apply a short-time exposure at a higher temperature (19). To evaluate the effect of the killing process at a temperature different from 121.1 °C, the following equation can be used when calculating the lethality factor, F_T :

$$F_T^z = \frac{F_0}{10^{(T-121.1)/z}} \quad (5)$$

in which D and Z values are known for a given microorganism, and F is the effectiveness of killing at the specified process temperature, T (°C).

Table I presents equivalent combinations of temperature and time, providing the F_0 value of 8- and 12-min equivalents. The use of 110 °C as a sterilizing temperature requires very long exposure times to reach the target F_0 values. Such exposure times would be unrealistic.

The biological and mathematical approaches are complementary to each other. Lethality using physical process data should be determined in conjunction with appropriate microbiological studies.

Qualifying an autoclave and validating the sterilization process

Absolute sterility cannot be practically demonstrated without the complete destruction of the load. For practical purposes, the industry and regulatory agencies approach the concept of sterility on a probabilistic basis. Validation must prove that the sterilization process delivers a treatment that ensures a certain statistical probability of sterility for an initially nonsterile unit. Therefore, any sterilization process must be validated before use and must be routinely monitored as required by many official texts (3,4,10).

It should be emphasized that the terms *qualification* and *validation* are not identical. Equipment is qualified, and processes are validated. Qualification provides assurance that the autoclave can consistently kill a microbial load of 1×10^6 colony-forming units per container during a defined cycle by using a specific loading configuration. The actual process validation of the cycles is performed by recording and interpreting the physical parameters (i.e., time, temperature, and pressure for steam sterilization) required to prove that the process will consistently yield a product that complies with previously established specifications.

During routine monitoring, the assurance of sterility is gained by demonstrating that validated conditions, known to produce the required level of microbial inactivation, have been attained. Therefore, the careful design and validation of the sterilization process enables sterility to be addressed with an increasing probability of success (12). Such a system is called *parametric release* and has been defined as a "sterility release procedure based upon effective control, monitoring, and documentation of a validated sterilization process in lieu of release based upon end-product sterility testing" (20).

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