Moist-Heat Sterilization Principles

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Definition of “sterility”

Absolute *vs* probabilistic (I): European

Ph. Eur. 10.0, 5.1.1, *Methods of preparation of sterile products*:

“Sterility is the absence of viable microorganisms, as defined by a sterility assurance level equal to or less than 10^{-6}.”

“The achievement of sterility for any one item in a population of items can neither be guaranteed nor demonstrated.”

“Sterilization process conditions are chosen to achieve the highest level of sterility assurance compatible with the drug product…”

“The SAL for a given sterilisation process is expressed as the probability of micro-organisms surviving in a product item after exposure to the process.”

Definition of "sterility"

Absolute *vs* probabilistic (II)

EN ISO 17665-1:2006, 3.49, 3.50 and 3.51:

"sterility: state of being free from viable microorganisms

NOTE In practice, no such absolute statement regarding the absence of microorganisms can be proven."

"sterility assurance level / SAL: probability of a single viable microorganism occurring on an item after sterilization

NOTE The term SAL takes a quantitative value, generally 10^{-6} or 10^{-3}. When applying this quantitative value to assurance of sterility, an SAL of 10^{-6} has a lower value but provides a greater assurance of sterility than a SAL of 10^{-3}."

"sterilization: validated process used to render product free from viable microorganisms

NOTE In a sterilization process, the nature of microbial inactivation is exponential and thus, the survival of a microorganism on an individual item can be expressed in terms of probability. While this probability can be reduced to a very low number, it can never be reduced to zero."

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Definition of “sterility”

Absolute *vs* probabilistic (III): USA 1

USP 43, <1211>, *Sterility Assurance - Introduction*:

“…an item is deemed sterile only when it contains no viable microorganisms … **Sterility cannot be demonstrated without the destructive testing of every sterile unit** … microbiological safety is achieved through the implementation of interrelated controls that in combination provide confidence that the items are suitable for use as labeled. It is the controls that provide the desired assurance from microbiological risk rather than the results of any in-process or finished goods testing. **The verification of safety of products labeled sterile is generally known as “sterility assurance”**…

The establishment of an effective sterility assurance program requires information about the material to be sterilized … the appropriate process provides a balance between conditions that are lethal to potential bioburden present in/on the item and those that preserve its essential quality attributes.”

Definition of "sterility"

Absolute *vs* probabilistic (IV): USA 2

USP 43, <1229>
Sterilization of Compendial Articles - Background and Scope:

"Sterility, therefore, is defined in probabilistic terms that establish an acceptable level of risk. Sterility can be accomplished only by the use of a validated sterilization process under appropriate current good manufacturing practices and cannot be demonstrated by reliance on sterility testing alone."

USP 43, <1229>
Ster. Comp. Art. - Establishing and Justifying Sterilization Processes:

"Articles intended to be sterile must attain a ≤10−6 *probability of a nonsterile unit.*"

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Probabilistic definition of “sterility”

PNSU *vs* SAL

American PNSU is referred to the *final product,* this means to the *Probability of a Nonsterile Unit* in a sample-tested lot of product

Theoretically at least, the European concept of *Sterility Assurance Level* is different: it means the *probability of micro-organisms to survive in an item* after a sterilization process and refers to the *trend of the process* of sterilization

But for explanation, the same Ph. Eur. 10.0, 5.1.1 adds:

“An SAL of 10^{-6}, for example, denotes a probability of not more than 1 non-sterile item in 1 x 10^{6} sterilised items of the final product.”

This “example” states the practical equivalence of the two definitions

Sterility and sterilization of medical devices in EN 556-1 (I)

EN 556-1:2001 (amended 2006)

Introduction: "…product items produced under standard manufacturing … may, prior to sterilization, have micro-organisms on them, albeit in low numbers. Such product items are non-sterile. The purpose of sterilization processing is to inactivate the microbiological contaminants and thereby transform the non-sterile items into *sterile* ones ."

Requirement 4.1: "For a terminally-sterilized *medical device* to be designated *"STERILE"*, the theoretical probability of there being a viable micro-organism present on/in the device shall be equal to or less than 1×10^{-6}."

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Sterility and sterilization of medical devices in EN 556-1 (II)

EN 556-1:2001 (amended 2006)

Requirement 4.2: “Compliance shall be shown by the manufacturer or supplier through provision of documentation and records which demonstrate that the devices have been subjected to a validated sterilization process fulfilling **4.1**.

NOTE 1 Evidence that a medical device is sterile comes from: i) the initial validation of the sterilization process and subsequent revalidations that demonstrate the acceptability of the process; and ii) information gathered during routine control and monitoring which demonstrates that the validated process has been delivered in practice.

NOTE 2 The achievement of sterility is predicted from the bioburden level on products, the resistance of the micro-organisms comprising that bioburden and the extent of treatment imposed during sterilization.”

Basic principles for control of sterilization processes (I)

USP 43, <1229>:

“The basic principles for control of sterilization processes, including method development, validation, and ongoing assurance, are as follows:

1. Sterilization process development that includes evaluation of the stability and compatibility of materials, container integrity, expected pre-sterilization bioburden, equipment method control parameters, etc.
2. Identification of sterilization process parameters that preserve the inherent properties of the materials yet inactivate or remove microorganisms.

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Basic principles for control of sterilization processes (II)

USP 43, <1229>:

“The basic principles for control of sterilization processes, including method development, validation, and ongoing assurance, are as follows:

3. Demonstration that the sterilization process and equipment are capable of operating within the prescribed parameters and corresponding to independent measurements of the critical parameters.

4. Performance of replicate studies that represent the operational range of the equipment and employ actual or simulated product. The use of biological indicators for correlation between the measured physical parameters and the expected lethality is recommended wherever possible.

Basic principles for control of sterilization processes (III)

USP 43, <1229>:

“The basic principles for control of sterilization processes, including method development, validation, and ongoing assurance, are as follows:

5. Maintenance and monitoring of the validated process during routine operation.

6. Assurance that the bioburden (number and type) of the materials is acceptable and is maintained within predetermined limits during routine operation.”

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Methods of sterilization – USP 1

USP 43, <1229> lists several methods:

a) by “destruction of microorganisms”:

Steam Sterilization by Direct Contact “with the load items (whether wrapped or unwrapped)” ⟨1229.1⟩

Moist Heat Sterilization of Aqueous Liquids: “the method of choice for aqueous parenteral products, in-process aqueous liquids, laboratory media, and biological waste materials … primarily in closed containers” ⟨1229.2⟩

Dry Heat Sterilization: “a process utilized for heat-stable items (glass, stainless steel, nonaqueous liquids, powders, etc.) that are unsuited for steam sterilization because of either an absence of water (nonaqueous liquids and powders) or requirements for absolute dryness following processing (product contact parts for nonaqueous products)” ⟨1229.8⟩

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Methods of sterilization – USP 2

USP 43, <1229> lists several methods:

Radiation Sterilization: "used extensively for the sterilization of medical devices and for a variety of other materials and products" ⟨1229.10⟩

Gaseous Sterilization: "commonly used for items that are susceptible to damage by heat or radiation processes" ⟨1229.7⟩

Liquid Phase Sterilization: "objects to be sterilized are immersed in the solution of a chemical agent *[aldehydes, acids, bases, and strong oxidants]*, after which the agent must be removed in a manner that preserves the sterilized object from recontamination … These agents are highly toxic" ⟨1229.6⟩

Methods of sterilization – USP 3

USP 43, <1229> lists several methods:

Vapor Phase Sterilization: "accomplished using sporicidal agents suspended in air *[vapors of hydrogen peroxide, peracetic acid, formaldehyde, and glutaraldehyde in aqueous solution]*. … They differ from sterilizing gases and liquids in that there are multiple phases within the vessel during sterilization … well suited for heat-sensitive materials and surface sterilization" ⟨1229.11⟩

b) by "physical removal" of microorganisms:

Sterilizing Filtration of Liquids: "The filtration process parameters that influence microbial retention include temperature, flow rate, volume, filtration time, differential pressure, and pressure pulsations" ⟨1229.4⟩

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Methods of sterilization – EP 1

Ph. Eur. 10.0, 5.1.1 lists the following methods:

"Steam sterilisation": "achieved by heat transfer during condensation of water"

"Dry heat sterilisation": "a terminal sterilisation method based on the transfer of heat … by means of convection, radiation or direct transfer" — Reference conditions: 2 hrs at 160 °C

"Ionising radiation sterilisation": "may be used for the terminal sterilisation of tissues and cells, or the sterilisation of materials or containers to be employed in aseptic processing" or "the surface sterilisation of materials upon entry to isolators" — Reference absorbed dose: 25 kGy

Methods of sterilization – EP 2

Ph. Eur. 10.0, 5.1.1 lists the following methods:

"Gas sterilisation (Vapor phase sterilisation)": "may be used for the sterilisation of primary packaging materials, equipment and some pharmaceuticals.
It is essential that penetration by gas and moisture into the material to be sterilised is ensured, and that it is followed by a process whereby the gas is eliminated … below concentrations that could give rise to toxic effects"

"Membrane filtration": "used for reduction of viable and non-viable particles in gases and fluid products that are not amenable to sterilisation by heat or irradiation … The principle of membrane filtration is not inactivation but removal of microorganisms … achieved by a combination of sieving and surface interaction"

EP does not list a method equivalent to USP "Liquid Phase Sterilization"

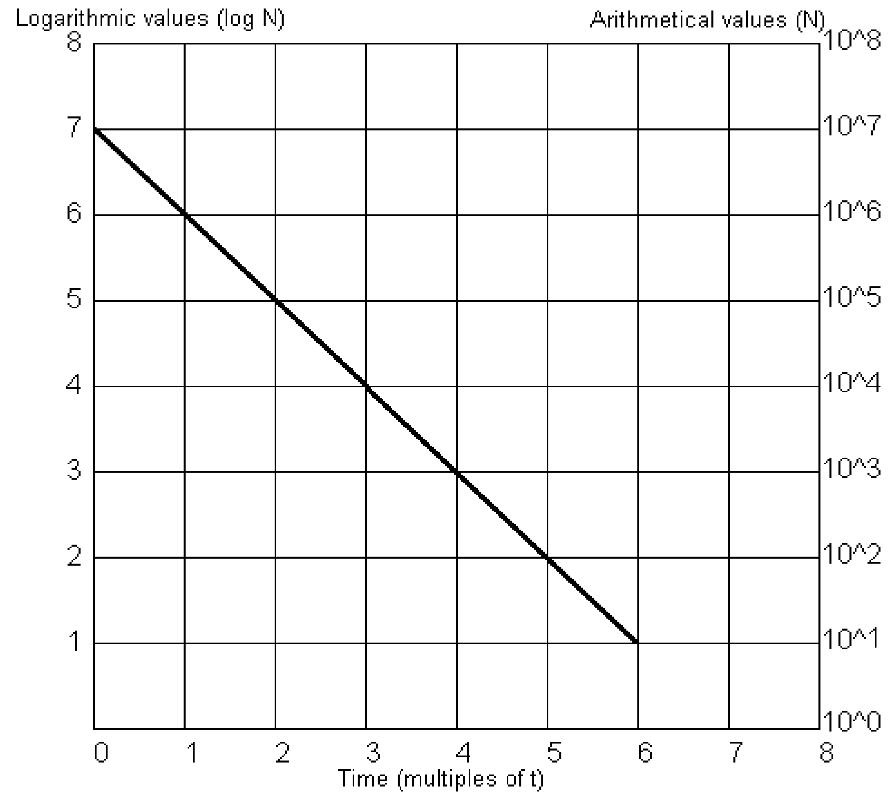
Asymptotic behavior of a sterilization process (I)

It is well known that most sterilization processes (except for filtration) "often approximates to an exponential relationship." (EN 556-1)

Most processes follows a decreasing asymptotic trend, that tends to zero: *in the same time interval* under the same conditions*, the same percentage of the microorganisms present at the beginning of that interval is destroyed, and the same percentage survives, complementary to the destroyed one*

This means that most sterilization processes proceed like a first-order chemical reaction: in this model (often named by Arrhenius), only a sterilization process of infinite duration could assure a complete destruction of all microorganisms initially present

Asymptotic behavior of a sterilization process (II)



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D-value: Decimal reduction time (I)

The rate of an asymptotic sterilization reaction is expressed by means of a parameter called *D*-value:

EN ISO 11138-7:2019, Def. 3.4: D-*value is the* **"time or dose required under stated conditions to achieve inactivation of 90 % of a population of the test microorganisms"**

D-value varies according to:

- the microbial "species" considered
- the "substrate" on which it lives
- the culture medium ("matrix")
- the recovery medium
- the test methodology
- the actual conditions of the sterilization process (temperature, concentration of the sterilant, humidity)

For moist-heat sterilization, the essential condition is the presence of liquid water in contact with the microorganisms. The *most common* reference temperature, both for process and D-values, is 121 °C (originally, 250 °F = 121.11 °C)

D-value: Decimal reduction time (II)

USP 43, <1229>, relevant to sterilization in general, defines:

"The D-value is the time (customarily in minutes) or radiation dose (customarily in kGy) required to reduce the microbial population by 90% or 1 log_{10} cycle (i.e., to a surviving fraction of 1/10) and must be associated with the specific lethal conditions at which it was determined."

EP 10.3, 5.1.5, relevant to "steam sterilization of aqueous preparations", defines the *D*-value as follows:

"The D-value (or decimal reduction value) is the time in minutes required at a defined temperature to reduce the number of viable test organisms by 90 per cent. It is only of significance under precisely defined experimental conditions."

So, both these Pharmacopoeias underline that the evaluation of the sterilizing effect of a process starts from a thorough knowledge of the conditions at which the resistance of the microorganisms has been defined

Meaning of *D*-value (I)

When saying that the moist-heat *D*-value at 121 °C of a specific microbial species in a specified culture medium is, for example, D = 1.5 minute, we mean that a population of that microbial species in that culture medium, exposed to moist-heat conditions at 121 °C for 1.5 minute = 90 seconds, is reduced to one tenth of its initial value:

At the beginning: 1,000 microorganisms, i.e. 10^3

after 90” @ 121 °C

➔ 10^2,

i.e. 100 microorganisms

after further 90” @ 121 °C

➔ ➔ 10^1,

i.e. 10 microorganisms

after further 90” @ 121 °C ➔ ➔ ➔ 10^0, i.e. only 1 microorganism

What happens after a further 90” @ 121 °C moist-heat?

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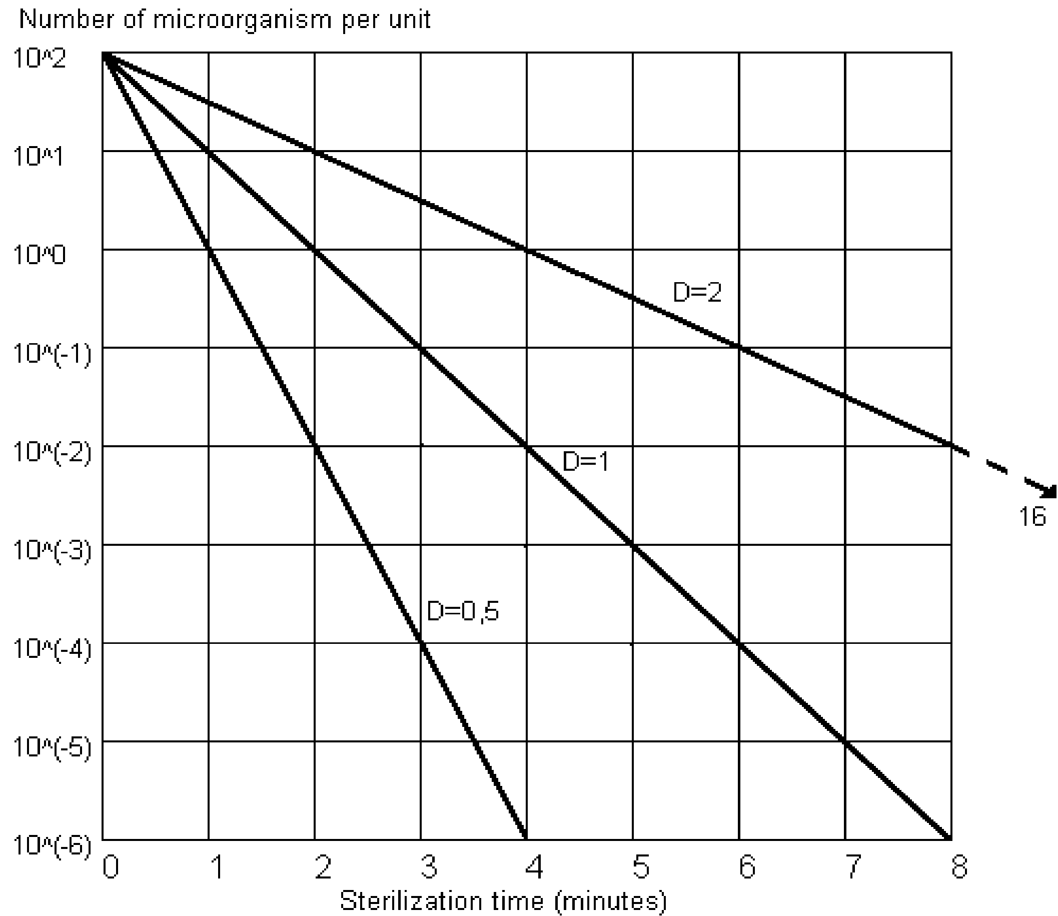
Meaning of *D*-value (II)

According to this model, after the sterilization has continued for additional 90 seconds under the same thermal conditions, the surviving units would be 10^{-1}, i.e., 1/10. It is apparent that the survival of one tenth of microorganism makes no true sense, physical or biological

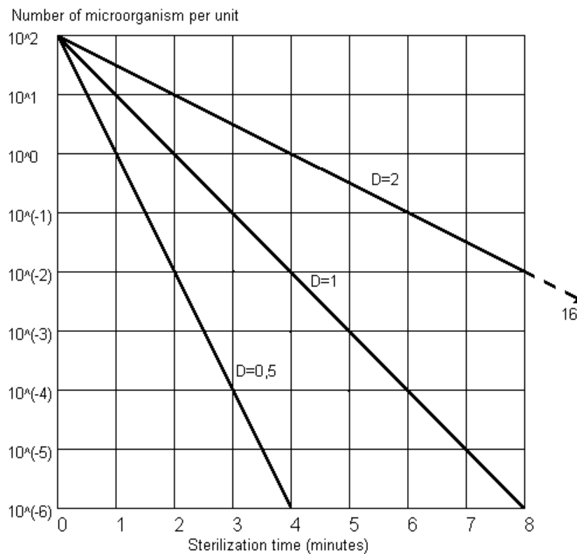
A possible explanation of this paradox could be that the mathematical model of steady decimal reduction is no longer applicable when only few microorganisms survive

But a useful meaning can be attached to this figure by saying that *the probability that the initial microbial population has been completely destroyed is 10 to 1*. If exposure to moist-heat continues for other 5 · D minutes, this probability becomes 1,000,000 to 1, i.e., 10^{-6}, and so on. As already said, the probabilistic definition of "sterile" as "correctly and reliably sterilized" is expressed in Europe by the "SAL" (**S**terility **A**ssurance **L**evel), in the United States by the "PNSU" (**P**robability of **N**on**s**terile **U**nit)

Meaning of *D*-value (III)



Mathematical expressions of *D*-value



$dN / dt = - k N \rightarrow N = N_0 \cdot e^{-kt}$

$D = (\log_e 10) / k = 2.3026 / k$

Any one of the next formulas corresponds to the definition of *D*-value for an asymptotic sterilization:

$$D = t / [\log_{10} (N_0 / N)]$$

$$N = N_0 \cdot 10^{-t/D}$$

$$\log_{10} N = \log_{10} N_0 - t / D$$

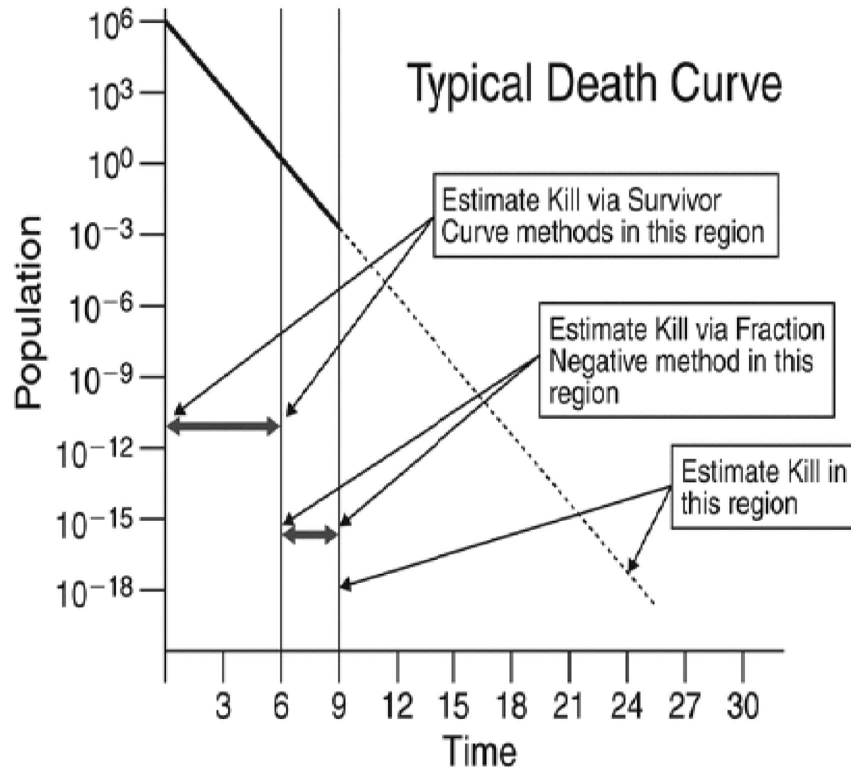
$$t = D (\log_{10} N_0 - \log_{10} N) = D \log_{10} (N_0 / N)$$

In these formulas:

N_0 = initial microbial population
N = actual microbial population after exposure time t

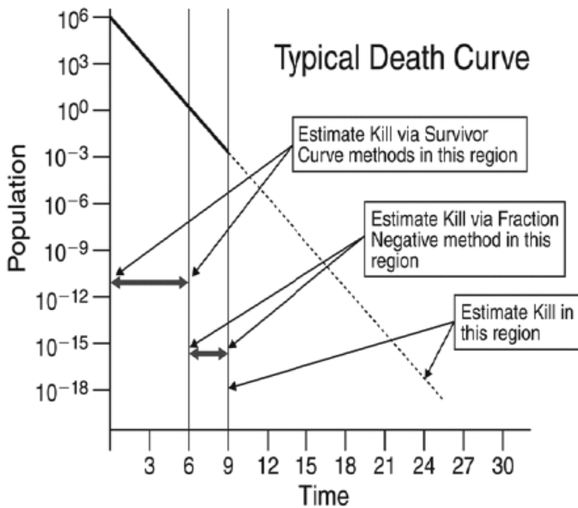
The ratio N_0 / N was called ***IF*** (Inactivation Factor) in EP 9.0, 5.1.5, but this definition has been removed from the current “informative” chapter EP 10.3, 5.1.5

Evaluation of the output of a sterilization process (I)



Source: USP 43, <1229>, Fig. 3

Evaluation of the output of a sterilization process (II)



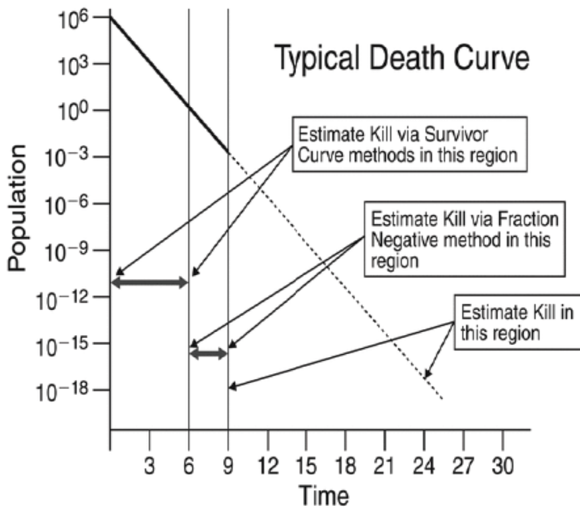
USP 43, <1229>:

"The death curve for microorganisms subjected to a sterilization process is comprised of three distinct regions:

1. *Survivor curve region*—Where viable microorganisms can be recovered and counted to determine the slope of the death curve.

Using survivor counts in short exposure periods, the first section of the death curve can be drawn to where the population is approximately 10 CFU.

Evaluation of the output of a sterilization process (III)



USP 43, <1229>:

The death curve for microorganisms subjected to a sterilization process is comprised of three distinct regions:

2. *Fraction negative region*—Where replicate studies with multiple biological indicators are used to estimate the slope. This can extend the demonstrable portion of the death curve to approximately 10^{-2} to 10^{-3}.

3. *Estimated region*—Where the death rate curve established by either the survivor curve method or fraction negative method is extrapolated to the desired PNSU. Below 10^{-3} the death curve is assumed to be linear and is depicted in *Figure 3* by the dashed line beyond the point assuming that the death of microorganisms continues at the same rate."

z-value: temperature coefficient (I)

Another evidence for heat sterilization is that the *D*-value decreases as the heat sterilization temperature increases, and *vice versa*. This fact is also in accordance with the point of view of chemical reactions involved in the inactivation of microorganisms

For whichever a process, the *reaction temperature coefficient* indicates how many temperature degrees involve a 10-fold change of the process rate. In the case of sterilization by heat, the temperature coefficient is named
z-value and represents *"the temperature difference that leads to a 10-fold change in the* D-*value of the biological indicator."* (EP 10.0, 5.1.2, 3-1-1)

In other words: *"The z-value is the change in temperature in degrees Celsius required to alter the* D-*value by a factor of 10 (the z-value relates the resistance of a micro-organism to changes in temperature)."* (EP 10.3, 5.1.5)

z-value: temperature coefficient (II)

The *z*-value for a microbial species in a temperature range T_1 to T_2 is given by the formula (EP 10.3, 5.1.5):

$$z = (T_2 - T_1) / (\log_{10} D_1 - \log_{10} D_2) = (T_2 - T_1) / (\log_{10} D_1 / D_2)$$

The calculation of a *z*-value demands the knowledge of (at least) two *D*-values D_1 and D_2 at temperatures T_1 and T_2, which will be the limits of the reliability range of the *z*-value so calculated.

Thus, as the *D*-values do, the *z*-value depends on:

- the microbial species considered
- the other factors listed for *D*-value
- the temperature range (this is too often forgotten!)

It would be a meaningless and dangerous extrapolation to utilize a *z*-value outside the range (T2 - T1) of which the *D*-values in the formula are the limits. This is also stated as a rule. (EP 10.0, 5.12, 3-1-1)

Practical meaning of z-value

For instance, with the assumption that z = 10 °C in all the temperature range 111 °C to 131 °C, and provided that the sterilizing conditions (in this case the presence of moist heat) are integrally and continuously maintained, one minute of exposure at 121 °C has the same effect of ten minutes at 111 °C, and one minute at 131 °C has the same effect of ten minutes at 121 °C

The above means that the same sterilizing effect of 8 minutes of moist heat at 121 °C would be obtained with only 0.8 minutes at 131 °C, but would require 80 minutes at 111 °C

If z = 10 °C, a change of a mere 1 °C entails a remarkable 26% change of the sterilization rate: in fact, $(1.2589\ldots)^{10} = 10$

Quite differently, the "Arrhenius temperature coefficient" for a tenfold change in rate of chemical reactions is in most cases about 23 °C, thus entailing a mere 10.5% change of rate per 1 °C. This remarkable difference may become very useful when sterilizing heat-sensitive products

Typical *D*-value and *z*-value (I)

In industrial sterilization practice, the *D*-value of microorganisms present in the bioburden to inactivate is usually remarkably lower than 1.0 minute at 121 °C. Likewise, the *z*-values of most microorganisms are included between 5 °C and 12 °C in the temperature range from 110 °C to 130 °C

Thus, if there are no reliable data on the microbial species in the bioburden (the most resistant one, in the common case of heterogeneous contamination), the *D*-value of it for moist-heat sterilization is often assumed to be 1.0 minute at 121 °C, and the z-value of it 10 °C

Typical *D*-value and *z*-value (II)

The combination of a hypothetical D_{121} = 1 minute and a hypothetical z = 10 °C has proven to offer acceptable safety margins, but *the final probability of microbial survival shall be evaluated by challenge with more resistant testing microorganisms* (i.e., by suitable Biological Indicators)

Very clearly, USP 43, <1229.5> states: "It must be established that the BI system provides a challenge to the sterilization process greater than the resistance of the native bioburden."

Microorganisms commonly in use for testing and validation (e.g., *Geobacillus stearothermophilus*) have definitely higher *D*-values than 1.0 minute, and the population of them in commercial BIs is much higher than the usual bioburden

"***Biological Indicator Challenge System (BI)***: A test system containing viable microorganisms of a pure, specified strain providing a defined resistance to a specified sterilization process." (PDA Technical Report No.1 rev. 2007, Glossary of Terms)

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Shared sterilization concepts, targets and requirements

- Sterility means being free of viable microorganisms
- This state can be recognized only as the probability to find a non-sterile item (PNSU) in a lot, or the probability to have a residual microbial contamination in an item (SAL)
- To deem an item "sterile", both pharma products and medical devices, these probabilities must be not higher than 10^{-6}
- Final tests on samples of finished products do not demonstrate sterility
- Only a documented routine compliance with a pre-validated production process can provide assurance of sterility
- Sterility is predictable on the base of the initial contamination of the product ("bioburden") and the characteristics of it (*D*-value and *z*-value of the most resistant microorganism expected on the product

Moist-heat sterilization temperature

USP 43, <1229.1> is very clear: "Moist heat sterilization process efficacy is not intrinsically linked to a target temperature of 121°, which is simply the Celsius conversion of 250°F, and other temperatures can be used."

This seems to indicate a complete freedom.

EP 10.0, 5.1.1 keeps valid the concept of a "reference cycle" ("15 min at 121 °C"), for which a less onerous documentation is requested by related guidelines, but states also clearly:

"Product- and load-specific cycles, e.g., applying another combination of time and temperature, may be adopted based on cycle development and validation. The minimum temperature acceptable for a steam sterilisation process is 110 °C."

So, EP prescribes a minimum temperature under which a moist-heat treatment is no longer deemed a method for achieving sterility

CEN ISO/TS 17665-2:2009 includes references to 121 °C only for testing purposes, and exemplification.

No reference to process temperature values can be found in EN ISO 17665-1:2006

Thank you

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