Moist heat sterilization principles

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PDA
Parenteral Drug Association

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Moist heat sterilization

- ✓ The methods of choice by all Pharmacopeias, Standards and Guidelines, for terminal sterilization

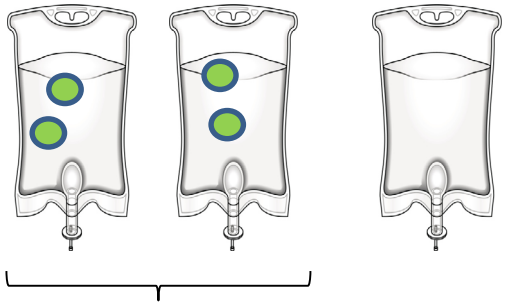
- ✓ Other methods can be used only if the material is not compatible with the temperature, humidity or pressure of steam sterilization

- ✓ It is carried out in an autoclave (pressure vessel: a chamber resistant to pressure)

What do sterility/sterile mean?

✓ Sterility

It is an absolute concept: total absence of any viable microorganism



Non-sterile

Sterile

What is a sterilization process?

✓ Sterility

Total absence of any viable microorganism

✓ Sterile item

An object free of any viable microorganism

Can we demonstrate the sterility of a product

and then use the sterilized product?

NO!

Because sterilization is a special process

To **demonstrate the absence of MOs**,
you **destroy the sterility** of the product

Sterilization: a special process

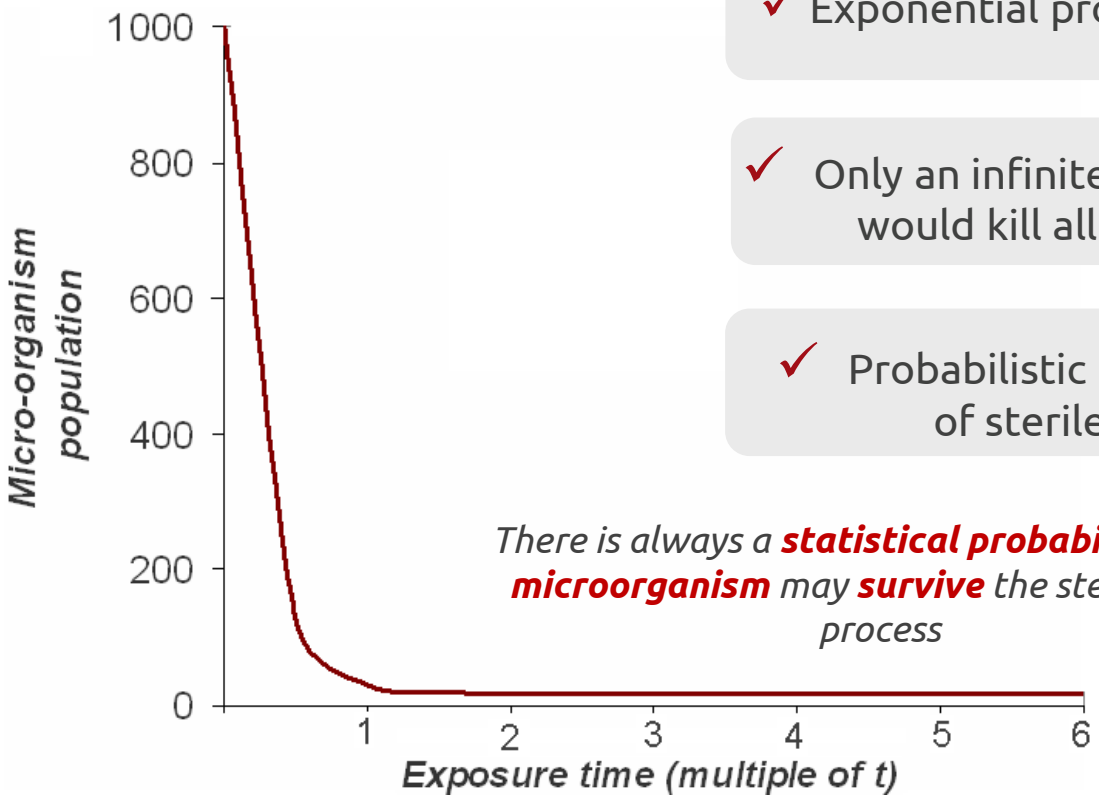
The outcome of a sterilization process (absence of viable MOs) cannot be proven without destroying the sterility of the sterilized product.

The achievement of sterility within any one item in a population of items submitted to a sterilization process cannot be guaranteed nor be demonstrated .
(European Pharmacopoeia, X Edition)

Sterilization: how does it work?

- ✓ Any sterilization process (= inactivation/destruction of a culture of microorganisms) approximates to an exponential relationship
- ✓ This means that any sterilization process proceeds with an asymptotic trend that tends to zero, but it never reaches zero
- ✓ There is always a statistical probability that a microorganism may survive the sterilization process

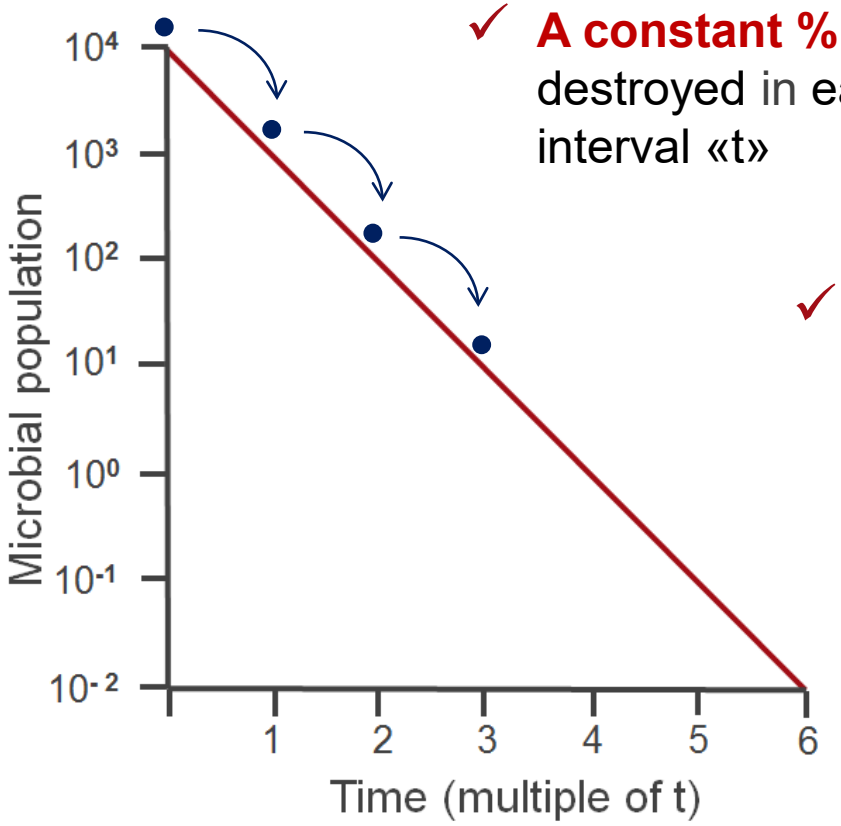
Sterilization: how does it work?



- ✓ Exponential process
- ✓ Only an infinite treatment would kill all the MOs
- ✓ Probabilistic meaning of sterile

*There is always a **statistical probability** that a **microorganism** may **survive** the sterilization process*

Sterilization: how does it work?



- ✓ **A constant % of MOs** is destroyed in each constant time interval «t»

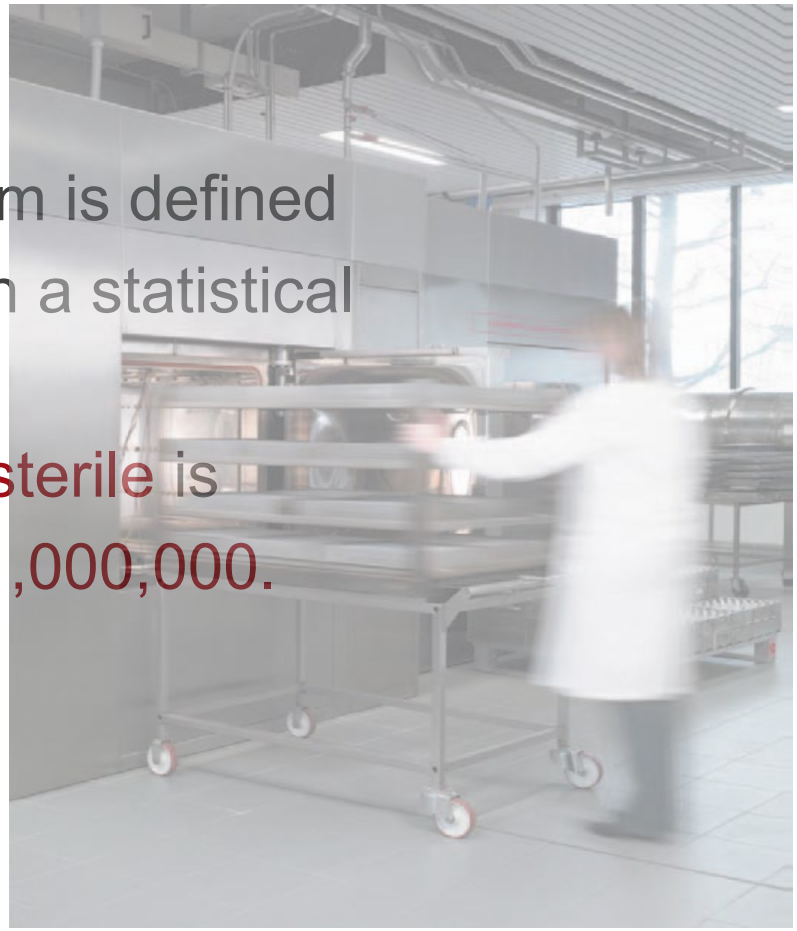
- ✓ A population of 10^{-2} (= 0.01) does **not** have any **physical meaning**, but **it has a probabilistic meaning**: the probability to find a contaminated unit is 1 to 100

Probabilist meaning of sterile

A population number which is lower than 1 does not have any physical/logic meaning, but it assumes a probabilistic meaning:

- ✓ 0.1 microorganism → The probability to find a contaminated unit is 1 to 10
- ✓ 0.001 microorganism → The probability to find a contaminated unit is 1 to 1000

In the healthcare industry, an item is defined sterile if it is possible to certify, on a statistical basis, that the probability that it is not sterile is equal to, or lower than 1 in a 1,000,000.



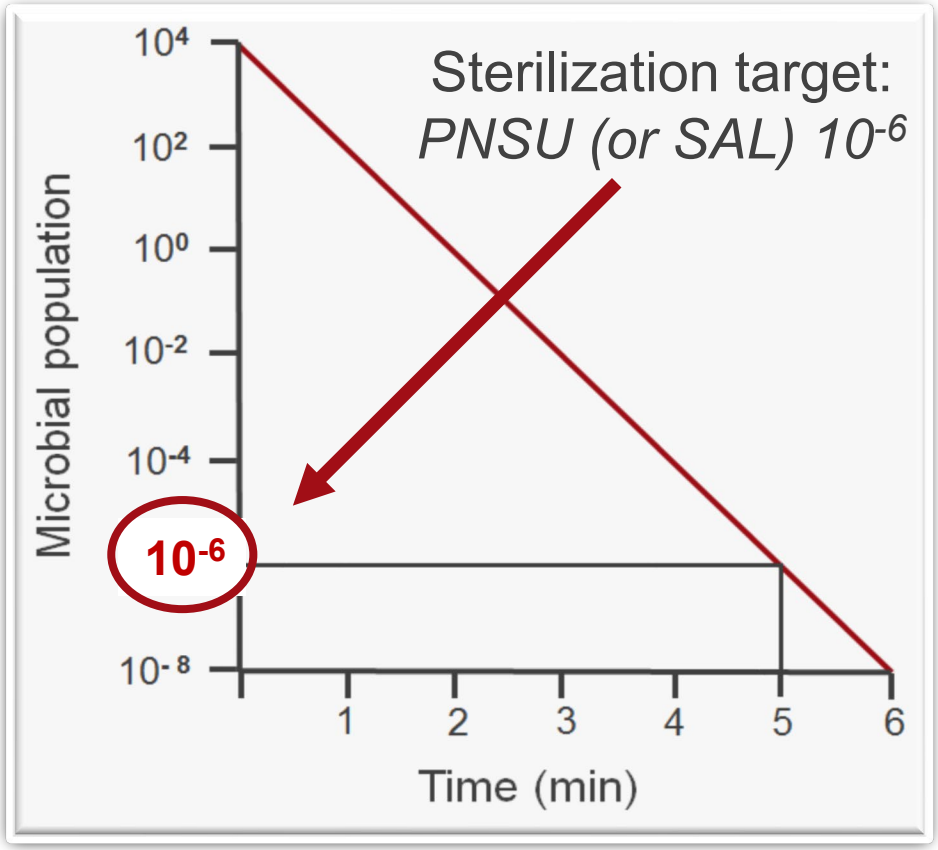
What is the target of a sterilization process?

- Probability of Non-Sterile Unit
  PNSU ≤ 10^{-6}

- Sterility Assurance Level
  SAL ≤ 10^{-6}

The probability to find a contaminated unit is 1 (or lower) in 1,000,000

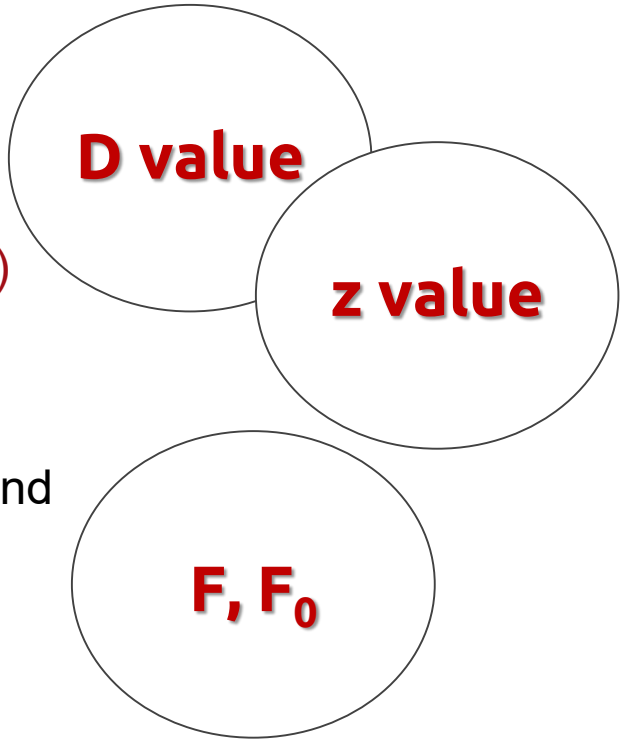
When an item is sterile?



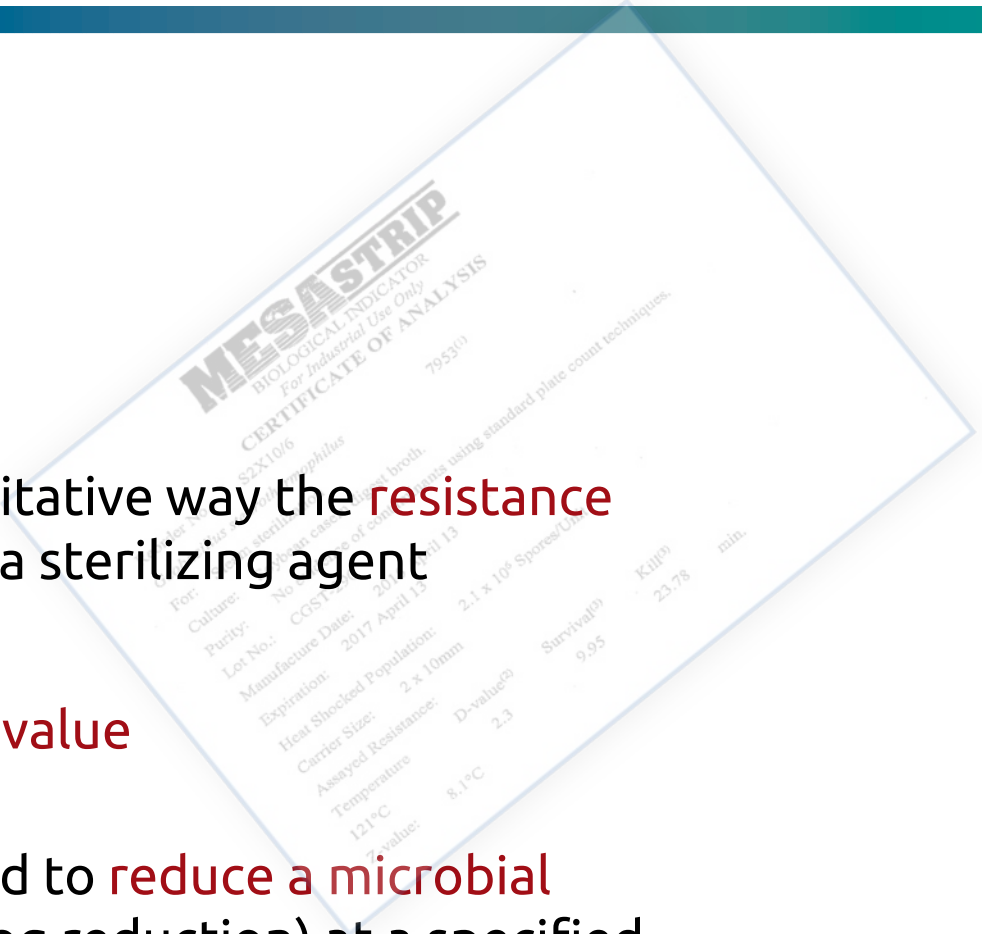
How can we demonstrate that a product has reached a SAL (PNSU) $\leq 10^{-6}$?

You should know…

- the microbial population (bioburden) that contaminates the product
- the sterilization method employed and its efficacy



D-value

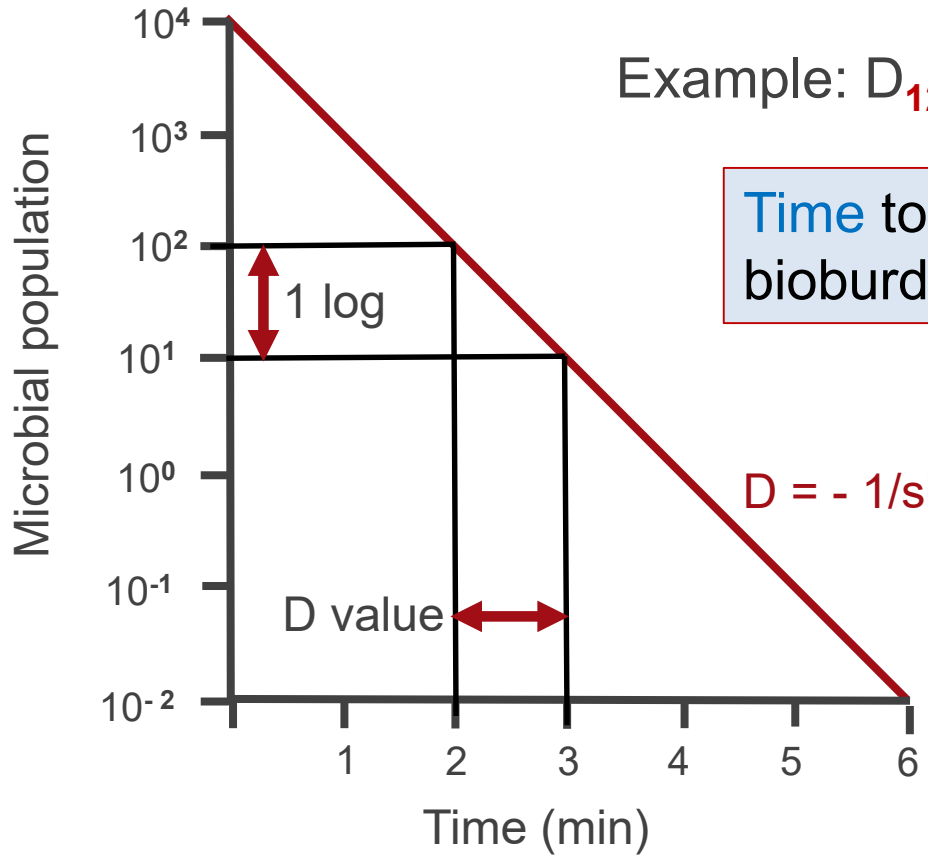


- ✓ It is physically a time
- ✓ It expresses in a quantitative way the resistance of a microbial species to a sterilizing agent
- ✓ PDA calls it resistance value
- ✓ The time (min) required to reduce a microbial population by 90% (= 1 log reduction) at a specified Temperature

D-value

- ✓ It varies according to:
  - the microbial species
  - the specific substrate
  - the process conditions (for steam sterilization: temperature and contact with water vapor)

D-value and microbial resistance

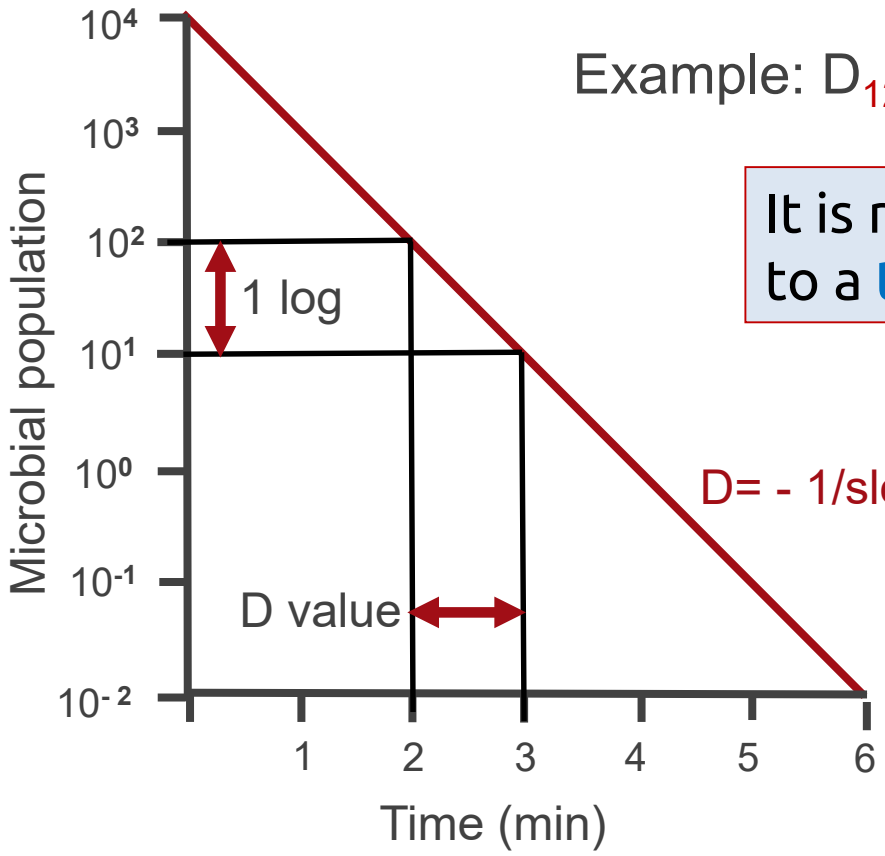


Example: $D_{121^\circ C} = 1'$

Time to reduce the bioburden by 1 log unit

D = - 1/slope

D-value and microbial resistance



Example: $D_{121^\circ C} = 1'$

It is referred always to a **temperature**

D= - 1/slope

D-value and microbial resistance

$$D_T = \frac{t_T}{LogN_0 - LogN}$$

It is based on experimental data and can not be predicted on a theoretical basis.

For each microorganism at a given temperature, the knowledge of its resistance value D_T derives from experimental evaluations of initial microbial population N_0 and the surviving population N after the exposure time t_T at the ideally constant temperature T.

D-value: influence of the substrate

Substrate Description	Organism	D_{121}-value (Moist heat sterilization)
Stopper A	G. stearothermophilus	1.2 minutes
Stopper B	G. stearothermophilus	1.4-minutes
Stopper C	G. stearothermophilus	1.7 minutes
Stopper D	G. stearothermophilus	1.8 minutes
Stopper E	G. stearothermophilus	1.9 minutes
Stopper F	G. stearothermophilus	2.0 minutes
Stopper G	G. stearothermophilus	2.3 minutes
Plunger H	G. stearothermophilus	2.3 minutes
	tearothermophilus	3.6 minutes
	stearothermophilus	6.1 minutes

If the substrate varies, D varies

✓ Initial microbial population (bioburden):
10^3 microorganisms of a microbial species having $D_{(121°C)}$=1 min

9 min @ 121 ° C	12 min @ 121 ° C
⇩	⇩
9 SLR	12 SLR
⇩	⇩
SAL =10^{-6}	SAL =10^{-9}

*SLR= Spore Log Reduction (referred to the microbial population)

Temperature coefficient: z-value

Does resistance value D change with the temperature?

YES!!!

z-value: represents the change of the microbial resistance with the change in temperature; it is the common way to express the dependence of D-value on temperature for a specified microorganism

Temperature coefficient: z-value

✓ It is defined as the number of degrees of sterilization temperature that causes a 10-fold variation of D or of the sterilization rate

✓ It is applicable in a specific range temperature for a specific microorganism!

Useful to compare the efficacy of two cycles operated at different temperatures

z-value: practical meaning

If near 121° C, z= 10° C …the same sterilizing effect can be obtained in …

- @ 121 ° C in 12 min
- @ 131 ° C in 1,2 min
- @ 111 ° C in 120 min

Temperature dramatically influences the sterilization rate (= time)

Temperature coefficient: z-value

The definition of z-coefficient involves that:

$$D_{(T-z)} = D_T * 10$$

Thanks to the definition of z, D value undergoes a ten fold variation if the temperature varies by z°C.
For 1°C, the variation of D is given by the formula

$$\frac{D_T}{D_{T-1}} = \sqrt[z]{10}$$

For instance: if z=5°C, the ratio of D-values at two temperatures which differes by 1°C is given by $\sqrt[5]{10}$ = 1.5849, i.e. D changes by more than 58% every degree centigrade.

Temperature coefficient: z-value

The fact that D varies by 10 times for a variation of 10°C, when z =10°C, does not mean that D varies by 1 time for a variation of 1°C!

It is a matter of finding the number which yields 10 when raised to the tenth power. This number is 1,2598…
D(121°C)= 1 min
D(120°C)= 1,26 min

Temperature coefficient: z-value

- z-coefficient for a specified microorganism within a given temperature range is calculated by the following formula from two experimental known values D_2 and D_1 at two temperatures T_2 and T_1

$$z= (T_2-T_1)/(\text{Log } D_1- \text{Log } D_2)$$

- It is worthy to repeat that a value of z-coefficient is reliable only within the temperature range to which the experimental known D-values used for the calculation of it are referred .

- The use of a z-coefficent outside this temperature range is not sound (extrapolations are always very risky).

Temperature coefficient: z-value

✓When there are no reliable experimental data on the microbial species contaminating the product (ex. heterogeneous contamination)…

✓…near 121° C (reference temperature for steam sterilization) the z-value is assumed to be 10° C and the D-value is assumed to be 1 min

✓This combination offers a good safety margins in determining the PNSU (or SAL) as regards the MO which we commonly dealt with

z-value and D-value: practical use

A sterilization at 115° C,

a known D value at 121° C (1'),

a z = 6° C

which is the D value at 115° C?

Often people use D(121°C) for a sterilization at different temperatures

$$D = D_0 \times 10^{(T0 - T)/z}$$

$$? = 1 \times 10^{(121 - 115)/6}$$

$$? = 10$$

Equivalent time

The equivalent time at the reference temperature will be the time that yields the same reduction of the microbial population as under the actual conditions.
This time is given by the formula:

$$F_{(T^0,z)} = t * 10^{(T - T_0)/z}$$

It calculates, by measuring physical parameters (time and temperature) the amount of heat (lethal dose) delivered to the product.

Equivalent time

How many minutes of sterilization at an ideally constant reference temperature (i.e. 121°C) are equivalent as t *minutes of sterilization at a different and / or variable temperature?*

$$F_0 = \sum \Delta t \times 10^{\left(\frac{T-121}{z}\right)}$$

Δt = suitably short interval of time between next measurements of actual exposure temperature T
T = actual sterilization temperature
z = temperature coefficient (10 ° C for F_0 calculation)

What is the lethality of a sterilization process?

Sterilization at a “x” temperature for a “x” time

Sterilization at standard conditions

$$F_0 \; when \; \mathrm{T} = 121°C \; and \; z = 10$$

F_0(PHYSICAL)

The F_0 aswers to this question:
how long must be the process (performed at a "x" temperature) to obtain the same lethality of a process at the standard temperature (121° C) for "y" minutes?

Sterilization at "x" temperature for "x" time	Sterilization at standard conditions (121° C)	
100° C, 10 h	4.8 min	→ F_0 = 4.8'
130° C, 15 min	116 min	→ F_0 = 116'

Temperature strongly influences the process

$F_{(PHYSICAL)}$

The “physical” equivalent time, FPHY, is that one calculated from the actual data of time and temperature measured during a sterilization process.

F_0 physical is a mathematical expression of the sterilization equivalent time at 121°C and it assumes a biological meaning just in moist-heat conditions.

If these conditions are not fulfilled, F_0 calculation has not a biological meaning.

$F_{(BIOLOGICAL)}$

The exposure time necessary to obtain the aimed reduction of a given microbial population at sterilization temperature T is called 'biological' equivalent time

$$F_{bio} = t_T = D_T * (LogN_0 - LogN)$$

From the definition of D-value:

$$D_T = \frac{t_T}{LogN_0 - LogN}$$

It is well described by European Pharmacopoeia, X edition, 5.1.1 Method of preparation of sterile products

$F_{(PHY)}/F_{(BIO)}$

F ‘physical’ can be compared with F ‘biological’.

A sterilization process achieves its goal when :

$$F_{phy} > F_{bio}$$

F_D

Another parameter has been introduced to “rectify” the actual F_{PHY}-value, in order to comply directly with the actual D-value of a reference microorganism: the ratio F_0 / D_0 has been called F_D.

$$F_D > \text{Log } N_0 - \text{Log } N$$

F (PHYSICAL)

To speak of equivalent sterilization time, it is always necessary that the essential conditions for the effectiveness of sterilization are complied with.

In the case of moist heat sterilization, the exposure to **temperature** is effective only as long as the microorganisms are in contact with liquid **H_2O**.

If this condition is not complied with, equivalent time becomes only a mathematical formula without any biological meaning and to use it is a non-sense.

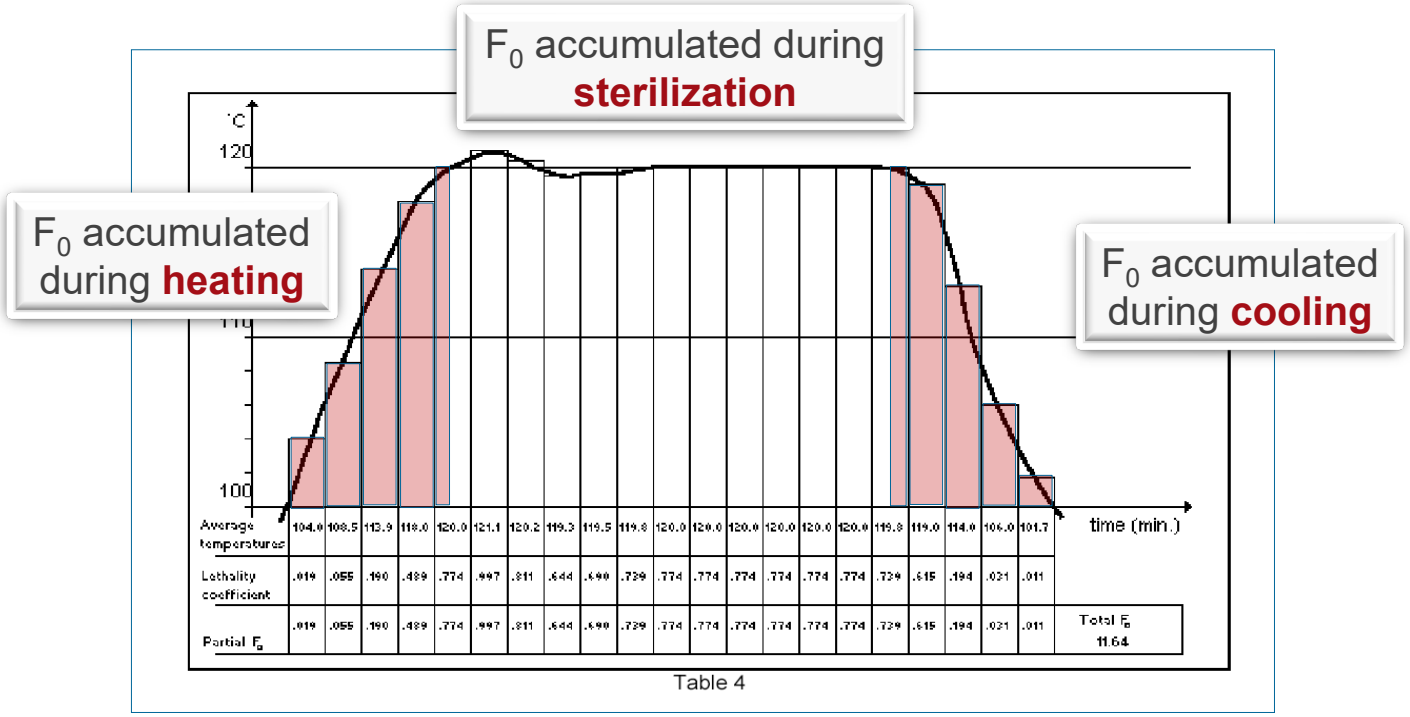
$F_{(PHYSICAL)}$

If *water* containing products are sterilized, e.g. ***saline solutions*** and most of the food specialities in sealed containers, *no doubt that liquid H_2O is present in contact with microorganisms*, because it is already contained in the bulk of the items to be sterilized.

The calculation of equivalent time may usefully include heating and cooling phases, even if temperatures lower than 100° C supply no appreciable lethality to load.

Aqueous solutions

The calculation of the equivalent time may usefully include heating and cooling phases, even if temperatures lower than 110° C supply no appreciable lethality to load.



Average temperatures	104.0	108.5	113.9	118.0	120.0	121.1	120.2	119.3	119.5	119.8	120.0	120.0	120.0	120.0	120.0	120.0	119.8	119.0	114.0	106.0	101.7
Lethality coefficient	.019	.055	.190	.489	.774	.997	.811	.644	.690	.729	.774	.774	.774	.774	.774	.774	.774	.729	.615	.194	.021	.011
Partial F_0	.019	.055	.190	.489	.774	.997	.811	.644	.690	.729	.774	.774	.774	.774	.774	.774	.774	.729	.615	.194	.021	.011

Total F_0 11.64

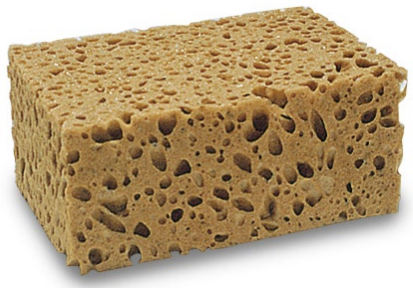
Table 4

Porous/hard goods

On the contrary, the sterilization of so called porous/hard goods is a surface sterilization process and the effective contact of the microorganisms with liquid H_2O (in this case condensing steam) may be obtained only after a complete removal of the air initially surrounding the load and by the presence of saturated steam in contact with the external surfaces, and internal if any, of the load.

Air removal for easy, fast penetration of

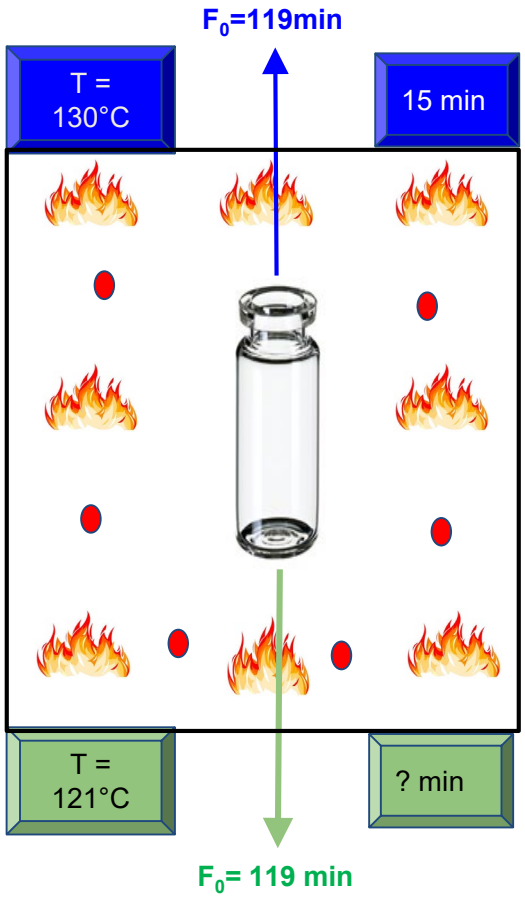
condensing steam!



Porous/hard goods

As the effectiveness of the sterilization of porous/hard goods does not depend only on temperature, it makes no sense to start the calculation of the equivalent time for such goods before all the sterilization conditions are fully attained, i.e., in advance of the so called plateau period, **when the no residual air is supposed to be any longer present in the chamber and around the load**. The equivalent time itself may be regarded in this case only as an additional instrument of control.

Practical example



T= 130°C
z= 10°C
t= 15'
F_0= ?

$$F_0 = 15 * 10^{\frac{130-121}{10}} = 119'$$

HOW LONG MUST BE A PROCESS AT A STANDARD TEMPERATURE TO OBTAIN THE SAME LETHALITY (F_0=119 MIN)?

Sterilization at standard conditions:
T= 121°C
z= 10°C
t= ? min
F0= 119'

t=119' ⟶ 119' to reach the same lethal effect.

Conclusions

- ✓ Sterilized item: item that has a specific probability to be free of any viable MO (PNSU $\leq 10^{-6}$)
- ✓ The temperature strongly influences the sterilization process rate (= time)
- ✓ D-value and z-value are related to the MO resistance to the process, and therefore they have an impact on the sterilization rate (= time)
- ✓ F_0 is a measure of the lethality of a moist heat sterilization process

Thank you

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