

← الشايفي بحكي عن فكرة وحدة :

انه قد ما حاولت أخلي المرليب

Streile

ما بهل نسبة ١٥٥٪

← بعد ما يدنا نحب 2 Values



اللهم إني استودعك كل ما قرأته وكل ما  
حفظته وتعلمته، فأسألك أن ترده إليّ عند  
الحاجة له، فأنت القادر على كل شيء.



دعواتكم ♥

# The design of sterilization processes

Chapter 18

لجان الدفاع

\* سول أكاديمي

# Survivor plots and sterility assurance levels

- ▶ It is neither possible to guarantee, nor to prove, that an article is sterile; it is possible to quote a probability that is sterile
- ▶ This probability is referred to in pharmacopoeias as the sterility assurance level and is usually quoted as  $10^{-6}$
- ▶  $10^{-6}$ : one surviving organism in one million items

زمان

المادة التي يترك  
اصغق

لما بقدر امكن انه كل المواد التي صنعها هي 1.000 ← sterile ← ومحبب التأكد من كل article انا صنعتي

لكن في

بكموها بالكتب و

بالفارماكوبيا

يعني كل مليون ابرة

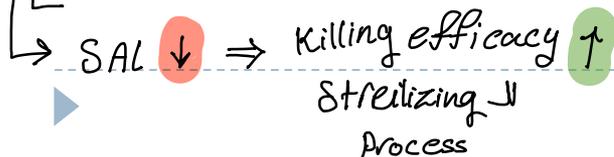
ممكن عندي ابرة واحدة مش sterile

$\frac{1}{1000000}$  → one surviving in one million items

# Sterility assurance level (SAL)

- ▶ **Sterility assurance level (SAL)** is the probability of a single unit being non-sterile after it has been subjected to sterilization.
- ▶ In microbiology it is impossible to prove that all organisms have been destroyed as the likelihood of survival of an individual microorganism is never zero, so **SAL** is used to express the probability of the survival. For example, medical device manufacturers design their sterilization processes for an extremely low SAL, such as  $10^{-6}$ , which is a 1 in 1,000,000 chance of a non-sterile unit.
- ▶ SAL also describes the killing efficacy of a sterilization process. A very effective sterilization process has a very low SAL.

المستلزمات  
الطبية



# Sterility assurance level and survivor plots

من طرف الـ Sterilization

- ▶ When exposed to heat or radiation bacteria normally die according to first-order kinetics ← متذكرون مادة first بالكائنات؟
- ▶ This means that the same proportion of the cells is killed in successive time intervals ← كل 1 min يقتل 90% من 100 الباقين من الدقيقة الأولى ← بعد 2 min يكون قتل 90% من 100 الدوى وهكذا الى ص
- ▶ The data represent the death of bacterial spores in 1 ml ampoules of injection being sterilized by steam in an autoclave (also called a 'steam sterilizer').



Simplified data illustrating bacterial death during steam sterilization.

Time of heat exposure	<sup>في</sup> Viable spore concentration CFU/ml <i>Colony forming Unit</i>	Log <sub>10</sub> viable spore concentration
0	10 000 <i>log 10000 = 4</i>	4
1	1000 <i>log 1000 = 3</i>	3
2	100 <i>log 100 = 2</i>	2
3	10 <i>log 10 = 1</i>	1
4	1 <i>log 1 = 0</i>	0

*المعروف بال 1st order*

*10٪ هوائي قتل عايش*

*Survival Plot*

5 min exposure  $\rightarrow \log 0.1 = -1 \rightarrow 0.1 \text{ CFU/ml} \rightarrow$  one whole spore in 10 ml  $\rightarrow$  9 amp sterile and one non sterile

# Sterility assurance level and survivor plots

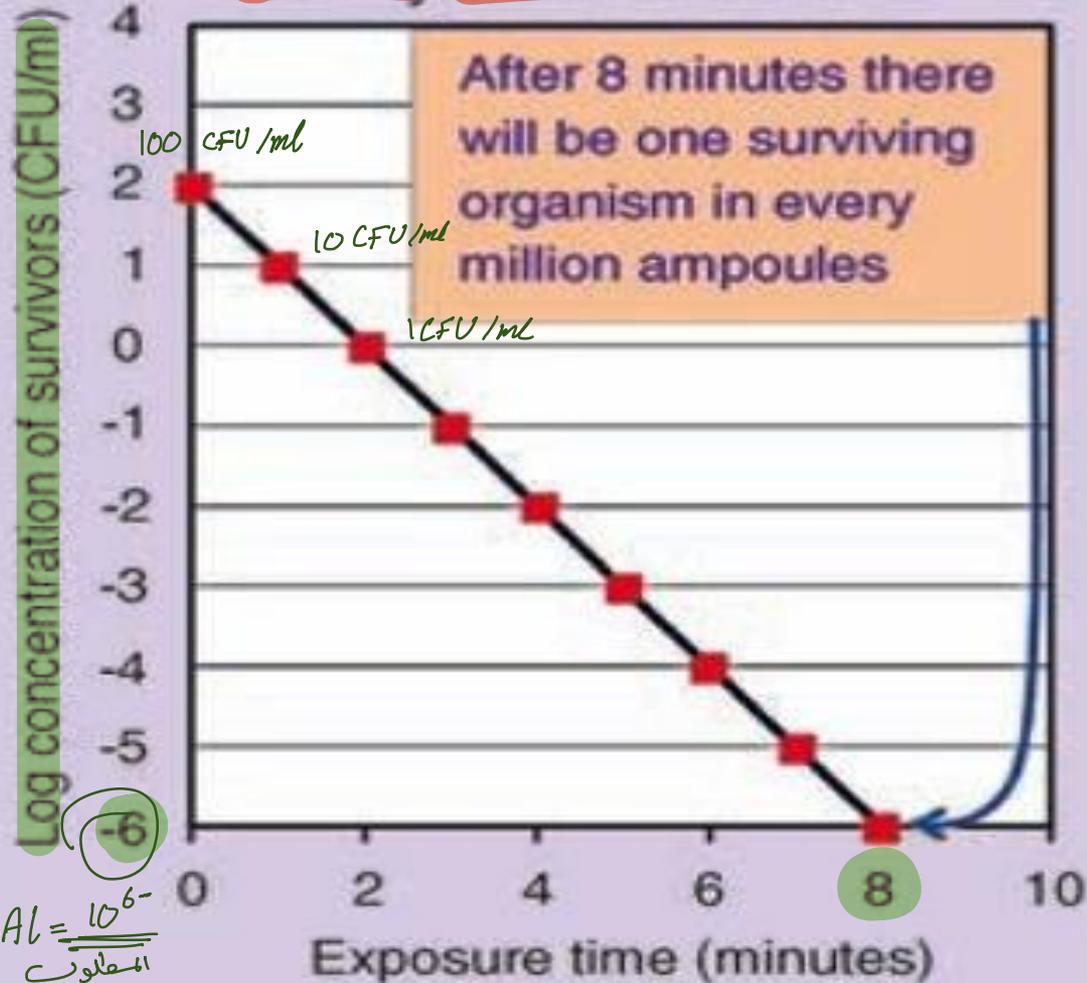
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- ▶ The next line in the data table would be 6 minutes' exposure → corresponding to one survivor in 100 ml, <sup>0.01</sup>
- ▶ then 7 minutes giving one survivor in 1000 ml, and so on.
- ▶ It is clear from this example that the data table could be extended indefinitely and zero survivors (sterility) would only arise after an infinite exposure time, so no matter how long the ampoules of injection are heated there is always a small but finite probability that there will be a surviving spore.
- ▶ This is the reason why it is not possible to guarantee sterility of an item selected at random from a sterilized batch of product.

فكرة  
المستطير



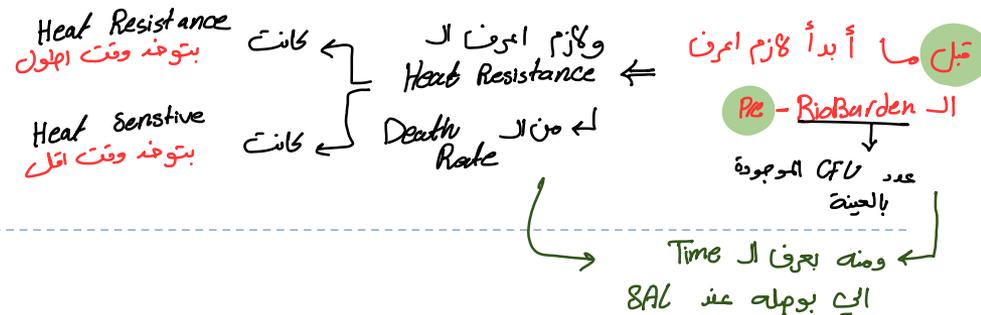
Survivor plot showing time to achieve required sterility assurance level



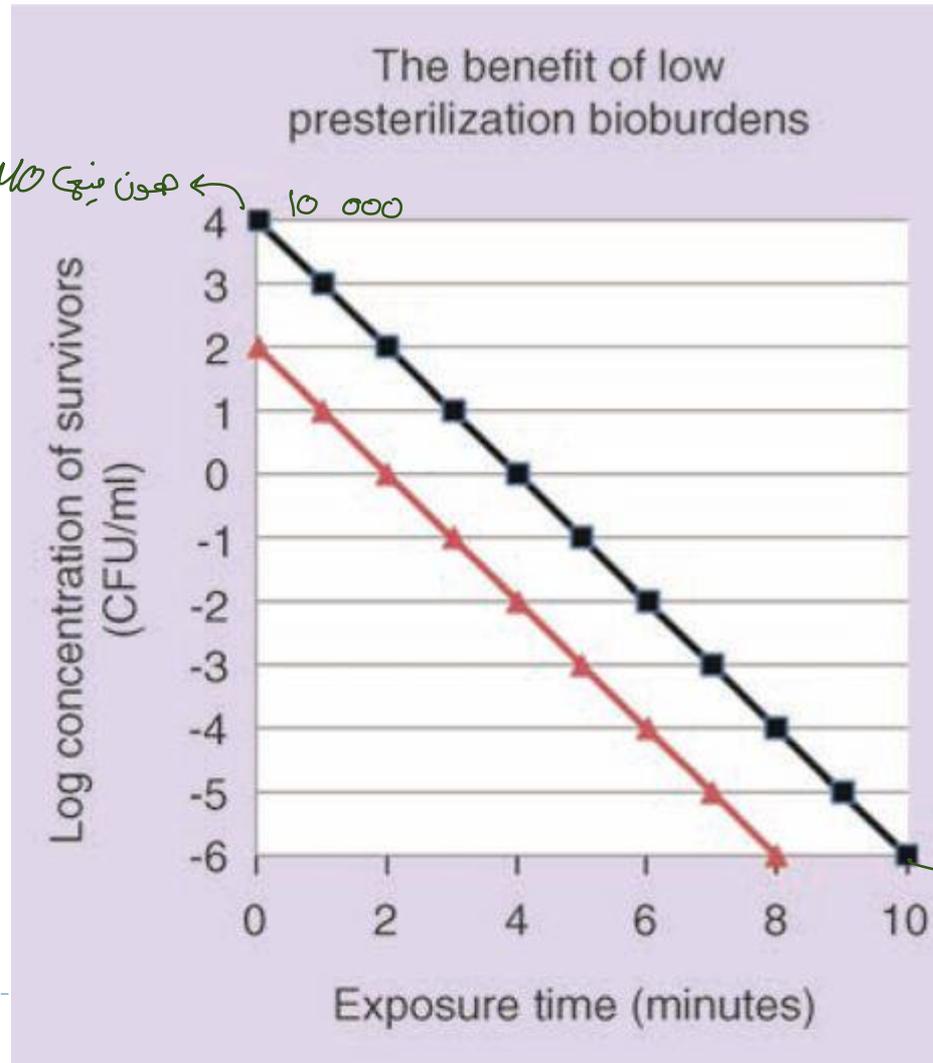
← وملتاج بعد 8 min

# Sterility assurance level and survivor plots

- ▶ Instead, a sterility assurance level (SAL) of  $10^{-6}$ , or better, is the target, and the exposure period in the autoclave is adjusted to achieve this because it can be calculated from knowledge of the pre-sterilization bioburden and the degree of heat resistance of the spores (from their death rate – in other words, the slope of the survivor plot).



- ▶ So, in the example above, the required SAL (a log concentration of survivors of  $-6$ ) would be achieved following exposure for 10 minutes.



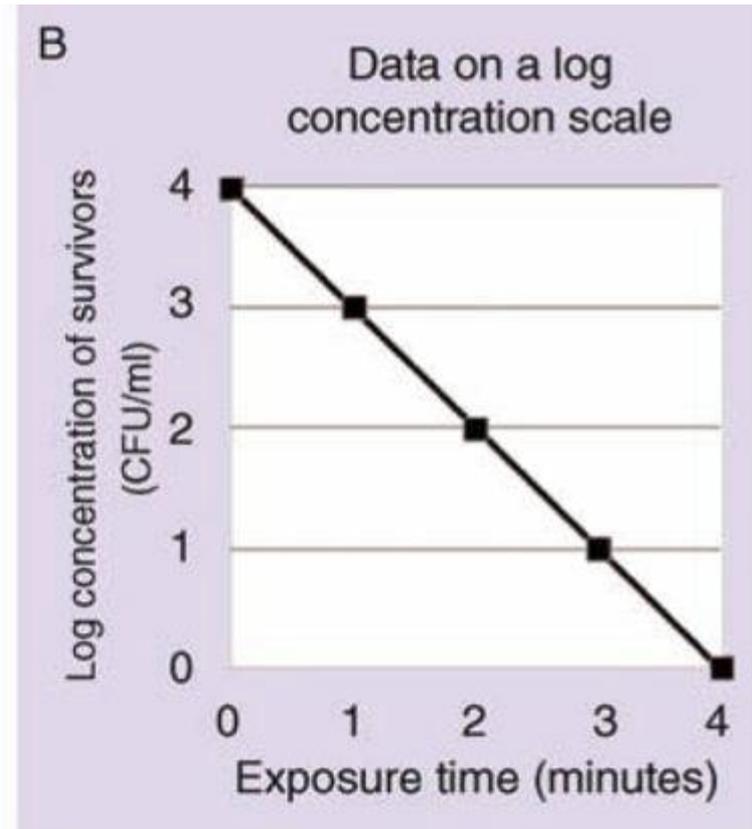
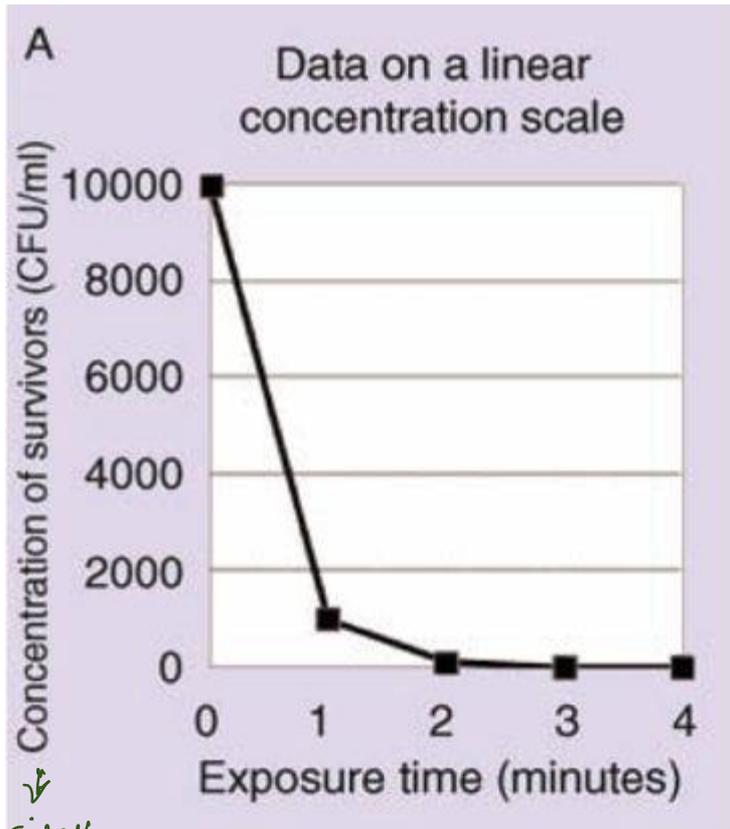
Pre - Bio Burden ← (كثري) ← بدون ميوحي MO ← 10 000

# استنتاج :-  
كل ما كانت Bio Burden  
اكبر يحتاج وقت  
الطول حتى اوصل

الوقت يحتاج Time  
الطول

▶ SAL

# Sterility assurance level and survivor plots



log

بزون

**A survivor plot or kill curve  
(semi log curve)**



نحو استفيد لما أبدأ على low Bio Burden

▶ Good manufacturing hygiene that results in lower levels of microbial contamination prior to sterilization therefore affords several benefits:

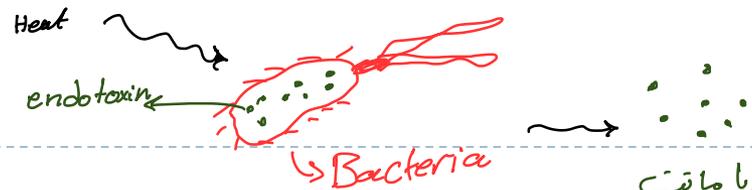
✓ shorter autoclaving times that reduce energy costs

زى امثال الي فوق لما بلبس بعد اقل استجبت و مت اوفر على اجزى ال Process  
بالنهي لطاقت اقل

✓ shorter heat exposure which is likely to reduce degradation of the active ingredient;

طبيع بالسخية المرابي  
بتأثر بالحرارة  
وما بدنا اياه يخرب

✓ fewer dead bacterial cells in the product after sterilization, which would reduce the risk of the injection failing the bacterial endotoxins test



البكتيريا ماتت

لكن ال endotoxin بعدها موجودة ← لو كان تركيزها عالي بتخرب المركب و بتصل مشاكل

# D-values

- ▶ The <sup>Time</sup> rate at which MOs are killed in a sterilization process is expressed as the D-value (decimal reduction time)
- ▶ D-value: is the time required to reduce the population to 10% of its initial value (time required to kill 90% of the population)
- ▶ D-values are often quoted for bacterial spores exposed to steam-sterilizing temperatures, and in this situation a subscript is added to the D-value to indicate the temperature at which it was measured.  
*كل ما تضررت عندي بوسني D-value جديده*  
*يستخدمها للـ Autoclave*

# D -value

- ▶ The D value is a measure of the heat resistance of a microorganism.
- ▶ It is the **time in minutes** at a **given temperature** required to **destroy 1 log cycle (90%)** of the target microorganism
- ▶ For example, a D value at 72°C of **1 minute** means that for each minute of processing at 72°C the bacteria population of the target microorganism will be reduced by 90%.

شرح بالعربي :-

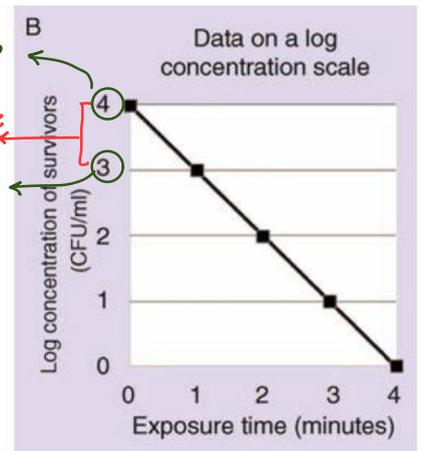
جوال Autoclave ← درجة الحرارة 72 ،

كل دقيقة يموت عدد من البكتيريا بنسبة 90% ← هائي النسبة

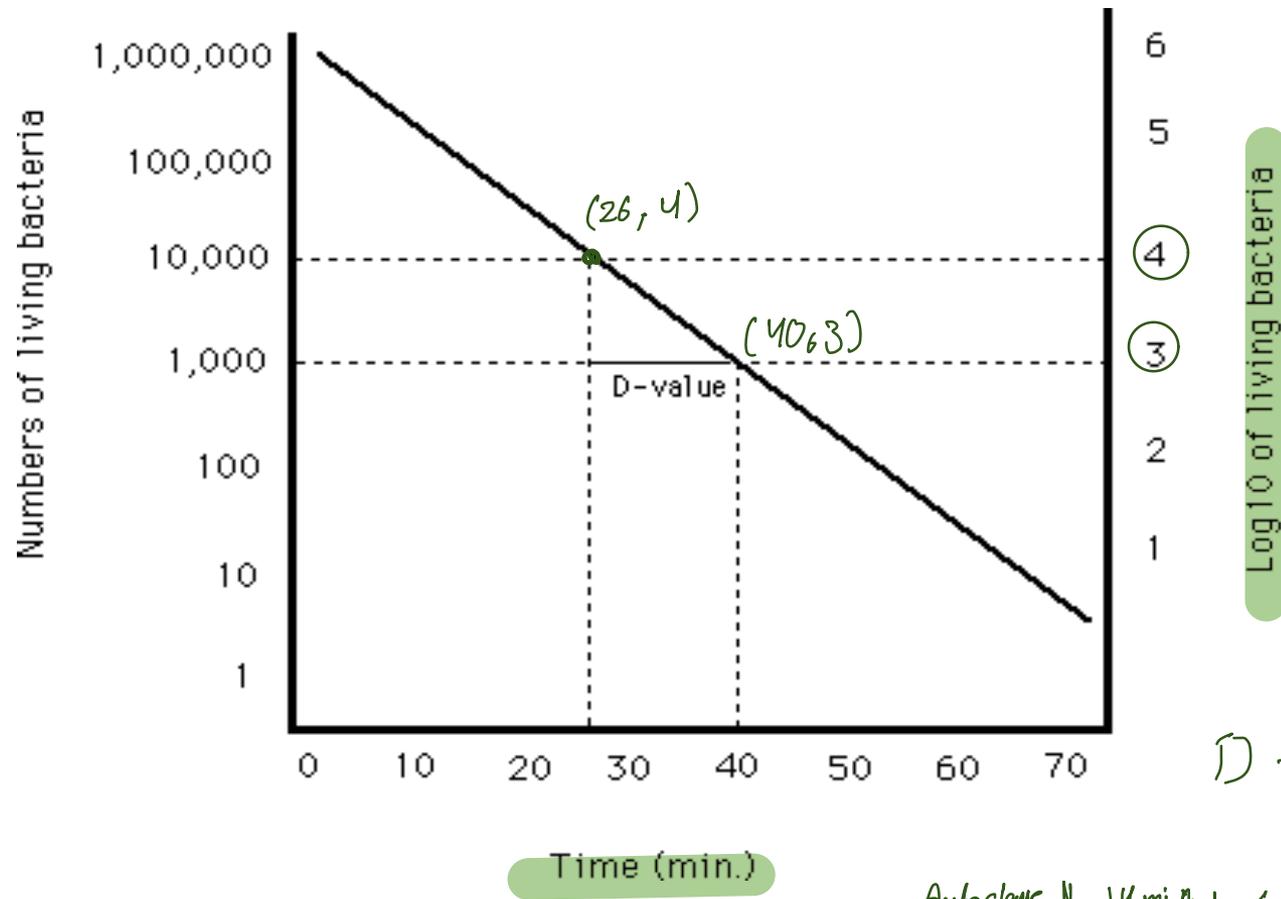
← لو قللت الحرارة عن 72 ، راح اصحاح وقت 1 log cycle =

▶ الأول من 1min ، لأنه D-value مرتبطة بال Temp

10000 كانت  
 10% عاشت  
 90% ماتت  
 1 log cycle  
 1000 كانت



A survivor plot or kill curve (semi log curve)



$$D\text{-value} = \frac{40 - 26}{4 - 3} = 14 \text{ min}$$

← يعني احرقت البكتيريا 14 min في Autoclave  
عشان اقل 90% منها

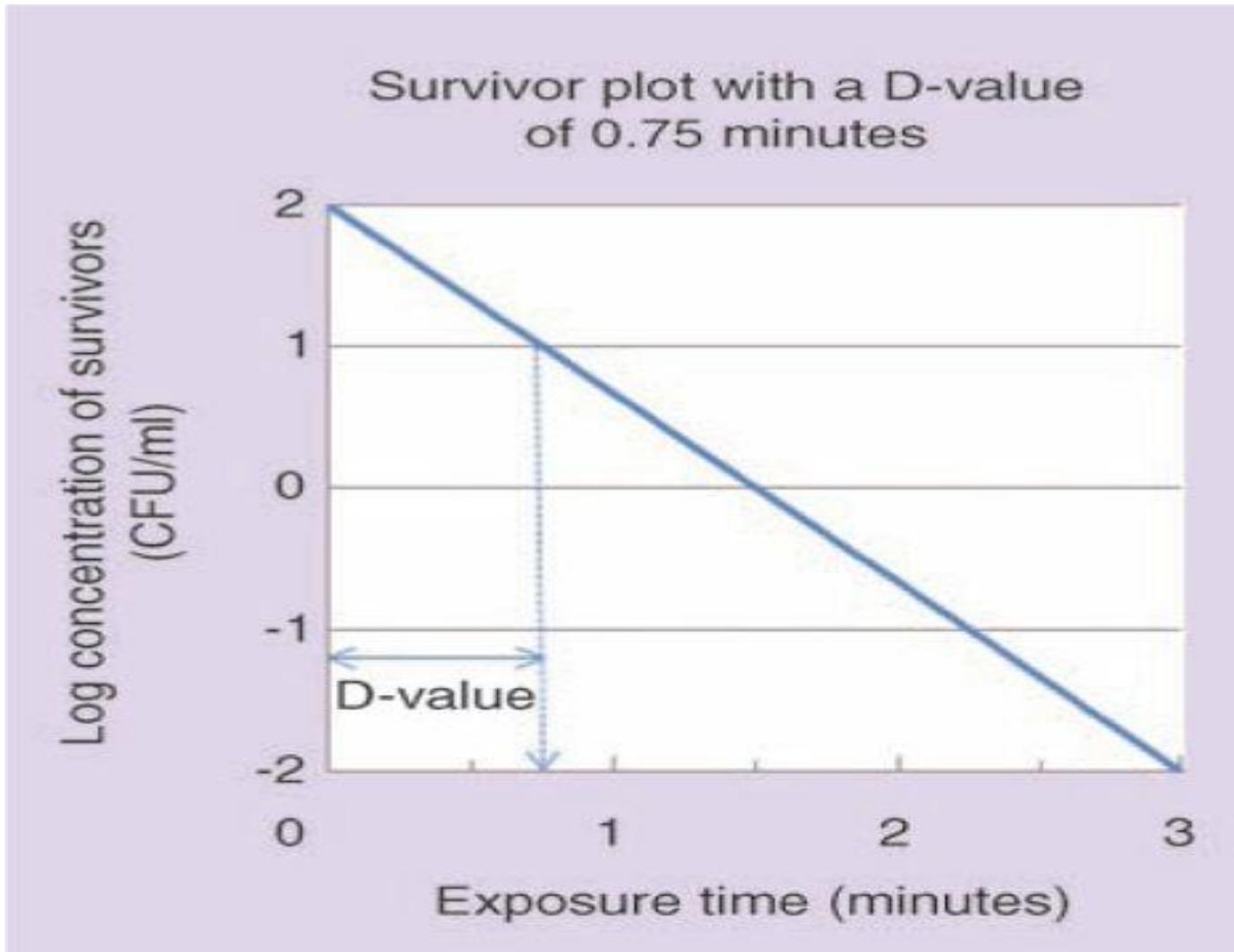
# D-value

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- ▶  $D_{121}$  values are the most commonly encountered because 121 °C is the most frequently used autoclaving temperature;
  - ▶ Spores of *Geobacillus stearothermophilus* (regarded as the most heat-resistant species) typically exhibit  $D_{121}$  values as high as 2.0 minutes or more.
- 



Survivor plot with a D-value of 0.75 minutes.



# Z-values

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- ▶ **Steam sterilization** (heating in an autoclave) is the most commonly **used terminal sterilization method**, and the **temperature-time combination of 15 minutes at 121 °C**, which is suggested in the pharmacopoeias, is the heat treatment commonly used  
تعليمات من ار ←
  - ▶ A pharmaceutical manufacturer is not obliged to use this standard autoclave cycle; it is permissible to use any other temperature-time combination, provided that it achieves the required SAL.  
ممن بالضرورة وبقدر اقل الـ Autoclave على درجة الحرارة الي بيدي ايها
- 



# Z-Value

- ▶ It is logical to expect that the rate at which contaminating organisms are killed would increase as the steam temperature increased, so it would be useful, when designing a sterilization process, to have a parameter which made it possible to calculate by how much the rate was changed for a given change in temperature
- ▶ The Z-value is a parameter which indicates how killing rate is influenced by temperature
- ▶ Z-value: is the number of degrees Celsius temperature change required to achieve a tenfold change in D-value

D Value → Time  $t_{90}$

90% Killing  
← 10%  $t_{90}$  ←

# Z-value:

for *G. stearothermophilus* spores.

Steam temperature (°C)	D-value (minutes)
99	120
110	12
121	1.2

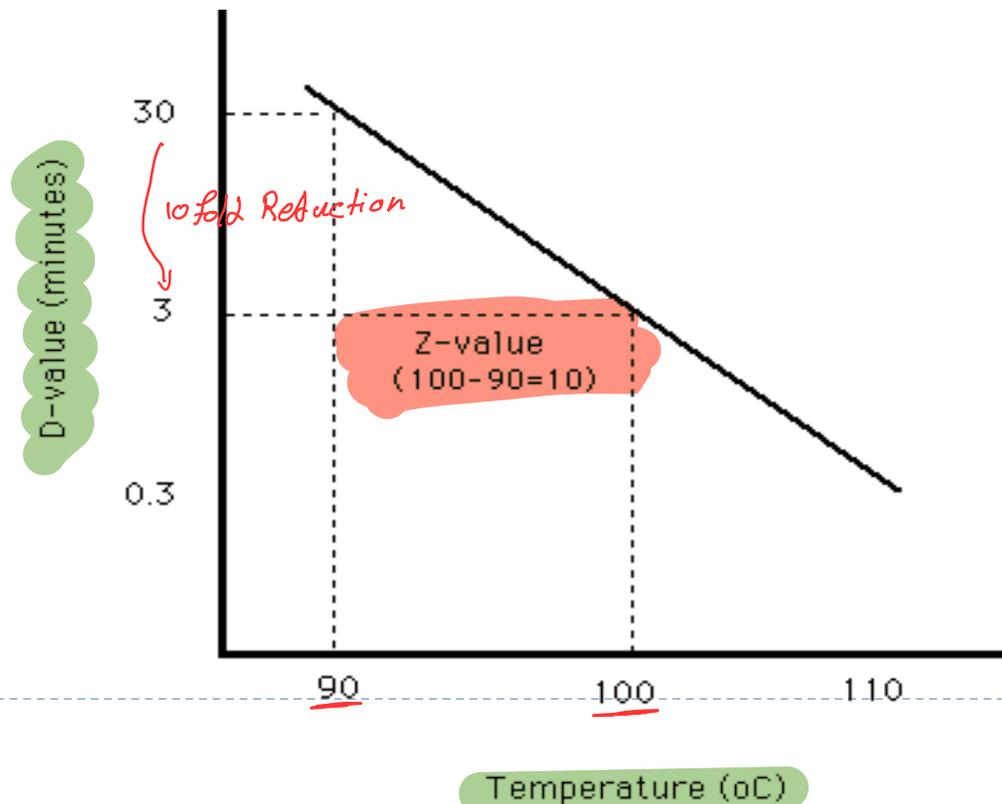
Handwritten annotations in red:

- Arrows pointing from 99 to 110 and 110 to 121 are labeled "الفروق 11°C" (11°C differences).
- An arrow from 120 to 12 is labeled "10 fold Reduction".
- An arrow from 12 to 1.2 is labeled "10 fold Reduction".
- An arrow from 120 to the right is labeled "يعني قدرت اقلك 90% من البكتيريا ب 120 min" (means you can kill 90% of bacteria in 120 min).

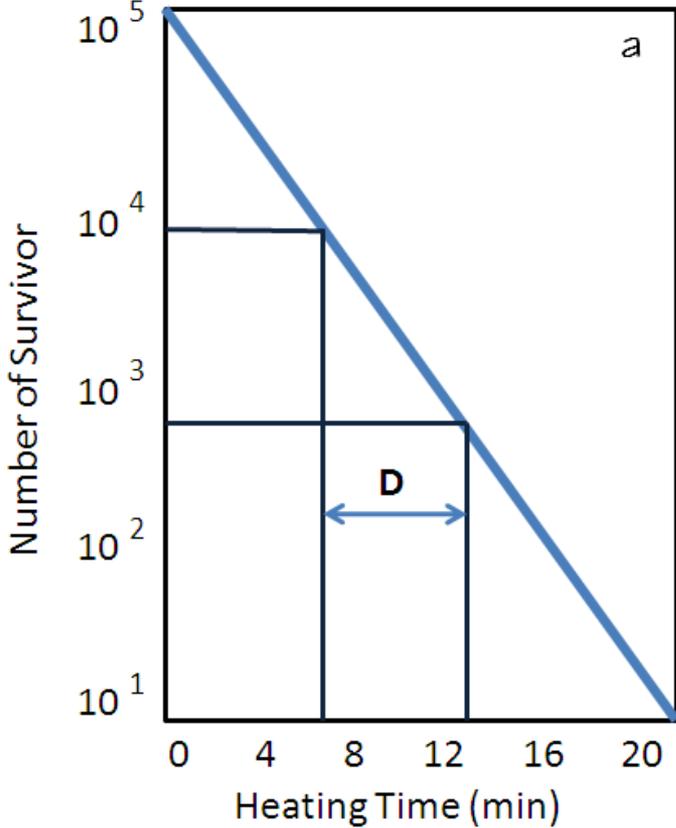
- ▶ In this example the Z-value is seen to be 11 °C, because the time required to kill 90% of the spores (D-value) is reduced tenfold as the steam temperature is increased from 99 to 110 °C, and again by a further tenfold from 110 to 121 °C



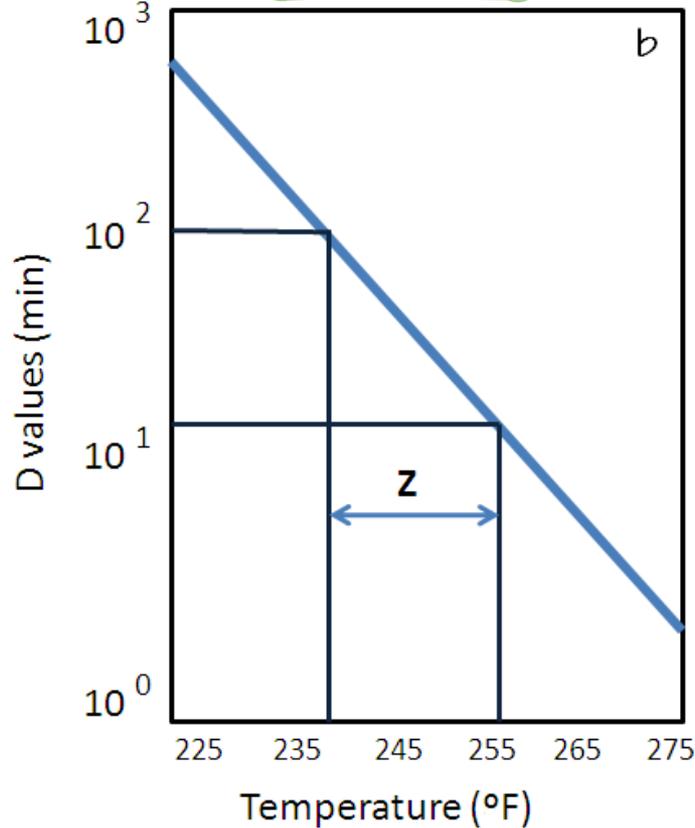
- ▶ The Z value reflects the temperature dependence of the reaction. It is defined as the temperature change required to change the D value by a factor of 10. In the illustration below the Z value is  $10^{\circ}\text{C}$ . *log survival - time*



D-value



Z-value



# Z-value:

- ▶ If both the Z-value and the D-value at one temperature are known, it would be possible to calculate the D-value at any other desired temperature from the equation below

- ▶ 
$$Z = \frac{T_2 - T_1}{\log D_1 - \log D_2}$$

$$10.5 = \frac{121 - 115}{\log 9 - \log x}$$

$$\Rightarrow 0.57 = 0.95 - \log x \Rightarrow 0.384 = \log x = 2.4 \text{ min}$$

- ▶ if a population of spores with a Z-value of 10.5 °C had a D-value of 9.0 minutes (D<sub>1</sub> in the equation) at 115 °C (T<sub>1</sub> in the equation) and it was necessary to know its D-value at 121 °C (T<sub>2</sub> in the equation) → pg

179

D<sub>2</sub> ??

## A working example of how to use D and Z values in pasteurization calculations:

- ▶ Pooled raw milk at the processing plant has bacterial population of  $4 \times 10^5$  /mL. It is to be processed at  $79^\circ\text{C}$  for 21 seconds. The average D value at  $65^\circ\text{C}$  for the mixed population is 7 min. The Z value is  $7^\circ\text{C}$ . How many organisms will be left after pasteurization? What time would be required at  $65^\circ\text{C}$  to accomplish the same degree of lethality?

الحل تحت من  
الاستاذ الفاضل  
Chat GPT

- ▶ At 79°C, the D value has been reduced by two log cycles from that at 65°C since the Z value is 7°C. Hence it is now 0.07 min. The milk is processed for  $21/60=0.35$  min, so that would accomplish 5 log cycle reductions to 4 organisms/mL. At 65°C, you would need 35 minutes to accomplish a 5 D reduction.

Let's solve this step by step. We're given:

- Initial bacterial population =  $4 \times 10^5$  organisms/mL
- Processing temperature = 79°C
- Holding time = 21 seconds
- D-value at 65°C = 7 minutes = 420 seconds
- z-value = 7°C

المعطيات

#### Step 1: Calculate the D-value at 79°C

Using the z-value formula:

$$\log \left( \frac{D_{T1}}{D_{T2}} \right) = \frac{T2 - T1}{z}$$

Let:

- $T1 = 65^\circ C,$
- $D_{T1} = 420 \text{ sec},$
- $T2 = 79^\circ C,$
- $z = 7^\circ C$

$$\log \left( \frac{420}{D_{79}} \right) = \frac{79 - 65}{7} = 2$$

$$\frac{420}{D_{79}} = 10^2 = 100 \Rightarrow D_{79} = \frac{420}{100} = 4.2 \text{ seconds}$$

## Step 2: Calculate the number of log reductions

Now use:

$$\text{log reduction} = \frac{\text{time at } 79\text{C}}{D_{79}} = \frac{21}{4.2} = 5 \text{ log reductions}$$

## Step 3: Determine remaining population

Initial count:  $4 \times 10^5$

$$\text{Final count} = \frac{4 \times 10^5}{10^5} = 4 \text{ organisms/mL}$$

## Step 4: Find time at 65°C for same lethality

We need 5 log reductions at 65°C. Each D-value at 65°C = 420 sec.

$$\text{Time} = 5 \times 420 = 2100 \text{ seconds} = 35 \text{ minutes}$$

## Final Answers:

- **Remaining organisms:** 4 organisms/mL
- **Equivalent time at 65°C:** 35 minutes

