MIRACLE Academy

التعقيم والتصنيع المعقم

لجان الدُّفعات

اللهم وفقني في دراستي، ونوّر بالكتاب بصري، واشرح به صدري، واستعمل به بدني، واطلق به لساني، وقوِّ به عزمي بحولك وقوتك، فإنّه لا حول ولا قوة إلّا بك يا أرحم الراحمين.

وفقكم الله...

The design of sterilization processes

Chapter 18

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

من الصعب جدا انه نقدر نحكي انه كل امبولة طالعة من المصنع هي 100% sterile ،عشان نقدر نحكي هيك ونعمل ال guarantee لازم كل امبولة ندرس ال sterility الها وهذا غير وارد لأن رح نخرب كل الbatch لكن اللي عنا بنقدر نعمله هو دراسة ال probability يعني احتمالية بتحكي عن ال sterility

Sterility assurance level (SAL) :
means that 1 MO surviving organism in one million item (1/1000000)
يعني اكم احتمال تكون ابرة وحدة مش sterile من بين مليون ابرة

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.**
  - يعني ما هي احتمالية يكون عنا single unit تكون non sterile في حالة تم تعريضها لل sterilizartion
- **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(such as syringe) 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.**
  - مستحيل نقدر نحكي انه ال sterilization method ادت لقتل كل ال MO الموجودة بالتالي there is zero MO صعب نحكي
- **SAL also describes the killing efficacy of a sterilization process. A very effective sterilization process has a very low SAL.**
  - بنحصل ال low SAL لما تكون ال Sterility method المستخدمة فعالة و قوية جدا

Give probability of sterility in batch

Describe the killing efficacy of sterilization process

Sterility assurance level and survivor plots

- When exposed to heat or radiation bacteria normally die according to first-order kinetics
- This means that the same proportion of the cells is killed in successive time intervals
- 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').

Slide note

The initial level of contamination in the liquid was 104 colony-forming units (CFU)/ml (which would be unrealistically high; presterilization bioburdens should normally be lower than this, but 104/ml is suitable for the purposes of this illustration). It can be seen from the middle column that in each successive one minute interval the spore concentration was reduced to 10% of the value at the start of that period.

First kinetics order : means that same proportion of cell is killed in successive time interval

البكتيريا لما تتعرض لحرارة او radiation رح يصير killing ل proportion معينة نفسها بكل time interval معينة يعني كل 1 min بصير killing ل 90% من ال MO و بعد دقيقة ثانية كمان 90%

Simplified data illustrating bacterial death during steam sterilization.

Time of heat exposure	Viable spore concentration CFU/ml	Log_{10} viable spore concentration
0	10 000	4
1	1000	3
2	100	2
3	10	1
4	1	0

5 min exposure→ log 0.1= -1→ 0.1 CFU/ml→ one whole spore in 10 ml→ 9 amp sterile and one non sterile

Slide note:

Logically, the next line of data in Table 18.1 would be 5 minutes' exposure corresponding to 0.1 CFU/ml and a log value of –1. One-tenth of a viable colony forming unit sounds like a contradiction in terms because one-tenth of a bacterial spore could not be viable, but 0.1 CFU/ml corresponds to one whole spore in 10 ml of liquid. Given that this example describes sterilization of 1 ml ampoules of an injection, one surviving spore in 10 ml corresponds to nine of the ampoules being sterile and the tenth being nonsterile because it contains the survivor.

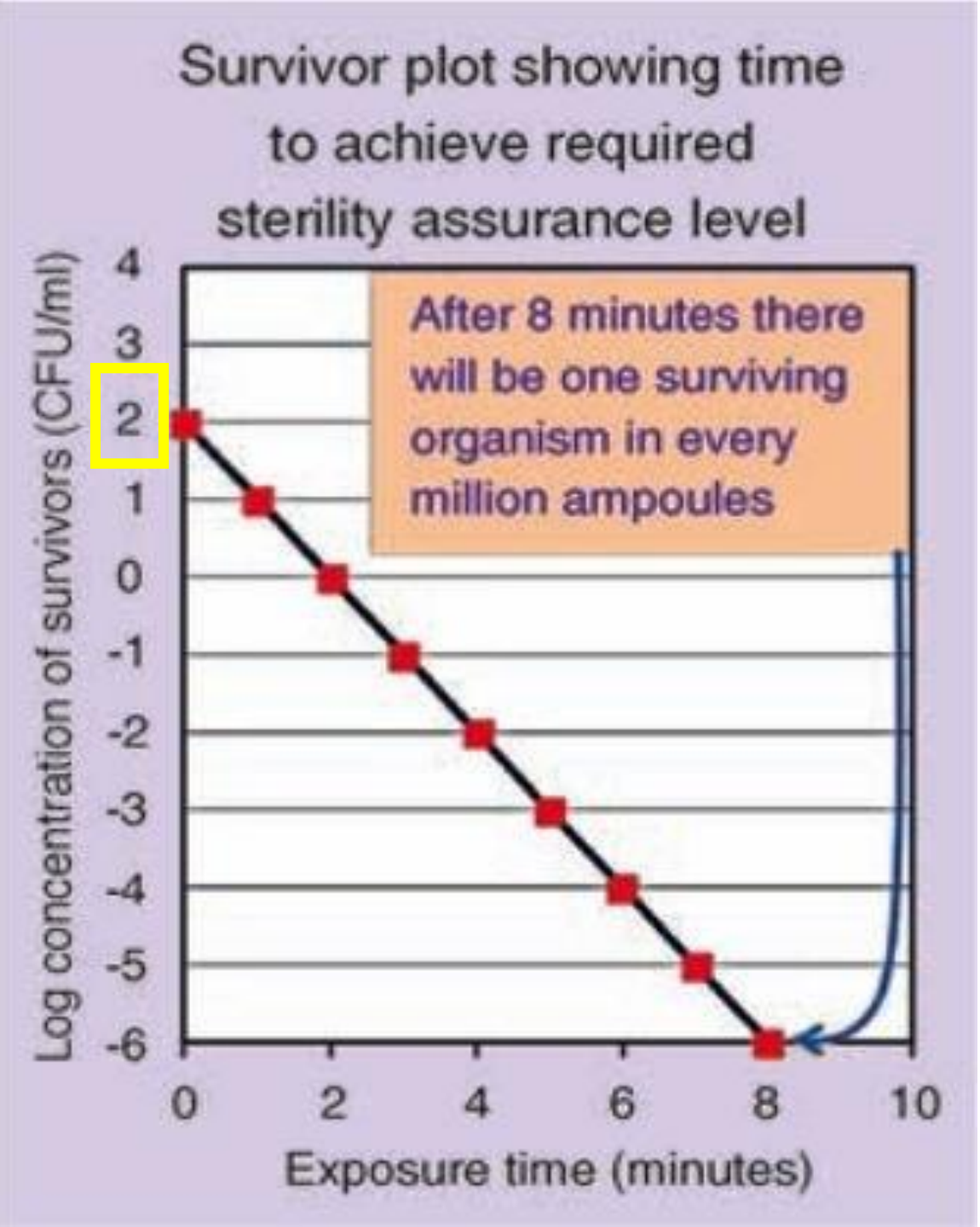
Time of heat exposure	Viable spore concentration CFU/ml	Log_{10} viable spore concentration
0	10 000	4
1	1000	3
2	100	2
3	10	1
4	1	0

هي 10% اللي ضلت من ال 10 الاف

عند رسم ال survival plot بتكون بين ال time & log viable cell عشان يعطي خط مستقيم

هذا الجدول يوضح ال Time اللي تعرضتله البكتيريا لل Steam sterilizer و ثم بعد دقيقة بنشوف اكم عايش و بعد دقيقتين و 3 و 4 وهكذا...حيث كل دقيقة يتم قتل 90% من البمتيريا و يتبقى 10%

هسا عند الدقيقة الخامسة ما في 0.1 خلية، لكن بدنا نوخد فرضا عنا 10 امبولات فهدول رح يعملوا 1 cell يعني 1 whole spore in 10 ml يعني 10 امبولات non sterile مقابل 10 امبولات sterile



survivor plot ? فنتذكر سوا شو ال
هو علاقة بين exposure time and
log survival

هسا هي الرسمة تمثل امبولات تعرضت
ل 8 دقائق ، حيث بنضل نشوف الوقت
اللازم لنحصل 10 to the power -6
يعني ال SAL
و بهالمثال هي 8 mins.

المربع الاصفر اللي عند 2 بال y-
axis يعبر انه عنا 100 CFU

Sterility assurance level and survivor plots

- **The next line in the data table would be 6 minutes' exposure → corresponding to one survivor in 100 ml,**
- **then 7 minutes giving one survivor in 1000 ml, and so on.**

1 survival in each 100 ml يعني 0.01 لو خليناها معرضة ل 6 دقايق في المثال السابق رح تنقص ل
يعني كل 100 امبولة فيها 1 survival

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.

ما بعمرنا بنقدر نحكي عنا **zero survival** و السبب بالأزرق
ببين شو السبب

This is the reason why it is not possible to guarantee sterility of an item selected at random from a sterilized batch of product

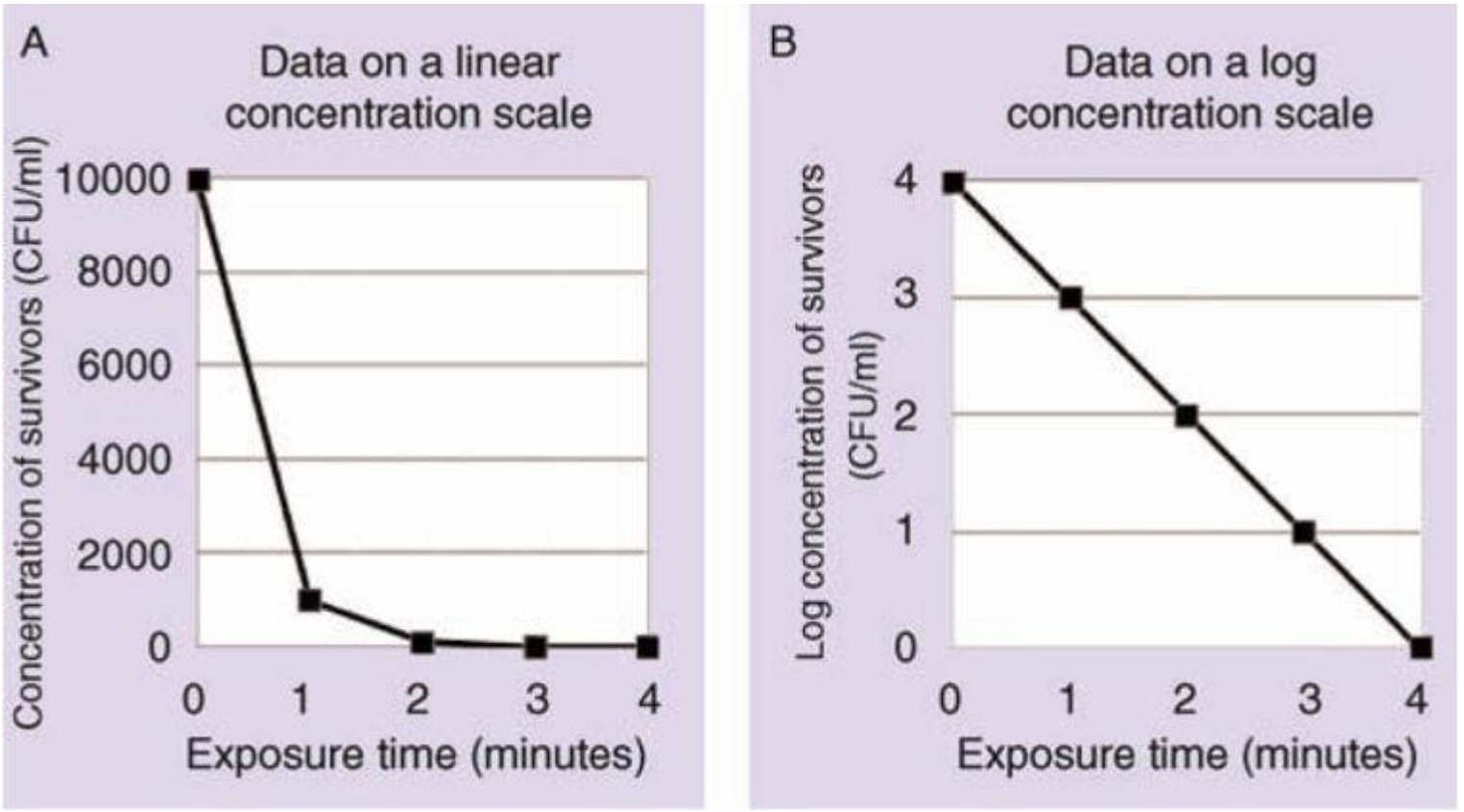
Sterility assurance level and survivor plots

- Instead, a sterility assurance level (SAL) of 10^{-6}(our target), or better, is the target, and the exposure period in the autoclave is adjusted to achieve this (بمثال قبل كانت 8 دقايق)because it can be calculated from knowledge of the presterilization bioburden and the degree of heat resistance of the spores (from their death rate – in other words, the slope of the survivor plot).

presterilization bioburden:

يعني اكم عدد MO ببداية ال sterilization (بمثال قبل كانت 100 اللي هي Log2)

Sterility assurance level and survivor plots

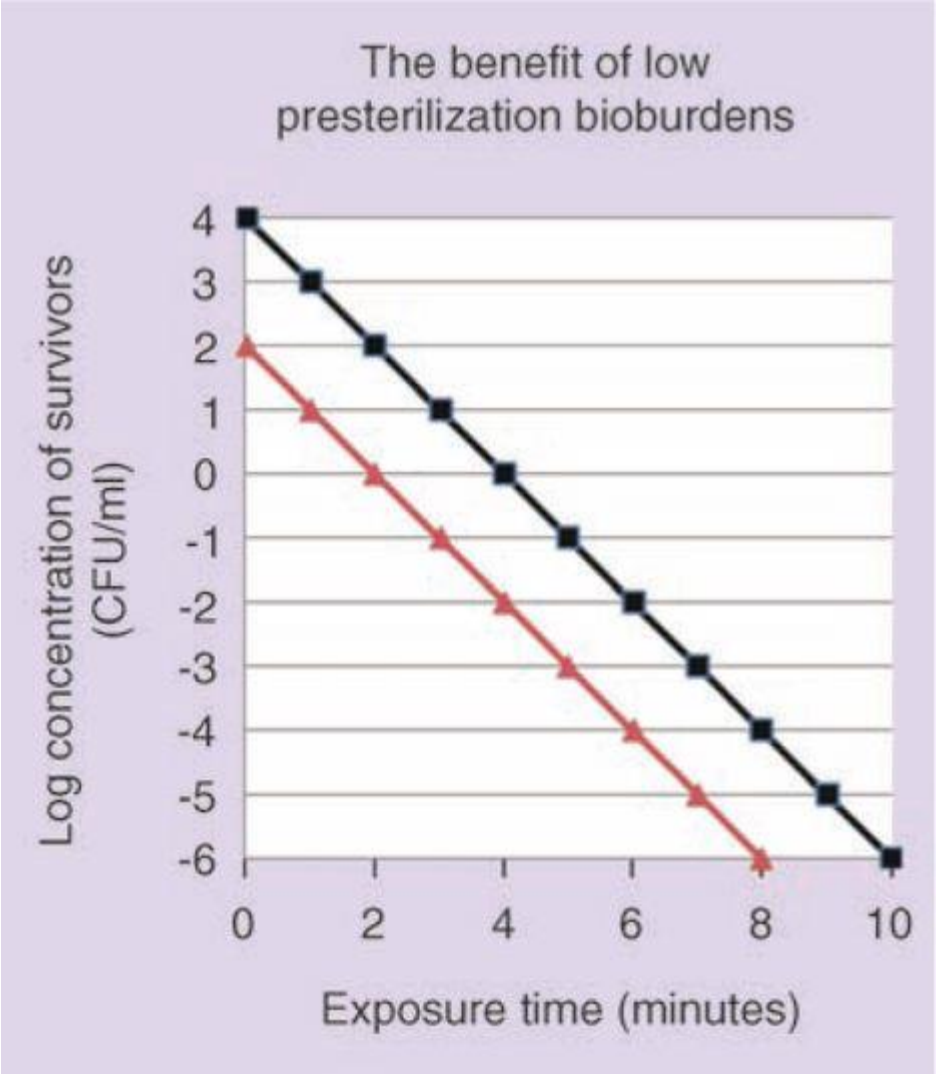


A survivor plot or kill curve (log curve)

Without log
مش خط مستقيم

Semilog
خط مستقيم

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



Slide note:

If, however, the presterilization bioburden were not 10 000 CFU/ml but 100 CFU/ml (which is a more realistic value) the plot would obviously start lower on the axis and the SAL would be achieved in 8 minutes rather than 10 (red triangles).
(Hanlon 177)

هسا توضيح الرسمة انه لو بلشنا من 100 CFU
سنحتاج لـ 8 mins .
هسا لو كان bioburdn اعلى يعني 10,000
رح نحتاج 10 mins لنحقق ال SAL

- **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**
  - ✓ **shorter heat exposure which is likely to reduce degradation of the active ingredient;**
  - ✓ **fewer dead bacterial cells in the product after sterilization, which would reduce the risk of the injection failing the bacterial endotoxins test**

- هسا زيادة ال MO تعني زيادة الوقت والحرارة والطاقة اللازمة لنحقق ال Sterility ، ف احسن method هو اللي بحقق ال sterility بحرارة و وقت اقل لانه هالشي بوفر طاقة فمثلا انه نحتاج 8 دقايق لل 100 CFU لكن 10000 تحتاج 10 دقايق و طبعا هاد بحمي المواد اللي بالمنتج من انها تخرب لما تتعرض لحرارة اقل و وقت تعرضها اقل.

- بدنا ننتبه لما نقتل ال MO داخل الامبولة لكن بقايا MO رح تضل ، فكلما كان عدد MO اكثر بالابرة بصير بقايا اكثر فهاد بزيد risk of injection failing the bacterial endotoxins test (حتى لو كانت مش حية هي البكتيريا برضه مش كسموح تزيد عن حد معين)

- و شي مهم جدا انه نبلش بعدد قليل من Contamination و ندخل على Sterilization لنحقق ال benifits

D-values

- **The 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.**

Slide note:

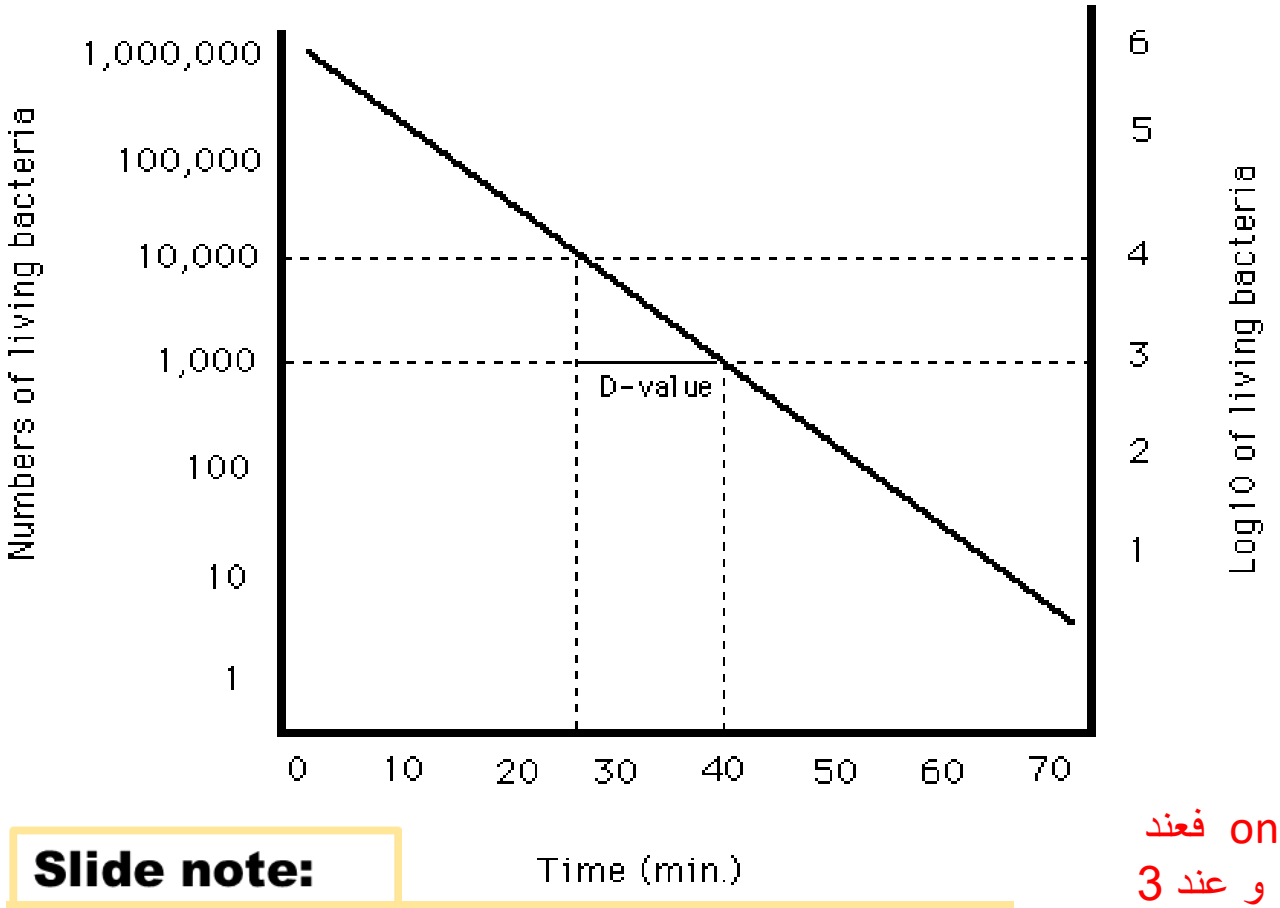
D-values should only be quoted where the survivor plot is linear, and this situation exists for both heat and radiation sterilization but it is frequently not the case when bacteria are killed by toxic chemicals like disinfectants and antibiotics. When the viable population has fallen tenfold (in other words when the value on the y-axis has fallen by 1) it is often referred to as a decimal reduction, or a fall of one log cycle; so Figure 18.3 shows four decimal reductions.

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%.**

- ✓ D value نستخدمها لمعرفة الوقت اللازم لقتل 90% من ال population و بالعادة نستخدمها بـ(steam sterilization (Autoclave
- ✓ البكتيريا لما ندخلها على autoclave على درجة 72 فكل دقيقة كافية لقتل90% من ال population .
- ✓ كل نوع spore له D value خاص فيه

Higher time needed to kill , so that's mean the bacteria is resistant



كيف رح نطلع ال D value ؟

بنروح عند لما ننقص one log cycle فعند 4 كان الوقت بساوي **26 mins** و عند 3 كان الوقت بساوي **40 mins** فبالتالي ال

D value= 40 -26 = 14 mins

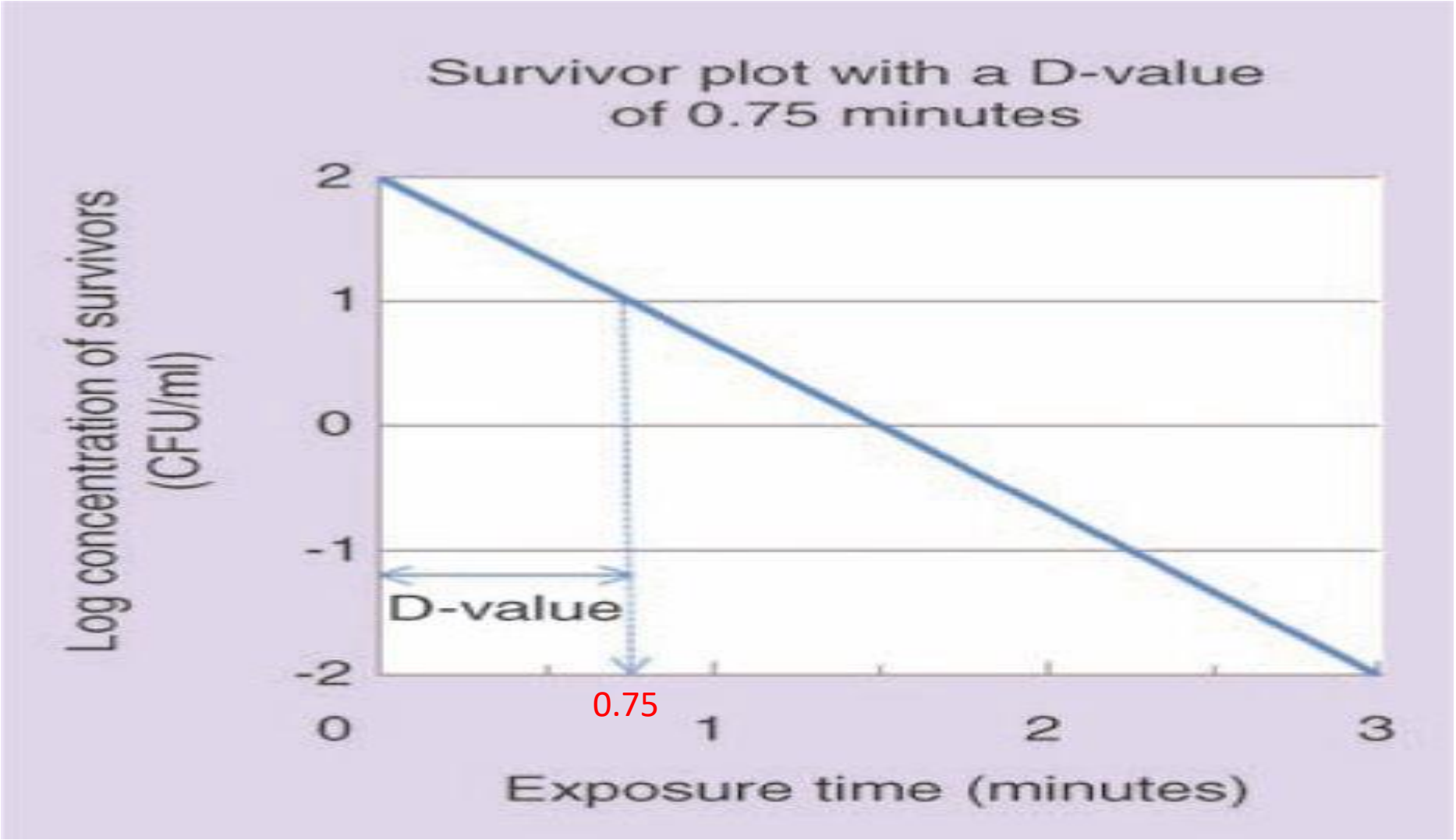
Slide note:

In the illustration below, the D value is 14 minutes (40-26) and would be representative of a process at 72°C. reduced by 90%. In the illustration below, the D value is 14 minutes (40-26) and would be representative of a process at 72°C.

D-value

- D_{121} (time need to kill 90% of MO at 121 °C) values are the most commonly encountered because 121 °C is the most frequently used autoclaving temperature;
- Spores of Geo-bacillus 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.



D value = 75%= 0.75

Z-values

- 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
- 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.

- ✓ In steam sterilization the most commonly used terminal sterilization method is combination of time = 15 - 20 mins & temperature = 121 C
  - ✓ هسا ال Z value بتعطي فكرة عن اكم درجة الحرارة تؤثر على ال Sterilization
  - ✓ هسا ال manufacture يا اما بستخدم المحطوط بال pharmacopeia او بنستخدم العلاقة بين temperature و time لنحقق ال sterility وهي العلاقة بنعرفها من ال Z value يعني بنعرف لو غيرنا ال Temperature اكم رح ينعكس على ال Rate of killing

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

طيب شو ال Z value اصلا ؟؟

هي اكم لازم نغير بدرجة الحرارة عشان نحقق ال 10 fold change in D value

Z-value:
for *G. stearothermophilus spores*.

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

- **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**

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

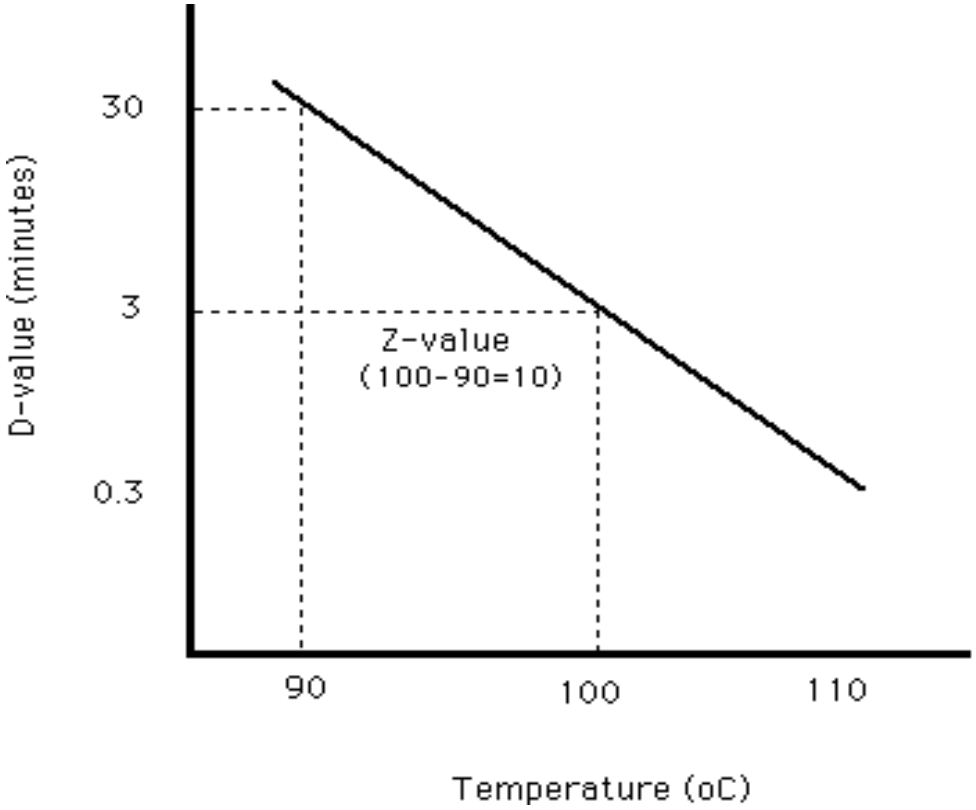
نحتاج 120 mins لقتل 90% من البكتيريا على درجة حرارة 99

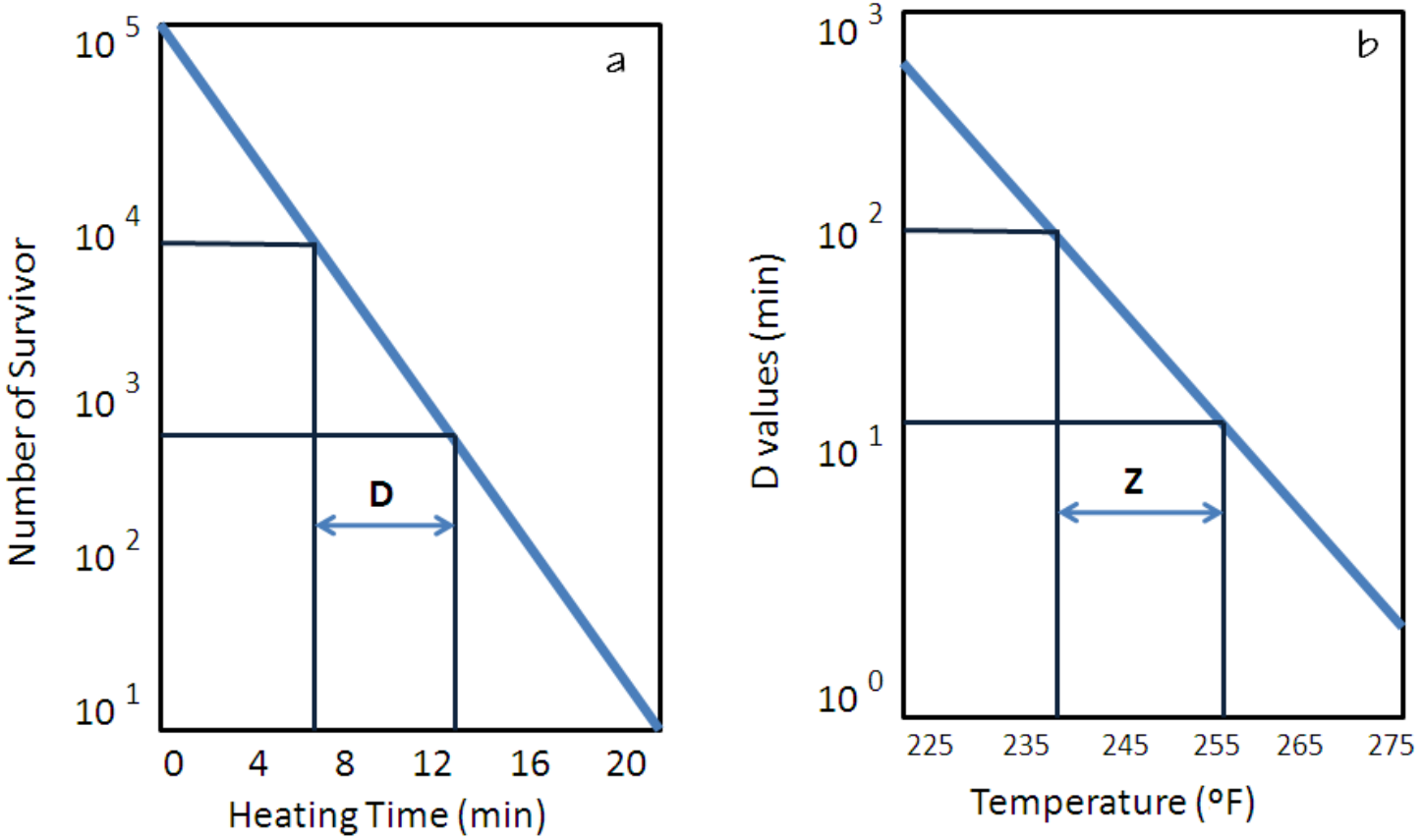
- ✓ Higher steam temoerature will decrease D value
- ✓ Z value هي ال temperature لما يصير 10 fold reduction (يعني من 120 دقيقة لـ 12 دقيقة هون صار 10 fold reduction) فبتطلع ال z value عن طريق نشوف الفرق بدرجة الحرارة و هو :

Z value = 110 – 99 = 11 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°C.**

صار ال 10 fold reduction من 30 ل 0.3
فبالتالي:
Z value = 10 C





هاد بخلينا نربط بين ال D value و ال Z value

نتذكر شي مهم :

- ✓ال D value نرسم العلاقة بين ال Log survive مع ال time
- ✓ال Z value نرسم العلاقة بين ال D value مع ال temperature

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
- $Z = \frac{T2 - T1}{LogD1 - Log\ D2}$

- if a population of spores with a Z-value of 10.5 °C had a D-value of 9.0 minutes (D_1 in the equation) at 115 °C (T_1 in the equation) and it was necessary to know its D-value at 121 °C (T_2 in the equation)→ pg 179

هاد المثال الدكتورة حكت رح تنزل حله ف هي حلي لكن بس تبعوا لما تنزل
الدكتورة الحل اذا في غلط بهالحل عدلوه عندكم

T1 = 115 C
T2 = 121 C
D1 = 9 mins
D2 = ??
Z value = 10.5 C

Answer:

Z= $(T2-T1)/(LogD1 - Log\ D2)$
10.5 =(121 -115)/(Log 9 – Log D2)
10.5(Log 9 – Log D2) = 6
0.57142 – Log 9 = - Log D2
-0.38282 = - Log D2 (take antilog for two sides)
D2 = 2.41445 mins

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 4x10exp5/mL. It is to be processed at 79°C for 21 seconds. The average D value at 65°C for the mixed population is 7 min. The Z value is 7°C. How many organisms will be left after pasteurization? What time would be required at 65°C to accomplish the same degree of lethality?

هون فكرة السؤال اجانا حليب raw من المزرعة كان عدد البكتيريا
4x10exp5/mL فعان يعقموه بحطوه على درجة حرارة 79 C لمدة 21 sec
هسا ال average D value هو 7 mins على درجة حرارة فبالتالي ال z
value هي 7 C يعني زيادة 7 درجات حرارة رح يعمل 10 fold D value
reduction

- 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 5D reduction.

حكت رح تنزل حله هاد كمان، تابعوا بس تنزله و على خير اذا فهمته
من حل الدكتورة بشكل كامل بنزل توضيح حله الكم بملف