تفريغ لاب مايكرو

محاضرة: Exp . 10

الصيدلاني\ة: ياسمين خليل 🌱

لجان الدفعات

Soul Academy

• اللهم علمنا ما ينفعنا وانفعنا بما علمتنا وزدنا علمًا

← تذكير مهم في آخر سلايد

Experiment 10

Data handling and interpretation associated with heat sterilization processes

The objective of this experiment is to familiarize you with data handling and interpretation associated with heat sterilization processes.

Bio-indicator

The general pattern of resistance of microorganisms to biocidal sterilization processes is independent of the type of agent employed (heat, radiation or gas), with vegetative forms of bacteria and fungi, along with the larger viruses, showing a greater sensitivity to sterilization processes than small viruses and bacterial or fungal spores. The choice of suitable reference organisms for testing the efficiency of sterilization processes is therefore made from the most durable bacterial spores; these are usually represented by *Bacillus stearothermophilus* for moist heat, certain strains of *B.subtilis* for dry heat and gaseous sterilization, and *B.pumilus* for ionizing radiation.

Bacillus stearothermophilus	moist heat
Bacillus subtilis	dry heat + gas
Bacillus pumilus	radiation (ionized)

Survivor curves

When microorganisms are exposed to a killing process, they lose their viability in an exponential pattern independent to the initial count.

Survivor curve: plots the logarithmic of the fraction of survivors against the exposure time or dose.

Typical curves have a linear portion which continuous (plot A) or modified by an initial shoulder (plot B) or by a reduced rate of kill at low survivor levels (plot C)

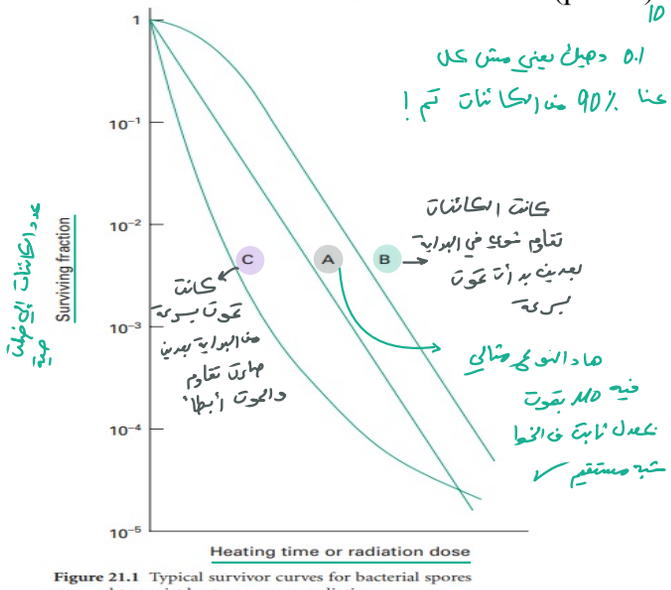


Figure 21.1 Typical survivor curves for bacterial spores exposed to moist heat or gamma radiation.

اللهم ارحم أبي وأمي واغفر له
وعافه واعف عنه وأدخله
وأهله الجنة والمسلمين

Sterility assurance level (SAL)

The term 'sterile', in a microbiological context, means no surviving organisms whatsoever. Thus, there are no degrees of sterility; an item is either sterile or it is not, and so there are no levels of contamination which may be considered negligible or insignificant and therefore acceptable. From the survivor curves presented, it can be seen that the elimination of viable microorganisms from a product is a time-dependent process, and will be influenced by the rate and duration of biocidal action and the initial microbial contamination level. It is also evident from survival curve A that true sterility, represented by zero survivors, can only be achieved after an infinite exposure period or radiation dose. Clearly, then, it is illogical to claim, or expect, that a sterilization procedure will guarantee sterility.

Thus, the likelihood of a product being produced free of microorganisms is best expressed in terms of the probability of an organism surviving the treatment process, a possibility not entertained in the absolute term 'sterile'. From this approach has arisen the concept of **sterility assurance** or a microbial safety index which gives a numerical value to the probability of a single surviving organism remaining to contaminate a processed product
For pharmaceutical products, the most frequently applied standard is that the probability, post-sterilization, of a non-sterile unit is no more than 1 in 1 million units processed (i.e. $\leq 10^{-6}$).

SAL also describes the killing efficacy of a sterilization process. So we can say that a very effective sterilization process has a very low SAL.

SAL ← هو مفهوم بنحتاجه ليه لإنه مستحيل نقيم مصنع بـ 100% شيء خالي بنسبة 100% من الـ 10 وهاد اشي منطقيا مستحيل في اختبارنا

نعبر بـ SAL ، نضمن التعقيم 100% يعني عم نضمن المنتج للحرارة أو الأشعة حتى الحياة بكل بساطة هاد مستحيل ، المهم بدنا الكلمة

مصنع تمامًا بـ احتمال بقاء كائن حي واحد بعد التعقيم 99.9% مصنع ، كائن واحد من مليون كائن ممكن ينجو بعد تعقيم المنتج = $SAL = 10^{-6}$

وأكيد كل ما قلت قيمة SAL رح يكون التعقيم أقوى ونتائجه أفضل مثلا يصير 10^{-3} بدل 10^{-6} يكون بيرفكت

← استنتاج : هل ممكن يوصل منحنى التعقيم إلى SAL يعني ؟ & ← إذا انحسب SAL بناءً على الانحدار بالمنحنى أو الوقت أو الجرعة المطلوبة للوصول لاحتمال نجاة واحد من مليون واحد

صفن معارفة ليه كل هاد
فراغ بين السهم اكتبوا :

سبحان الله ، الحمد لله
ولا إله إلا الله ، الله أكبر
ولا حول ولا قوة إلا بالله

D value [وقت]	Z value [حرارة]	F value
الوقت بالدقائق اللازم لقتل 90% من MO (يقل عددهم $\frac{1}{10}$) عند حرارة معينة 100 000 ← 2min 10 000 ← 4min 1 000 ← 6min	عدد الدرجات المئوية المطلوبة لتغيير D-value بعدار عشرة أضعاف (ten folds) مدى حساسية MO للتغير في الحرارة، لو زدت 10 درجات حرارة مع تقل D value 10 مرات (MO عموت أسرع) والعكس صحيح.	هو وقت مكافئ للتعقيم عند درجة حرارة معينة لقتل نفس عدد MO تحت شروط معينة

Sum

Expressions of Resistance

-

1. D-value.
2. Z-value.
3. F-value.

D-value

أقلها 1 log = عشرة أضعاف ذي الأرقام في الجدول فوقه

-

For heat treatments, D-value is defined as the time taken at a fixed temperature required to achieve a 90% reduction in viable cells (1 log cycle reduction in survivors).

D-value ← الكائن الحي بكون سهل صعوبة ① ← الكائن الحي بكون فطري بكون سهل صعوبة

The calculation of the D-value assumes a linear type A survivor curve, and must be corrected to allow for any deviation from linearity with type B or C curves.

② هو عبارة عن كائنات تقاوم بعد ين فترة عموت بسرعة وهكسها C ← ③ هاي تناقصات تتمرض في عدد الكائنات الي ظهلت حية

High D-value reflects an increase in the resistance of the microorganism to the killing process.

D value عني دقيقة ← الكائن الحي بكون مقاوم بقيمة

اللهم إني أسألك الجنة وأعوذ بك من النار

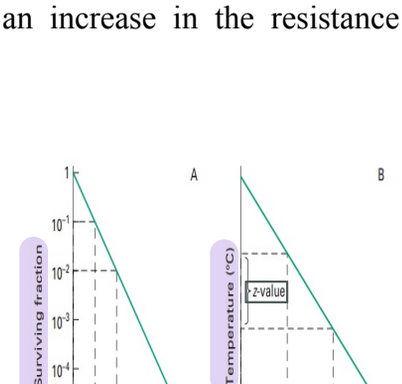


Figure 21.2 Determination of: (A) D-value; (B) Z-value.

هو ← D-value

D value = 1 min ← الحاجة دقيقة واحدة عشان تقتل 90% من MO بشكل تام

Table 21.1 Inactivation factors (IF) for selected sterilization protocols and their corresponding biological indicator (BI) organisms

Sterilization protocol	BI organism	*D*-value	Log IF
Moist heat (121 °C for 15 min)	*B. stearothermophilus*	1.5 min	10
Dry heat (160 °C for 2 h)	*B. subtilis var. niger*	Max. 3 min	Min. 40
Irradiation (25 kGy)	*B. pumilus*	1.9 kGy	13.2

z-value

صدى تأثير الحرارة على مقاومة الكائنات

[1]

For heat treatment, a D-value only refers to the resistance of a microorganism at a particular temperature.

فهمنا ← 1 min ← عند حرارة 120°c ولكن بس رح تقل نفس العدد عند 110°c ← لقتل 90% (D value = 10 min) شكلها تحتاج

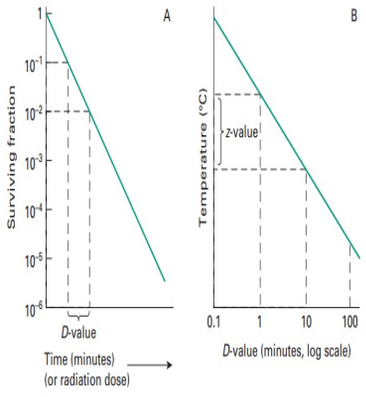
In order to assess the influence of temperature changes on thermal resistance, a relationship between temperature and log D-value can be developed, leading to the expression of a Z-value, which represents the increase in temperature needed to reduce the D-value of an organism by 90% (i.e. 1 log cycle reduction).

إذاً Z value = 10°c عشان تتغير D-value ten fold

[2]

High Z-value reflects an increase in the resistance of the microorganism to heat.

For bacterial spores used as biological indicators for moist heat (*B. stearothermophilus*) sterilization processes, mean *Z*-values are given as 10 °C.



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F-value

In any heat sterilization process, the articles to be treated must first be raised to sterilization temperature and this involves a heating-up stage. In the traditional approach, timing for the process (the holding time) then begins. It has been recognized, however, that during both the heating-up and cooling-down stages of a sterilization cycle, the product is held at an elevated temperature and these stages may thus contribute to the overall biocidal potential of the process.

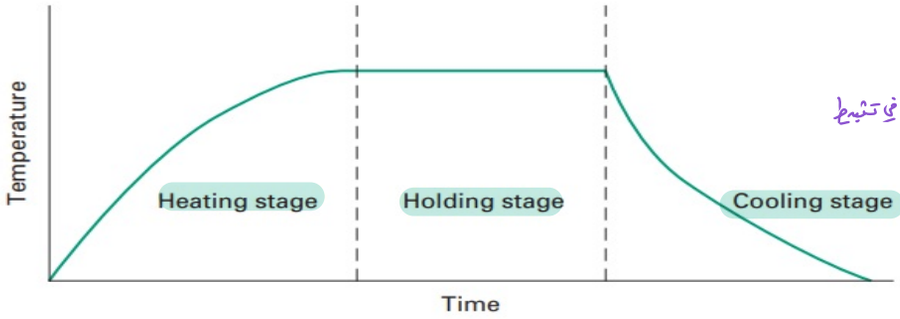

Figure 21.4 Typical temperature profile of a heat sterilization process.

Typical temperature profile of a heat sterilization process

During the heating, sterilizing and cooling stages of a moist heat (steam) sterilization cycle the temperature–time combinations can be converted to the equivalent time at 121 °C.

Using this approach, the overall lethality of any process can be deduced and is defined as the ***F*-value**; this expresses heat treatment at any temperature as equal to that of a certain number of minutes at 121 °C.

In other words, if a moist heat sterilization process has an *F*-value of *x*, then it has the same lethal effect on a given organism as heating at 121 °C for *x* minutes, irrespective of the actual temperature employed or of any fluctuations in the heating process due to heating and cooling stages.

The *F*-value of a process will vary according to the moist heat resistance of the reference organism.

When the reference spore is that of *B. stearothermophilus* with a *Z*-value of 10 °C, then the *F*-value is known as the F_0-value.

A relationship between *F*-and *D*-values, leading to an assessment of the probable number of survivors in a load following heat treatment, can be established from the following equation:

$$F = D(\log N_0 - \log N)$$

Then

$$\log N = \log N_0 - \left(\frac{F}{D}\right)$$

Where *D* is the *D*-value at 121 °C, and N_0 and *N* represent, respectively, the initial and final number of viable cells per unit volume.

The *F*-concept enables a sterilization process to be individually developed for a particular product. This means that adequate sterility assurance can be achieved in autoclaving cycles in which the traditional pharmacopoeial recommendation of 15 minutes at 121 °C is not achieved.

The holding time may be reduced below 15 minutes if there is a substantial killing effect during the heating and cooling phases, and an adequate cycle can be achieved even if the 'target' temperature of 121 °C is not reached.

Thus, *F*-values offer alternative sterilizing cycles and a mechanism by which over-processing of marginally thermos-labile products can be reduced without compromising sterility assurance.

F_0 values calculated by using the following equation:

$$F_O = 10^{\frac{T-121}{Z}} \times \Delta t$$

where Δ *t* is the time interval between temperature measurements, *T* is the product temperature at time *t*, and *Z* is (assumed to be) 10 °C.

Example:

If we assume sterilization lasts 15 minutes, constantly at 111°C, we instead obtain:

$$F_0 = 15 * 10^{\frac{111-121}{10}} = 15 * 10^{\frac{-10}{10}} = 15^{-1} = 1.5'$$

Therefore, a 15 minutes' sterilization at 111°C is equivalent, in terms of lethal effect, to 1.5 minutes at 121°C; this can be easily expected if z = 10.

Similarly, if we assume a 15 minutes' sterilization constantly at 124°C, we have:

$$F_0 = 15 * 10^{\frac{124-121}{10}} = 15 * 10^{\frac{3}{10}} = 29'$$

If the heating process involve multi stages (heating, holding and cooling) F_0 total will equal to:

$$F_0 = F_0 \text{ heating} + F_0 \text{ holding} + F_0 \text{ cooling}$$

يلزم توصيل الماء
عند الوضوء للمرفق
وتتبع أصابعك مها يعني هكذا

يلزم توصيل ماء الوضوء
للكعب هاتنسوا
تطبعقها وتذكروا عيونكم.