General Information about biological indicators (BI)

The population, D-value, F_{Bio}-value and z-value are important variables for BIs.
The population indicates the number of microorganisms of the BI.
The D-value describes the resistance of the germ and is given in a unit of time, such as minutes. However, this value is only valid for a specific sterilization process at a precisely specified temperature and only valid for a defined batch.
The F_{Bio}-value is calculated from the D value together with the population and is also given in a unit of time, such as minutes. It reflects the "total resistance" of the BI.
The z-value indicates the change in the D-value for other temperatures of the same sterilization process and is expressed in Kelvin (K).

Population:

The population indicates the number of viable "spores" on a BI. It is expressed in the unit CFU (colony forming unit) and written in exponential notation of 10^6 and has a correction factor, e.g. 2.5 x 10^6 CFU.
The unit has this name because each viable spore, once placed on a culture medium plate and incubated at the correct temperature, begins to divide until the large quantity of bacteria is visible to the naked eye as a "colony" on a Petri dish.

Decimal reduction time = D-value:

1.) Logarithmic kill kinetics
If microorganisms, such as bacteria, are subjected to a sterilization process, they do not die in a linear order, but the kill kinetics correspond to a logarithmic decay curve.
It can be easily understood by the following example, which is also shown in the table:
Assuming a biological indicator has 100,000 bacteria spores, 10^5 spores in exponential notation, which has a D-value of 2 min at 121°C. After sterilizing 2 min at the given temperature (121°C), only 10 % of the original quantity of bacteria is still present, i.e. 10,000 germs. After a further minute the remaining 10 % have been killed, but rather 1,000 germs are still alive, i.e. again 10 % of the previous original quantity. With each further D-value duration, the population is reduced by exactly 1/10 (lat.: deci-), the "decimal reduction time" or 10 %. This means for each D-value unit, the decimal point in the population is simply moved one place to the right in our decimal system.
See also table 1 for details.

Table 1: the example starts with 100,000 germs with a given D value of 2 min at 121°C (steam sterilization):

min	Number of germs left after sterilization time	Sterilization Time	Sterility Assurance Level (SAL)	Definitions
	100.000	Start		new biological indicator
2,0	10.000	1 D = 2 min		
2,0	1.000	2 D = 4 min		
2,0	100	3 D = 6 min		$(F_{Bio} - 2)$ = growth of BI
2,0	10	4 D = 8 min		
2,0	1	5 D = 10 min	F_{Bio}-value = log Pop x D = strength of BI strip	
	1 of x packs = non-sterile			
2,0	x = 10	6 D = 12	$1/10 = 10^{-1}$	
2,0	x = 100	7 D = 14	$1/100 = 10^{-2}$	
2,0	x = 1.000	8 D = 16	$1/1,000 = 10^{-3}$	
2,0	x = 10.000	9 D = 18	$1/10,000 = 10^{-4}$	F_{Bio} + 4 = kill of BI
2,0	x = 100.000	10 D = 20	$1/100,000 = 10^{-5}$	
2,0	x = 1.000.000	11 D = 22	$1/1,000,000 = 10^{-6}$	= sterile according EN 556-1

2.) Spores in biological indicators are more difficult to inactivate than pathogenic microorganisms and are therefore suitable to simulate the most difficult sterilization situation ("worst-case") of pathogenic germ inactivation:

After each D-value unit, only 10 % of the original number of microorganisms is still present. The D-value, varies heavily depending on the type of germ, and even with the same species, the resistance values often differ considerably. The pathogens with the highest resistance to sterilization processes are bacterial spores, which is the reason they are also used as biological indicators as a "worst-case" model. The germs used as indicators are much more resistant than pathogens and are non-pathogenic themselves.

3.) Kill probability:

After 10 minutes, only one bacterium is still present. If a further minute is sterilized, the decimal point is shifted one place to the right again and 1/10 bacterium in purely mathematical terms is obtained. This means that the probability of still finding a viable bacterial spore there is 1 in 10 [without taking the Gaussian distribution into account]. If the experiment is repeated 10 times or 10 packages with 100,000 spores each are sterilized for 10 minutes, on average one living bacterium would be found in one of the packages, since there would be 10^6 spores in all packages together.

The logarithmic kill kinetics does not allow to reach zero, no matter how long the sterilization is carried out. It has been agreed that in Europe products are defined as "sterile" according to EN 556 if the probability of finding a living germ is 1 in 1 million. It is defined as the “Sterility Assurance Level” (SAL) of 10^{-6} SAL. In the table, this probability of 1 in 1 million described in the standard is reached after 22 minutes of sterilization time. The time needed to reach this value depends, of course, on the initial population ("bioburden") and the D-value of the contamination. The SAL can’t be tested, but must be extrapolated from the population and D-value.

F_{Bio}-value: the total resistance of a biological indicator

The F_{Bio}-value combines both the population and the resistance of the individual BI and defines the overall strength of a biological indicator.
The F_{Bio}-value is the time required to reduce any biological indicator from the initial population to just a single spore and provides a definition of the total resistance of a BI.

In table 1 going 5 "decimals" down only one of the original 100,000 germs are obtained.
Since the decrease of each decimal step takes the time of one D-value, the calculation of the F_{Bio}-value is quite simple:

$$F_{Bio}\text{-value} = \log \text{population} \times \text{D-value}$$

In the example of the first table:

$$F_{Bio}\text{-value} = \log 10^5 \times 2 \text{ min} = 5 \times 2 \text{ min} = 10 \text{ min}$$

The resistance enters directly into the equation, whereas in the case of the population only the number of powers of ten, i.e. the decadic logarithm of the population, is included.
A small change of the population is therefore much less significant than a change in the resistance of the D-value. This leads to the fact that biological indicators with higher population do not necessarily have the higher overall resistance, although they are always more expensive due to higher production costs.
The following example ins shown on the two tables:

Table 2. and 3.: two examples of biological indicators with different F_{Bio}-value:

Example 1: Biological indicator with 10^6 CFU and a $D_{121°C}$-value: 1.5 min		
Time in autoclave at 121°C	Population: Number of spores of the biological indicator	
0 min	10^6	1,000,000
1.5 min	10^5	100,000
3 min	10^4	10,000
4.5 min	10^3	1,000
6 min	10^2	100
7.5 min	10^1	10
9 min	10^0	1
F_{Bio} = 6 x 1.5 = 9 min		

Example 2: Biological indicator with only 10^5 CFU but a higher $D_{121°C}$-value: 2.0 min		
Time in autoclave at 121°C	Population: Number of spores of the biological indicator	
0 min	10^5	100,000
2.0 min	10^4	10,000
4 min	10^3	1,000
6 min	10^2	100
8 min	10^1	10
10 min	10^0	1
F_{Bio} = 5 x 2 = 10 min		

Although the population of the left example is 10 times higher than of the right example and the D-values differ by only 30 seconds, the cheaper 10^5 biological indicator has a higher overall strength, i.e. a higher F_{Bio} value, or it takes longer until it is inactivated.

z-value:

The z-value can be used to calculate the resistance of a biological indicator, at another temperature. The process itself remains the same, only the temperature changes.
The z-value is expressed in K, and is determined experimentally for each batch of spores. If the temperature is increased by the amount of the z-value, the killing process works 10 times faster. If the temperature is decreased by the z-value amount, the D-value increases by a factor of 10, so it takes 10 times as long to achieve the same kill rate.
In steam sterilization processes, the D-value of commonly used indicator spores is usually around 10 K and must not be less than 6 K according to the ISO standard 11138-3.

<u>Example:</u>
The D value of a bacterial spore is 2 min at 121°C (steam).
The measured z-value is 8 K.
I.e. at 129°C the new calculated D-value would be just 0.2 min, i.e. 24 seconds. At 113°C, the new D-value would be 20 min. The temperature has a very great influence on the sterilization speed. The higher the temperature, the shorter the sterilization time.

For other temperatures, the calculation is not difficult:

$$\text{D-value}_{new} = \frac{\text{D-value}_{old}}{10^{\left(\frac{T_{new} - T_{old}}{\text{z-value}}\right)}}$$

D-value_{new}: calculated D-value for the „new“ temperature
D-value_{old}: the old, known D-value of the certificate
T= Temperature in °C

<u>Examples: BI with z = +/-8 K, D_{121}-value = 2 min:</u>

Temperature increase: + 8 K
New D-value at 129°C:

$$D_{129} = \frac{2\text{ min}}{10^{\left(\frac{129°C - 121°C}{8°C}\right)}} = \frac{2\text{ min}}{10^{\left(\frac{8°C}{8°C}\right)}} = \frac{2\text{ min}}{10^{1}} = 0.2\text{ min}$$

Temperature decrease: -8 K
New D-value at 113°C:

$$D_{113} = \frac{2\text{ min}}{10^{\left(\frac{113°C - 121°C}{8°C}\right)}} = \frac{2\text{ min}}{10^{\left(\frac{-8°C}{8°C}\right)}} = \frac{2\text{ min}}{10^{-1}} = 20\text{ min}$$