CLEANING
STERILIZATION

PRODUCT CATALOGUE

Biological Indicators and Accessories for sterilization processes

Spore Strips, Ampoules, Suspensions
Self-contained Mini-Bio-Plus BI

to monitor steam, formaldehyde, hydrogen peroxide, ethylene oxide and dry heat sterilization processes as well as room disinfection processes

Content

General Information

Today, sterilization in industry and hospitals has reached a high level of quality, for the benefit and safety of patients and operators alike. If the great effort required to carry out and monitor sterilization - and the associated costs - which is common today is criticised, it must be countered that the reliability of sterilisation is the basic prerequisite for asepsis in the complicated operations possible today. Irrespective of the moral obligation to do everything possible to avoid infections, medical device (MP) legislation requires the validation of all sterilisation processes in industry and healthcare, as well as their monitoring and documentation.

The validation of sterilisation processes can be carried out with the help of biological and/or chemical indicators and/or by physical measurements.

EN ISO 11138-1 to 5 describe biological indicators for dry heat, steam, LTSF and ethylene oxide sterilisation processes, but not yet for hydrogen peroxide (H2O2)/plasma sterilisation processes.

Besides industry, healthcare facilities must follow the same validation, monitoring and documentation procedures. Validation and monitoring of sterilization processes is carried out by parametric, chemical and/or biological tests. The validation using biological indicators is necessary if:

- the structure of the goods to be sterilized is such that physical sensors cannot be applied (e.g.: small holes, gaps, sealed areas, coatings with oils etc.)
- lumens of hollow devices are so tiny that the temperature difference between non-condensable gases (inside) and steam (outside) is not detectable. Gases in such small lumens of several 100 µl heat up very quickly to the steam-temperature-level.
- the presence of water condensate cannot be detected by physical means (e.g.: If the temperature gradient in the process is so slow that encapsulated non-condensable gases have time to heat up and do not show a detectable temperature difference.)
- the surface structure of the medical devices requires specific testing (e.g.: porous rubber stoppers)
- the sterilizing agent, the goods to be sterilized and/or packaging contain salts. The salts may get dissolved in the condensate film and cause big changes of the resistance characteristics.
- the condensate contains substances changing the pH-value (e.g.: corrosion-inhibitors) or the material of medical instruments (e.g.: aluminium surfaces) may react with water creating basic hydroxides.

In above cases all surfaces or liquids have to be inoculated with biological indicator suspensions. After a validated population determination, reduced process cycles have to be carried out to achieve survivor curves to determine the kill kinetics on/in those critical areas. For porous loads and hollow process challenge devices (PCDs) biological indicators may be used to monitor the process conditions in such critical internal areas.

Biological indicators are defined in the European and International Standards EN ISO 11138 parts 1 to 5. For most of the commonly used sterilization processes special reference biological germs have been selected, such as *Geobacillus stearothermophilus* for steam, formaldehyde and hydrogen peroxide sterilization processes, *Bacillus atrophaeus* for ethylene oxide and dry heat sterilization processes and *Bacillus pumilus* for radiation sterilization processes.

Depending on the type of sterilization process, a special resistance characteristic of biological indicators is required to prove the success of a defined sterilization process. During such a sterilization process the spore population always decreases due to the exponential kill characteristic called reaction kinetics first order. The population however, will never reach an absolute 0-value. Therefore modern definitions of goods declared “sterile” do not specify the absolute absence of biological activity, but determine aseptic conditions with the certain probability, called Sterility Assurance Level (SAL).

Resistance of biological indicators

According to the European Standard EN 556 the minimum SAL has to be 10^{-6} CFU/per part or better. This means that out of 1 million units, no more than 1 unit may show growth.

Both the kill kinetics and the penetration characteristics of a sterilization process have to be monitored. The kill kinetics is monitored by the right type of bacteria with the total resistance of a biological indicator.

The total resistance of a biological indicator depends on the population and resistance of each individual germ. The resistance of each individual germ is defined by the decimal reduction value which is the time needed to reduce the population of a biological indicator to one tenth of the original population. The total resistance of a biological indicator is expressed by the F_{BIO} value:

$$\mathbf{F_{BIO} = D_{121°C}\ value \times \log (population)}$$

This fact may be demonstrated by the 2 examples below in the table.

Example	Population [CFU/strip]	D_{121}-value [min]	F_{Bio}-value [min]
1	10^6	1.0	6
2	10^5	2.0	10

As seen above, the D-value of a given strain is never constant and depends on growth and process condition. Therefore, for each batch of biological indicators certificates must be associated to the product indicating the population, individual resistance and the total resistance of a biological indicator.

GKE offers its Steri-Record® biological indicators according to EN ISO 11138 series. All biological idnicators contain a certificate with all necessary information mentioned above.

After the biological indicator has passed the sterilization process, all treated spore strips have to remain in the glassine envelopes. They should be sent with one marked untreated spore strip to a microbiological lab. All strips should be aseptically transferred into Tryptic Soja broth (TSB) and developed for at least 7 days. If there is any doubt about the spore type, 1 ml of solution may be developed on TS agar plates (TSA) to determine the spore type. TSA vials without a spore strip should not show any growth, the untreated spore strip should show vital growth. Growth of treated spore strips have to be determined individually (see our technical information TI 730-067). GKE offers growth medium test tubes with pH-indicator for faster evaluation.

Self-contained biological indicators contain growth media in a separate vial and may be developed directly at the user's site. They must not be used in dry heat processes. For information in more detail, please see our data sheet "self-contained biological indicators".

The penetration characteristics are monitored using Process Challenge Devices (PCDs) representing the "worst-case" penetration characteristics of a load. PCDs as described in EN ISO 11140-6 "Hollow Load Test" and in DIN 58921 may be used. Biological indicators are used inside to check the penetration of the sterilization agent.

GKE biological indicators are available with different D-values. If a particular D-value is required, that differs from the BI available in stock, it is advisable to check if the BI can be produced with the particular characteristics.

Self-contained Biological Indicators

1. GKE Steri-Record® Self-contained biological indicators

Mini-Bio-Plus self-contained biological indicator (SCBI) uses a plastic vial containing a spore plate and glass ampoule with a growth medium and pH-indicator inside. It is used for validation and routine monitoring of most sterilization processes without using a microbiological laboratory. For a better differentiation of the SCBI versions all have different coloured caps. They can also be used inside GKE process challenge devices (Bio-C-PCD®s), see 1.5. All SCBI fulfil the requirements according to EN ISO 11138-1.

1.1. for steam sterilization processes

G. Stearothermophilus available with population of 10^5 and 10^6, on paper carrier according to EN ISO 11138-3.

Three versions are available:

1.1.1. Standard SCBIs with incubation time of 24 hours

Art.-No.	Quantity	Product Code	Colour of cap	Population
324-501	10	B-S-MBP-10-5	Light blue	10^5
324-505	50			
324-510	100			
324-601	10	B-S-MBP-10-6	Dark blue	10^6
324-605	50			
324-610	100			

1.1.2. Instant-SCBIs for immediate release

The 121°C and 134 °C Instant-Mini-Bio-Plus SCBI contain a type 5 chemical indicator allowing that the result of steam sterilization processes can be instantly evaluated at the end of the steam sterilization process at 121-124°C or 132-137°C. Therefore, it is not necessary to wait for the result of the SCBI incubation since the type 5 indicator provides equivalent or better information about the result of the sterilization process according to EN ISO 11140-1. The Instant-Mini-Bio-Plus SCBI has to be selected according to the sterilization temperature.

Art.-No.	Quantity	Product Code	Colour of cap	Population	Sterilization Temperature
324-521	10	B-S-MBP-I-10-5-SV5	Light green	10^5	121-124 °C
324-525	50				
324-621	10	B-S-MBP-I-10-6-SV5	Dark green	10^6	
324-625	50				
324-551	10	B-S-MBP-I-10-5-SV4	Light orange	10^5	132-137 °C
324-555	50				
324-550	100				
324-651	10	B-S-MBP-I-10-6-SV4	Dark orange	10^6	
324-655	50				
324-650	100				

Self-contained Biological Indicators

1.1.3. Activation Control SCBI (AC-SCBI®)

NEW

The SCBI with activation control contains a glass ampoule with colourless growth medium that only turns coloured after activation of the SCBI, thus preventing misapplication.

Art.-No.	Quantity	Product Code	Colour of cap	Population
324-591	10	B-S-AC-MBP-10-5	Light blue	10^5
324-595	50			
324-590	100			
324-691	10	B-S-AC-MBP-10-6	Dark blue	10^6
324-695	50			
324-690	100			

1.1.4. Activation Control 134°C Instant-SCBI (134°C Instant AC-SCBI®)

Combination of an Instant-SCBI at 134 °C (1.1.2) and an AC-SCBIs (1.1.3).

Art.-No.	Quantity	Product Code	Colour of cap	Population
324-581	10	B-S-AC-MBP-I-10-5 (132-137 °C)	Light orange	10^5
324-585	50			
324-580	100			

1.2. for formaldehyde (LTSF) sterilization processes

G. stearothermophilus available with population of 10^6, paper carrier according to EN ISO 11138-5.
The growth medium also contains a neutralization agent for remaining formaldehyde, so that the pretreatment with Na_2SO_3 is not required as described in EN ISO 11138-5.

Art.-No.	Quantity	Product Code	Colour of cap
325-601	10	B-F-MBP-10-6	Yellow
325-605	50		

Self-contained Biological Indicators

1.3. for hydrogen peroxide/plasma sterilization processes

G. stearothermophilus available with population 10^6, on different carrier materials

Art.-No.	Quantity	Product Code	Carrier	Colour of cap
327-601	10	B-V-G-MBP-10-6*	Glass fiber	Light grey
327-605	50			
327-610	100			
337-601	10	B-V-T-MBP-10-6*	Tyvek	Colourless
337-605	50			
347-601	10	B-V-ST-MBP-10-6*	Stainless steel	Dark grey
347-605	50			
357-601	10	B-V-P-MBP-10-6*	PET	Purple
357-605	50			

(*) For hydrogen peroxide sterilization processes four different versions are available using exactly the same germ and population. It shows that the resistance of biological indicators in hydrogen peroxide sterilization processes depends not only on population and spore type but also extremely on the carrier material used.

1.4. for ethylene oxide sterilization processes

B. atrophaeus available with population 10^6, on paper carrier according to EN ISO 11138-2 and EP.

Art.-No.	Quantity	Product Code	Colour of cap
326-605	50	B-E-MBP-10-6	Red
326-610	100		

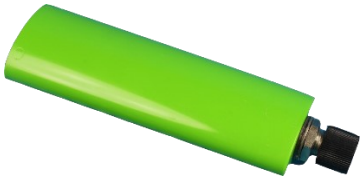
PCDs and Accessories

1.5. Process Challenge Devices (PCD) for self-contained biological indicators

Bio-C-PCD®s, colour: green, to be used with all Mini-Bio-Plus SCBIs described before, for validation and routine monitoring of steam, ethylene oxide, formaldehyde and hydrogen peroxide sterilization processes or Helix-PCD according to EN 1422 for ethylene oxide sterilization processes.

It is recommended to use the round versions in large and the oval versions in small sterilizers. A PCD with SCBI placed inside is called a type 2 indicator system according to EN ISO 11140-1.

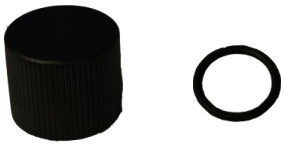
Each PCD comes along with 5 seal rings in addition for replacement in the screw cap.



Art.-No.	Product Code	PCD-Version	Penetration Characteristics
300-031	B-PM-OCPCD-0	oval	Very low requirements for air removal
300-032	B-PM-RCPCD-0	round	
300-033	B-PM-OCPCD-1	oval	Minimal requirements for air removal
300-034	B-PM-RCPCD-1	round	
300-035	B-PM-OCPCD-2	oval	Low requirements for air removal
300-036	B-PM-RCPCD-2	round	
300-037	B-PM-OCPCD-3	oval	Air removal less difficult than Hollow Load Test according to EN ISO 11140-6
300-038	B-PM-RCPCD-3	round	
300-039	B-PM-OCPCD-4	oval	Air removal equal to Hollow Load Test according to EN ISO 11140-6
300-040	B-PM-RCPCD-4	round	
300-041	B-PM-RCPCD-5	round	Air removal more difficult than Hollow Load Test according to EN EN ISO 11140-6
300-042	B-PM-RCPCD-6	round	
300-028	B-E-PM-HPCD	Helix	Type test according to Line/ Pickerill (EN 1422 EO monitoring)

1.6. Accessories

1.6.1. Replacement parts for PCDs



Art.-No.	Product Code	Quantity
300-005	Replacement screw cap (M14x1 thread)	5
300-006	Replacement seal kit for all PCDs listed above	5

1.6.2. Crusher for SCBIs

to activate all GKE SCBIs. The GKE incubator already includes a crusher.

Art.-No.	Product Code	Material	Quantity
224-002	I-C	Stainless steel	1
224-004	I-PC	Plastic	10

Incubators and Accessories

2. GKE Steri-Record® Incubators and accessories

The incubator is available in four versions with different temperatures. The incubation temperature is visible in the display. All incubators are either available with an aluminium block to incubate SCBIs or alternatively without aluminium block. In this case an aluminium block available for different applications (see 2.2 accessories) has to be ordered separately. The plug contains a CE conformity for the low voltage directive.

2.1. Dry Bath Incubators

Art.-No.	Product Code	Temperature	Application
With aluminium block for 12 SCBIs (hole diameter 10 mm)			
610-119	I-37-AB-MBP	37	to incubate *B. atrophaeus* biological indicators
610-120	I-57-AB-MBP	57	to incubate *G. stearothermophilus* biological indicators
610-121	I-V-AB-MBP	30 - 60	variable temperature selection
610-122	I-V-T-AB-MBP		variable temperature selection and programming of the incubation time
Without aluminium block			
610-109	I-37	37	to incubate *B. atrophaeus* biological indicators
610-110	I-57	57	to incubate *G. stearothermophilus* biological indicators
610-111	I-V	30 - 60	variable temperature selection
610-112	I-V-T		variable temperature selection and programming of the incubation time

2.2. Accessories

Aluminium blocks to insert SCBIs, Stearo-Ampoules or growth medium tubes (12 pcs each).

Art.-No.	Product Code	Diameter	Application
610-113	I-AB-MBP	10 mm	for all GKE Mini-Bio-Plus SCBIs
610-114	I-AB-AMP	11 mm	for all GKE Mini and Stearo-Ampoules
610-115	I-AB-CM	16.5 mm	for all GKE growth medium tubes

3. GKE Steri-Record® Stearo-Ampoules

to monitor extreme wet steam or liquid sterilization processes. The ampoules are available in two different sizes. It contains a 1.5 or 0.2 ml *G. stearothermophilus* suspension with growth medium and pH-indicator, available with nominal populations of 10^5 or 10^6 CFU. They comply with EN ISO 11138-1 + 3, EP and USP.

Art.-No.	Quantity	Product Code	Population [CFU/Amp.]	Diameter/ Height
225-550	50	B-S-AMP-10-5	10^5	11 / 45 mm (1.5 ml)
225-650		B-S-AMP-10-6	10^6	
235-510	100	B-S-MAMP-10-5	10^5	5 / 25 mm (0.2 ml)
235-610		B-S-MAMP-10-6	10^6	

Suspensions and Ampoules

4. GKE Steri-Record® Suspensions

All spore suspensions are delivered in 10 ml glass bottles with a septum, suspended in 40 % ethanol/water and comply with EN ISO 11138-1.

4.1. for dry heat and ethylene oxide sterilization processes

The suspension *B. atrophaeus* is delivered with certificate which states nominal population and D-value for ethylene oxide EN ISO 11138-2 EP and USP. If the suspension shall be used in dry heat or hydrogen peroxide sterilization processes, this must be already stated with the order, since a special manufacturing procedure is necessary. The D-value for dry heat (art.-no. 226-999) and hydrogen peroxide (art.-no. 226-997) can be determined at extra cost.

Art.-No.	Product Code	Population [CFU/ml]	Population/bottle
226-107	B-E-H-SUS-10-7	10^7	10^8
226-108	B-E-H-SUS-10-8	10^8	10^9
226-109	B-E-H-SUS-10-9	10^9	10^{10}

4.2. for steam, formaldehyde and hydrogen peroxide sterilization processes

The suspension *G. stearothermophilus* will be delivered with certificate which states nominal population and $D_{121°C}$-value for steam according to EN ISO 11138-3 or hydrogen peroxide. A standard for H_2O_2 does not yet exist. If the suspension shall be used in hydrogen peroxide sterilization processes, this must be already stated with the order, since a special manufacturing procedure is necessary. The D-value for formaldehyde according EN ISO 11138-5 (art.-no. 228-998) can be determined at extra cost.

Art.-No.	Product Code	Population [CFU/ml]	Population per bottle	Sterilization Process
228-107	B-S-F-SUS-10-7	10^7	10^8	Steam, Formaldehyde
228-108	B-S-F-SUS-10-8	10^8	10^9	
229-107	B-V-SUS-10-7	10^7	10^8	Hydrogen Peroxide
229-108	B-V-SUS-10-8	10^8	10^9	

Growth Media and Spore Strips

5. GKE Steri-Record® Growth media

Test tubes with aluminium screw cap (diameter: 16.1 mm) filled with TSB and pH-indicator.
The test tubes have optimized dimensions and volume to fit all kind of spore strips and spore discs. If germs are growing the pH-indicator changes its colour and allows a quick evaluation of the result.

Art.-No.	Quantity	Product Code	Process	Germ
221-010	10	B-E-H-CM	ethylene oxide, dry heat	*B. atrophaeus*
221-100	100			
223-010	10	B-S-V-CM	steam, hydrogen peroxide	*G. stearo-thermophilus*
223-100	100			
330-010*	10	B-F-CM	formaldehyde	
330-100*	100			

* on demand only

6. GKE Steri-Record® Spore strips

The biological indicators (6 x 38 mm) inoculated with bacteria spores contain a certificate which states nominal population and D-value. All spore strips can be also used inside of process challenge devices (PCD). Alternatively the bacteria spores are inoculated on discs with 7 mm diameter and are packaged individually or together in a blister bag.

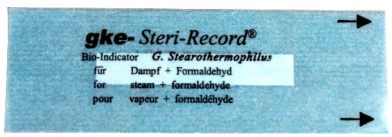
6.1. for dry heat and ethylene oxide sterilization processes

Spore strips *B. atrophaeus*, packaged in glassine envelopes, according to EN ISO 11138-2+4.
The resistance determination according to EP (art.-no. 221-999, see 1.4) is available at extra cost.

Art.-No.	Quantity	Carrier	Product Code	Population
221-601	100	Paper	B-E-H-SS-10-6	10^6
221-605	500			
221-610	1,000			

6.2. for steam and formaldehyde sterilization processes

Spore strips *G. stearothermophilus*, packaged in glassine envelopes, according to EN ISO 11138-3. The resistance determination for LTSF (formaldehyde) according to EN ISO 11138-5 is available at extra cost (art.-No. 223-998).



Art.-No.	Quantity	Carrier	Product Code	Population
223-501	100	Paper	B-S-SS-10-5	10^5
223-505	500			
223-510	1,000			
223-601	100		B-S-SS-10-6	10^6
223-605	500			
223-610	1,000			

SPORE DISCS AND GROWTH MEDIA

6.3. for hydrogen peroxide decontamination and sterilization processes

To decontaminate isolators and rooms, vaporized hydrogen peroxide processes are used. Unpackaged biological spore discs can be placed in critical locations inside the room or in equipment.

For resistance determinations there is currently no EN or ISO standard available. The D-values are determined by exposure of spore suspensions to hydrogen peroxide solutions. Spore suspensions analyzed in this way are used to inoculate carriers. Therefore, the influence of the carrier material on the resistance of the BI suspension remains unconsidered.

6.3.1. as strip (6 x 38 mm)

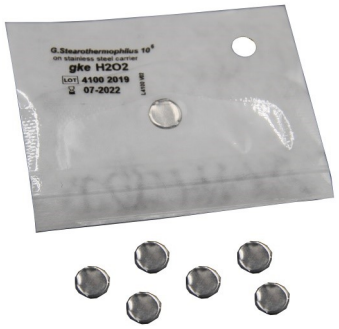
The biological indicators consist of *G. Stearothermophilus* bacteria spores inoculated on different carriers with the size of 6 x 38 mm and packaged individually (in Tyvek envelope of 94 x 65 mm) or in bulk in a blister box. They also contain a certificate which states nominal population and D-value. All spore strips can be also used inside of process challenge devices (PCD).

Art.-No.	Quantity	Packaged	Carrier	Product Code	Population
332-407	100	individually	Stainless Steel	B-V-ST-SS-10-4	10^4
332-507	100	individually		B-V-ST-SS-10-5	10^5
332-607	100	individually		B-V-ST-SS-10-6	10^6
332-608	40	bulk			
332-601	100	individually	PET	B-V-P-SS-10-6	
332-604	40	bulk*			
332-602	100	individually	Glas fiber	B-V-G-SS-10-6	
332-605	40	bulk*			
332-603	100	individually	Tyvek	B-V-T-SS-10-6	
332-606	40	bulk*			

* not in stock, available on request.

6.3.2. as discs (7 mm diameter)

The *G. Stearothermophilus* bacteria spores are inoculated on discs with 7 mm diameter (available with different carriers) and are packaged individually (in Tyvek envelopes of 60 x 65 mm) or in bulk in a blister box.



Art.-No.	Quantity	Packaged	Carrier	Product Code	Population
332-417	100	individually	Stainless Steel	B-V-ST-DIS-SP-10-4	10^4
332-517	100	individually		B-V-ST-DIS-SP-10-5	10^5
332-617	100	individually		B-V-ST-DIS-SP-10-6	10^6
332-415	110	bulk		B-V-ST-DIS-SP-10-4	10^4
332-515	110	bulk		B-V-ST-DIS-SP-10-5	10^5
332-615	110	bulk		B-V-ST-DIS-SP-10-6	
332-612	100	individually	PET	B-V-P-DIS-SP-10-6	
332-614	110	bulk*			
332-616	100	individually	Glas fiber	B-V-G-DIS-SP-10-6	
332-611	110	bulk*			
332-618	100	individually	Tyvek	B-V-T-DIS-SP-10-6	
332-613	110	bulk*			

* not in stock, available on request.

Basic Principles in Kill Kinetics and Design of Sterilization Processes

Author: Dr. Ulrich Kaiser

Content

1. Kill kinetics in sterilization processes

The mathematical laws for the inactivation of microorganisms are very similar in most sterilization processes under the condition that the physical and/or chemical parameters remain constant during the sterilization procedure. Even if the sterilization conditions are constant, the resistance of the same strain may be quite different depending on the vegetative growth and sporulation conditions. Even spores of identical strain with the same reference number (i.e. *G. Stearothermophilus*) may be quite different and may vary by a factor up to 10.

Under the condition using identical germs and sterilization processes the velocity of kill is only dependent on the existing amount of alive germs measured in colony forming unit (CFU). The kill kinetics equation has been proven valid for dry heat, steam, formaldehyde, ethylene oxide and hydrogen peroxide sterilization processes.

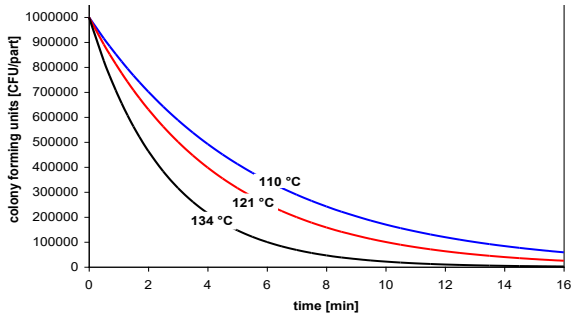


Diagram 1: *Survival curve for steam sterilization at different temperatures*

1.1. Definition of reaction kinetics first order

The kill velocity in sterilization processes is described by equation 1 and describes the reduction of the germ amount N over the time t and is called reaction velocity.

$$\frac{dN}{dt} = -k' * N \qquad (1)$$

t = Sterilization time [min]
N = Nominal population on a medical device [CFU]
k' = Reaction kinetics constant using the natural logarithm [min^{-1}]

The reaction velocity [dN/dt] is always proportional to the current existing amount of alive germs in the process. The proportional constant k' is called reaction kinetics constant. k' describes the kind of sterilization process. The constant is dependant in thermal processes on temperature, in chemical processes also on the gas concentration.

If equation 1 is integrated and the natural logarithm is exchanged against the decade logarithm the new reaction kinetics constant k is defined:

$$\lg \frac{N_0}{N_F} = k * t \qquad (2)$$

t = Sterilization time [min]
N_0 = Number of germs when starting the process [CFU]
N_F = Number of germs after sterilization [CFU]
I_F = Inactivation Factor [number]
k = Reaction kinetics constant [min^{-1}] (valid for the decade logarithm)

1.2 Inactivation factor

In diagram 1 the colony forming units [CFU] are plotted on a linear scale showing e-function curves. If the same diagram is plotted on a half logarithmic scale, the curves become a straight line for the same type of germs if steam, ethylene oxide, dry heat and LTSF sterilization processes are used. If the line is not straight, the same population may contain germs of the same strain but with different resistance. Due to their complex chemical process hydrogen peroxide sterilization processes do not form a straight line.

Equation 2 can be changed to:

$$\lg N_0 - \lg N_F = k * t = IF \qquad (3)$$

The term inactivation factor (IF) describes the efficacy of a sterilization process. If a sterilization starts with 10^6 [CFU] and finishes with 10^2 [CFU], there is a population reduction of the power of 4 or has an inactivation factor IF = 4.

1.3 Decimal reduction factor (D-value)

The decimal reduction factor, quite often called D-value, represents the resistance characteristic of an individual germ for a defined sterilization process. The D-value determines how long a germ must be inside of a sterilization process to reduce the starting population by 90 % of the starting bioburden. In steam, ethylene oxide, formaldehyde, dry heat and hydrogen peroxide sterilization processes the D-value is expressed in a time scale [min]. If a radiation sterilization process is used, it is expressed in the radiation dose [Mrad]. The D-value may be experimentally determined by plotting the logarithm of the still remaining population in the sterilization process against the time, the reciprocal slope of the straight line is the definition of the D-value. The D-value is only valid for a defined sterilization process and a defined germ. In a steam sterilization process the D-value contains in the index the sterilization temperature. The certificate of a biological indicator must always specify under which conditions the D-value is tested. The D-value is very temperature dependent as shown in diagram 2.

$$D_T = \frac{1}{k} \qquad (4)$$

D_T = Decimal Reduction Factor [min] or [Mrad] at a tested temperature [t]
k = Reaction kinetics constant of the decimal logarithm [min^{-1}]

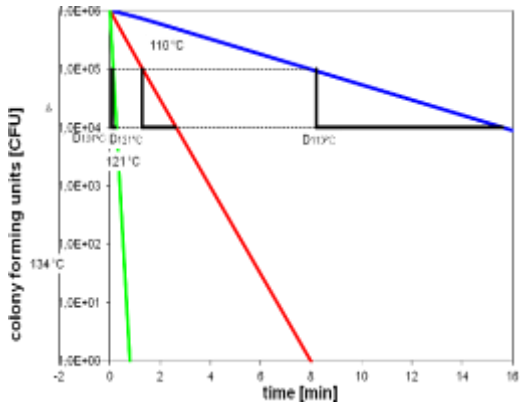


Diagram 2: *Definition of the D-value at different temperatures*

If equation 4 is put into equation 3, the result is:

$$\lg N_0 - \lg N_F = \frac{t}{D_T} = IF \qquad (5)$$

N_0 = Starting bioburden [CFU]
N_F = Number of germs after sterilization [CFU]
D_T = Decimal reduction factor [min] or [Mrad] (D_T-value)
t = Sterilization time [min]
IF = Inactivation factor [number] (Decimal reduction level)

The coefficient of sterilization time divided by the D-value provides also the inactivation factor equivalent to the number of decimal reduction steps.

A D-value time equivalent reduces the population by 90 % or one decimal reduction step.
If the D-value is known, it is possible to calculate the sterilization time to reduce the population by defined amount of decimal reduction steps independent from the starting population.

If the starting population N_0 is changed, the final population is changing accordingly, if the same sterilization process is used. Therefore the starting population, also called bioburden, determines the result of the final number of germs N_F. To get the necessary sterilization time, equation 5 can be changed accordingly:

$$t = (\lg N_0 - \lg N_F) * D_T = IF * D_T \qquad (6)$$

1.4 Experimental determination of the resistance (D_T) of a biological indicator

The resistance (D-value) may be determined with two methods according to EN ISO 11138-1 (see Annex C and D) or with the determination of the survival kill window (see Annex E of the standard).

1.4.1 D-value determination using the MPN-Method (most probable number)

Biological indicators with a defined population are put in several steam sterilization processes with modified sterilization times where all other process variables remain constant except the time. For each sterilization time a minimum of 20 biological indicators is required. After sterilization the biological indicators are checked for growth. A minimum of 7 different sterilization times is to be tested.

a) minimum 1 sterilization time where all biological indicators are growing
b) minimum 4 sterilization times where at least some biological indicators are growing
c) minimum 2 sterilization times where no growth of biological indictors is detected.

Using the above results the D-value will be calculated using the equations below.

Sterilization time	Number of trials	Number of trials without growth
$[t_i]$	$[n_i]$	$[r_i]$
t_1	n_1	$r_1 = 0$
t_2	n_2	r_2
t_3	n_3	r_3
t_4	n_4	r_4
t_5	n_5	r_5
t_6	n_6	r_6
t_7	n_7	r_7

$_{t1}$ is the shortest sterilization time where all BIs should grow. The sterilization times t_1 – t_7 are increasing sterilization times using the result of t_6 and t_7 should not show alive BIs.

Using these data the factors x and y are calculated for the sterilization times $t_1 - t_7$.

$$x_i = \frac{t_i + t_{(i+1)}}{2} \qquad (7)$$

$$y_i = \frac{r_{(i+1)}}{n_{(i+1)}} - \frac{r_i}{n_i} \qquad (8)$$

For t_1 where all samples show growth $r_1 = 0$. In this case y_i is determined by:

$$y_i = \frac{r_{(i+1)}}{n_{(i+1)}} \qquad (9)$$

Using the calculated values of x_i and y_i the sterilization time μ_i may be calculated:

$$\mu_i = x_i * y_i \qquad \text{[min]} \qquad (10)$$

The mean sterilization time $\overline{\mu}$ which does not show growth may be calculated summarizing all μ_i:

$$\overline{\mu} = \sum_{i=0}^{i=6} \mu_i \qquad \text{[min]} \qquad (11)$$

If the interval d between the sterilization times is constant, and the same number of tests for each sterilization time is used, the mean value for no growth $\overline{\mu}$ may be calculated using the following equation:

$$\overline{\mu} = t_6 - \frac{d}{2} - \frac{d}{n} \sum_{i=1}^{i=6} r_i \qquad \text{[min]} \qquad (12)$$

The mean D-value will be calculated using the equation:

$$D = \frac{\overline{\mu}}{0{,}2507 + \lg N_0} \qquad \text{[min]} \qquad (13)$$

where N_0 is the starting population CFU/test.

1.4.2 D-Value Determination using the survivor curve

Biological indicators have to be sterilized with different sterilization times where all process variables have to remain constant except the time.

5 different sterilization times should be used:

a) One exposure in which the sample is not subjected to the sterilant (e.g. 0 time exposure)
b) At least one exposure in which the viable population is reduced to 0.01 % of the original inocula (4 $\log_{10}$ reduction)
c) A minimum of three exposures covering the intervals between exposure a) und exposure b) above.
d) Not less than four test samples shall be used for each exposure in each determination.
e) The same number of replicates shall be used for each exposure.

A minimum of 2 consecutive tests shall be carried out. For each test a minimum of 4 biological indicators shall be used. After sterilization the population of the biological indicator is determined using the method provided by the manufacturer. The logarithm of the remaining germ population is plotted against the sterilization time. The reciprocal slope provides the D-value in minutes provided that the reaction kinetics is first order.

min	Number of germs left after sterilization time	Sterilization Time	Sterility Assurance Level (SAL)	Definition
2,0	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	10^{-1}	
2,0	x = 100	7 D = 14	10^{-2}	
2,0	x = 1.000	8 D = 16	10^{-3}	
2,0	x = 10.000	9 D = 18	10^{-4}	F_{Bio} + 4 = kill of BI
2,0	x = 100.000	10 D = 20	10^{-5}	
2,0	x = 1.000.000	11 D = 22	10^{-6}	= sterile according EN 556-1

Diagram 3: *Time to reduce the amount of germs in a steam sterilization process at 121°C with a D-value of 2 min*

1.4.3. Survival/kill window

The survivor/kill window is defined with the guaranteed survival of a biological indicator. A biological indicator shall contain a minimum of 100 germs, if it has been inside of a sterilization process with the initial population N_0 for the following sterilization time:

Survival time = (log N_0 - 2) x D [min]

The guaranteed kill of a biological indicator occurs after the following sterilization time at 121°C:

Kill time = (log N_0 + 4) x D [min]

This sterilization time determines a sterility assurance level of SAL = 10^{-4} (every 10.000^{th} germ may remain alive).

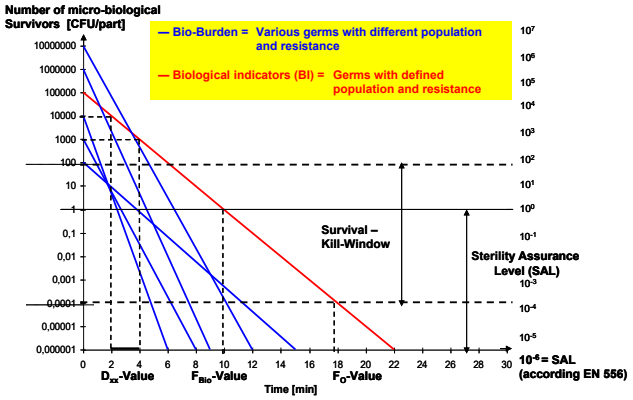


Diagram 4: *Definition of: Bio-Burden, SAL, Biological indicator, F_{Bio}-Value, F_0-Value*

2. Definition of the sterility assurance level (SAL)

The number of germs decreases during a sterilization process first order with each D-value time unit by a power of ten or 90 % of the previous value. After the number of germs has reached 1 CFU with each D-value sterilization time unit the population is decreased by another power of ten reaching 0.1 CFU. Values below 1 do not determine the number of alive germs on a part but determine the probability how many parts still have alive germs. If 10 parts which contain one germ each are sterilized for another D-value time unit, again 90 % of the germs are inactivated. Therefore the value 0.1 CFU expresses that 9 out of 10 parts become sterile and one part is still non-sterile. The value 0.01 or 10^{-2} means accordingly that out of 100 parts 99 parts are sterile and one part is non-sterile. Population values < 1 do not determine the number of germs but the sterility assurance level. This is the ratio between non-sterile and sterile products in one process.

2.1 Definition of a sterile product according to the European standard EN 556-1

The classic definition of sterility determines that non-viable germs are inside a sterile product. The kill kinetics law first order however demonstrates that the SAL-level may be reduced as longer the sterilization process is carried out, however the SAL will never reach zero. That means the probability of sterility may be increased as longer the sterilization is carried out, however the absolute sterility cannot be achieved. Since absolute sterility cannot be achieved, goods may be labelled sterile according EN 556-1 if the SAL ≤ 10^{-6} is reached for terminal sterilized products. For sterile liquid fillings in part 2 of EN 556 an SAL of ≤ 10^{-3} is accepted since the production processes cannot achieve better results. If a sterility assurance level of ≤ 10^{-6} is achieved, those products according to EN 556-1 may be labelled sterile in Europe. In other countries outside of Europe the accepted SAL-level is different depending on the application and defined by local regulations. The direct biological proof for such values cannot be achieved by experimental tests but is available by extrapolation of the straight line of the kill kinetic equation.

3. Temperature dependence of sterilization processes

3.1 Arrhenius equation

As reported in part 1 the constant k' and k and also the D-values are temperature-dependent. This dependency is described by the Arrhenius equation:

$$k = k_0 * e^{\frac{-Ea}{RT}} \qquad (14)$$

R = General gas constant [8,314 J/mol K]
T = Temperature [K]
k = Reaction kinetics constant of the decimal logarithm [min^{-1}]
k_0 = Reaction kinetics constant defines a sterilization process [min^{-1}]
Ea = Activation energy of the process [J/mol]

The constant k_0 depends only on the type of sterilization process, is independent from the temperature and may be experimentally achieved. The activation energy Ea is the energy amount to start the kill reaction. Using the Arrhenius equation the experimental change of the D-value versus the temperature may be derived. This dependence is expressed with the z-value (see diagram 4).

3.2 Definition of the z-value (Temperature coefficient of the D-value)

The z-value describes the dependence of the kill velocity of microorganisms with changing temperature. Mathematically the z-value is the necessary temperature difference to change the D-value by a factor of 10 keeping all other sterilization conditions constant. If D-values are achieved at different temperatures and are put in a half-logarithmic D-value scale against the temperature a straight line is achieved where the reciprocal slope determines the z-value.

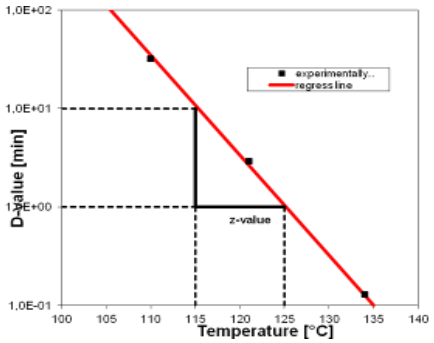


Diagram 5: *Determination of the z-value*

If the z-value is known, D-values at given temperatures may be converted to D-values with another temperature.

$$\frac{1}{z} = -\frac{\lg D_{T1} - \lg D_{T2}}{T_1 - T_2} \qquad (15)$$

4. Sterilization equivalence value ($F_{(T,z)}$-value)

Referring to equation 6 the sterilization time can be achieved multiplying the decimal reduction and inactivation factor. Since the D-value is valid only for one temperature, the sterilization time at different temperatures during the come-up time has to be adjusted to one defined temperature. This sterilization time at one temperature is defined as the equivalent time ($F_{T,z}$) using the index of the temperature and the z-value of the sterilization process. This F-value determines the sterilization time at a constant temperature. The F_0-value expresses the sterilization power of a defined sterilization process and is usually expressed in minutes at a given temperature. The inactivation factor alone is no value for the sterilization power, since germs with low resistance are killed much quicker in comparison to germs with high resistance or D-values.

As shown above the sterilization time at a given temperature may be calculated, if the starting bioburden (N_0) is known to achieve a defined final SAL. In reality a sterilizer is heating up over a certain period until the sterilization plateau temperature of for example 121°C is reached. During the come-up and go-down time between 100 and 121°C germs are killed already .

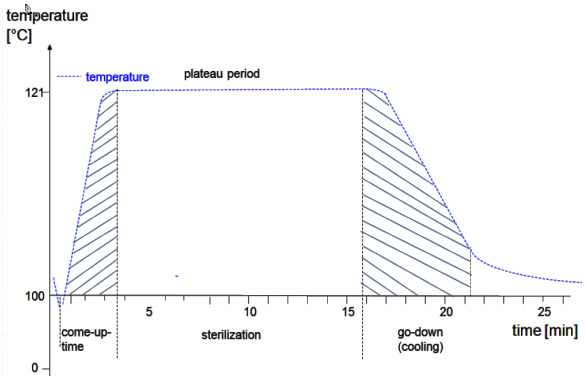


Diagram 6: *F_0-value integral of all time above 100 °C*

This inactivation has to be added to the plateau sterilization time. Is the z-value known, the additional sterilization times outside the plateau period may be recalculated to the temperature at the plateau period. The summary of all time integrals may be added to the total sterilization time of 121°C and is a definition of the equivalence time.

The F-value is a sterilization time at <u>one</u> defined temperature, in radiation sterilization it is defined by a radiation dose.

$$F_{T,z} = (\lg N_0 - \lg N_F) * D_T = IF * D_T \qquad (16)$$

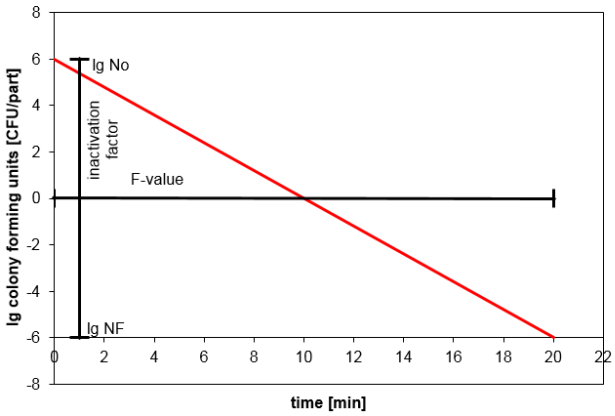


Diagram 7: *Illustration of the F-value*

4.1. Definition of F_0-value

The F_0-value is defined at a sterilization temperature of 121°C and a z-value of 10°C and is used in industry as a reference for sterilization processes.

4.2. Other $F_{T,z}$-values

Other F-values may be defined with other temperatures and z-values. In the metric system the F_c-value is defined at 120°C and z = 10°C.

5. Design of a sterilization process

Before the validation of a sterilization process can be carried out, the sterilization starting conditions have to be known (sterilization type, goods to be sterilized, packaging, etc.). Hydro- and thermo-stable products may be sterilized in steam sterilization processes. Non temperature stable products are sterilized in low temperature sterilization processes in industry with EO or radiation sterilization processes, in health care with Formaldehyde sterilization processes. After the sterilization process is defined, the sterilization process parameters have to be defined that the SAL ≤ 10^{-6} has to be achieved at the end. If the starting bioburden including other constant starting conditions which are available in industry sterilizing new products, the F_0-values can be determined using the starting bioburden and the SAL which has to be achieved. If constant starting conditions cannot be guaranteed like in health care units, a so-called overkill-process is used.

To take care of the sterilized goods and to minimize sterilization times the sterilization time and temperature should be adapted to the necessary kill values only. To achieve this goal not only the necessary process parameters need to be calculated but also it is necessary that all process parameters are kept constant during sterilization.

5.1. Process design with known starting bioburden values

If the starting bioburden of the products to be sterilized is known (types, population and resistance of all germs), the most resistant germs including the z-values have to be determined. If these data are available, the sterilization parameters may be calculated as demonstrated at the following example:

Starting conditions for exercises 1-4:

Starting germ number:
$N_0 = 10^3$ CFU

Expected SAL: $N_F = 10^{-6}$ CFU = SAL = 10^{-6}
D_{121}-value = 1,5 min
z-value = 10°C

Exercise 1:
Calculate the inactivation factor necessary:

IF= lg N_0 – lg N_F
IF = lg 10^3 – lg 10^{-6} = 3 – (-6)=9

The inactivation factor has a value of 9 decimal reduction steps to reach the sterility assurance level SAL = 10^{-6}.

Exercise 2:
Which sterilization time at 121°C is required:

F_0 = (lg N_0 – lg N_F) • D_T

F_0 = (3+6) • 1.5 min = 13.5 min

The necessary equivalence sterilization time for this process at 121°C is 13.5 min.

Exercise 3:
Since the sterilization goods are not stable at 121°C, a sterilization temperature of 110°C should be used. How long is the required sterilization time $F_{110°C,\ z\ =\ 10\ K}$?

1. Calculation of the D-value at 110°C using equation 16:

$$\frac{1}{z} = -\frac{\lg D_{T1} - \lg D_{T2}}{T_1 - T_2}$$

$$z * (\lg D_{T2} - \lg D_{T1}) = T_1 - T_2$$

$$\lg D_{T2} = \lg D_{T1} + \frac{(T_1 - T_2)}{z}$$

$$\lg D_{110°C} = \lg D_{121°C} + \frac{11°C}{z}$$

$$\lg D_{110°C} = \lg 1{,}5 + 1{,}1 = 1{,}276$$

$$D_{110°C} = 10^{1{,}276} = 18{,}8 \quad \text{[min]}$$

2. Calculation of the sterilization time using equation 6:

$F_{110°C,10}$ = (lg N_0 – lg N_F) • D_T
$F_{110°C,10}$ = (3+6) • 18.8 min = 170 min

The sterilization time at 110°C is 2 h, 50 min.

Exercise 4:
Which temperature has to be used that the sterilization time should not be longer than 3 min? The temperature may be above 121°C.

1. Determination of the D-value:

$$D_T = \frac{t}{\lg N_0 - \lg N_F}$$

$$D_T = \frac{3\,\text{min}}{3+6} = 0{,}33\,\text{min}$$

2. Determination of the sterilization temperature

$$\frac{1}{z} = -\frac{\lg D_{T_1} - \lg D_{T_2}}{T_1 - T_2}$$

$$T_1 = z * (\lg D_{T_2} - \lg D_{T_1}) + T_2$$

$$T_1 = 10°C * (\lg 1{,}5 * \lg 0{,}33) + 121°C$$

$$T_1 = 127{,}56°C$$

For a sterilization time of 3 min a temperature of 127.6°C is required.

In diagram 8 all 3 processes are plotted.

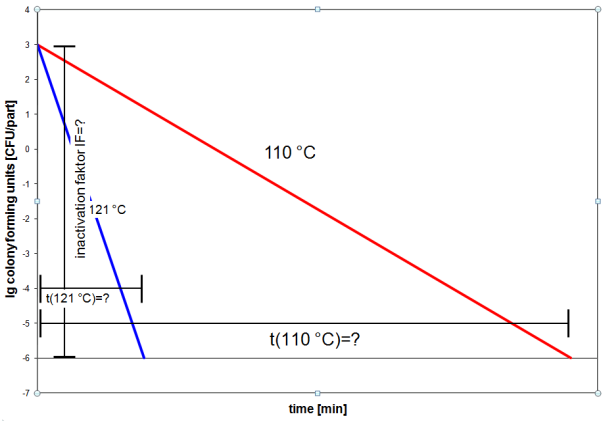


Diagram 8: *Illustration of the examples*

5.2. Process design with unknown or changing starting bioburden values (overkill process)

If the starting conditions like in healthcare units are unknown because of changing load configurations and changing bioburdens, the process has to be developed using worst case conditions. For those conditions minimum F_0-values are described in the European Pharmacopeia (EP) and the US Pharmacopeia (USP). For steam sterilization processes two alternative sterilization times and temperatures are given, however having two different F_0-values:

Temperature [°C]	Time [min]	F_0-value [min]
121	15	15
134	3	> 60

The reason for a much higher F_0value at 134°C is that temperature equilibration times are added. Short sterilization times below 1 min bear the risk that the temperature is not achieved at all locations within the load. Therefore a longer sterilization time is used as required for the F_0-value.

Comparing the same F_0-values at 121°C with 15 min it would be at 134°C only about 0.75 min using a z-value of 10°C. The time of 0.75 min is the at 134°C only. In addition all other times have to be added to reach 134°C. It is not absolutely sure if the sterilization time is reached in the chamber or reached at all surfaces of the goods to be sterilized because non-condensable gases (NCG) may hinder the homogenous heat-up of the goods. If extreme short sterilization times are used this potential problem may occur. Therefore the sterilization time at 134°C has been extended as a safety margin.

6. Requirements and selection of biological indicators for validation and routine monitoring

If there are changing parametric sterilization conditions, like changing steam quality, a validation using parametric release is impossible. In this case only the direct inoculation with biological indicator suspension at worst case conditions can be carried out. In low temperature sterilization processes biological indicators are exclusively used for validation.

6.1. Selection of strain

Depending on the sterilization process non-pathogenic germs are selected having a higher resistance in comparison to pathogenic germs. The international standard EN ISO 11138 recommends individual germs for different sterilization processes. Preferably spore generating germs with defined populations are produced. They remain their population over several years. Only biological indicators with certificate should be used stating the germ, population, D-value, manufacturer and expiry date and are manufactured according to above standard. The certificate also should refer from which culture collection the strain is coming from.

Like:

DSM = Deutsche Sammlung für Mikroorganismen (German collection of Microorganisms)
ATCC = American Type Culture Collection,
NCTC = National Collection of Type Culture (London)

The following table lists most popular strains for different sterilization processes:

Name	**ATCC No:**	**Sterilization process**
Atrophaeus	9372	Ethylene oxide, dry heat
Stearothermophilus	7953	Steam, Formaldehyde, H_2O_2
Pumilus	27142	γ and β radiation

6.2. Resistance of biological indicators:

The total resistance of a biological indicator depends on the population and resistance of each individual germ. The resistance of each individual germ is defined by the decimal reduction value which is the time needed to reduce the population of a biological indicator to one tenth of the original population. The total resistance of a biological indicator is expressed by the F_{BIO} value:

F_{BIO} = $D_{121°C}$ value x log (population)

This fact may be demonstrated by the 2 examples below in the table.

Example	Population [CFU/unit]	D_{121}-value [min]	F_{Bio}-value [min]
1	10^6	1,5	9
2	10^5	2	10

As seen above, the D-value of a given strain is never constant and depends on growth and process condition. Therefore, for each batch of biological indicators certificates must be associated to the product indicating the population, individual resistance and the total resistance of a biological indicator.

6.3. Selection of biological indicators for routine monitoring

For routine monitoring biological indicators have to be selected according the requirements of the international standards and need to be adopted to the F_0-value of the sterilization process. To monitor overkill processes in steam sterilization processes the F_{Bio}-value should be selected that the SAL of the biological indicator at the end of the sterilization process should reach 10^{-4}. Therefore the F_{Bio}-value can be calculated:

$$F_0 = F_{Bio} + 4 \cdot D_{121}$$

$$F_{Bio} = F_0 - 4 \cdot D_{121}$$

The sterility test according EN 556 with biological indicators is directly not possible since it is not feasible to make tests with one million biological indicators. To check if the SAL ≤ 10^{-6} is achieved, the F_{Bio}-value of the biological indicator should be above the bioburden of the load (see diagram 9).

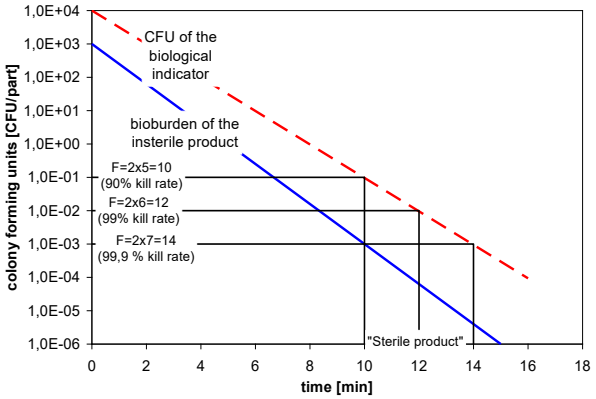


Diagram 9: *Selection of biological indicators*

6.4 Positioning of biological indicators

Biological indicators should never be placed outside of packs. In the standards of biological indicators there are no recommendations given where to position the biological indicators inside of a sterilization load. Biological indicators have to be placed always at the worst case location according to the validation standard, e.g. for steam sterilization processes EN ISO 17665-1, which may be inside of a package with solid goods or inside of instruments containing hollow lumens and/or splits. If biological indicator strips cannot be placed into hollow devices or splits, direct inoculation with biological indicator suspensions have to be made or they have to be placed inside process challenge devices (PCDs).

On top the small load effect and non-condensable gases inside of the sterilizer chamber have to be recognized. Non-condensable gases (NCG) mixed with steam inside the sterilization chamber are transferred in a single pack creating dangerous amounts of NCG inside.

7. Glossary of symbols used within the text

Symbol	Unit Description	Unit	Abbr. Unit	Application Description
CFU	Number of germs	Colony forming unit	number	Amount of germs on a biological indicator
D_T	Decimal reduction factor (D-value) at temperature T	time or dose	[min]	Describes the resistance of a biological indicator, describes the time necessary to kill 90 % of the starting bioburden or reduces population by one decade
E_0	Activation energy of the reaction	Reaction energy	[J/mol]	Reaction energy to start chemical reaction
$F_{(T,z)}$	Equivalence time of a sterilization process	time	[min]	Correlation of all sterilization times at different temperatures to a given reference temperature, expresses the sterilization effort, given as a time at a defined temperature
F_0	Equivalence time of a sterilization process under standardized conditions (i.e. steam 121°C)	time	[min]	For steam sterilization processes 121°C and a set value of 10°C, expresses the sterilization effort
IF	Inactivation factor	amount	N	Population reduction during a sterilization process, expressed by the number of decimal reduction numbers (log difference of population)
k	Reaction kinetics constant of the decimal logarithm	1/time	$[min^{-1}]$	Used if the decade logarithm is used
k'	Reaction kinetics constant of the natural logarithm	1/time	$[min^{-1}]$	Used if the natural logarithm is used
k_0	Temperature dependent factor of the reaction kinetics constant	1/time	$[min^{-1}]$	Specific for individual sterilization processes
N	Nominal population on a medical device	number of germs	[CFU/part]	Number of germs on an instrument
N_F	Number of germs on a MD after a sterilization cycle	number of germs	[CFU/part]	Number of germs on a medical device after a process with sterilization time F has been carried out
N_0	Starting bioburden on a medical device	number of germs	[CFU/part]	Bioburden of a MD before sterilization
PCD	Process challenge device			
R	General gas constant	constant value	[J/mol K]	= 8,314 [J/mol K]
SAL	Sterility Assurance Level			
t	Sterilization time	time	[min]	Time elapsed during sterilization
z	Temperature coefficient	temperature	[°C]	Describes the modification of the D-value depending on the temperature

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