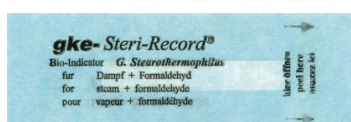


Product Catalogue

for **gke Steri-Record®**
Biological Indicators, Process Challenge
Devices and Accessories



**Spore Strips,
Ampoules, Suspensions,
Self-contained Mini-Bio-Plus Indicators**

to monitor steam, formaldehyde, hydrogen peroxide,
ethylene oxide and dry heat sterilization processes

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General Information about biological indicators

Sterilization procedures in hospitals have reached a high standard of quality. Necessary monitoring procedures are costly, however important, to ensure long-term asepsis in all fields of surgical operations.

International and local standards and directives like the European Medical Device Directive (MDD) require validation, batch monitoring and documentation of sterilization 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.: aluminum 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 1-5. 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 sterilisation processes, *Bacillus atrophaeus* for ethylene oxide and dry heat sterilisation processes and *Bacillus pumilus* for radiation sterilisation 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).

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:

$$F_{BIO} = D_{121^{\circ}C} \text{ value} \times \log (\text{population})$$

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.

gke offers its *Steri-Record*® biological indicators according to EN ISO 11138 series. All packs contain a certificate with all necessary information mentioned above. More information is available from our technical data sheet. Each individual package contains a direction for use.

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). **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 or formaldehyde sterilization processes. For information in more detail, please see our enclosed 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 867-5 "Hollow Load Test" and in DIN 58921 may be used. Biological indicators are used inside to check the penetration of the sterilization agent.

1. **gke Steri-Record® Self-contained biological indicators**

Mini-Bio-Plus self-contained biological indicators (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-PCDs), see 1.5. All SCBI fulfill the requirements according to EN ISO 11138-1.

1.1. **for steam sterilization processes according to EN ISO 11138-3**

G. Stearothermophilus, available with population of 10^5 and 10^6 , paper carrier
Two versions are available:

STEAM

1. **Standard version with incubation time of 24 hours**



Art-No.	Product Code	Colour of cap	Population	Quantity
Standard version (incubation time 24 h)				
324-501	B-S-MBP-10-5	Light blue	10^5	10
324-505				50
324-510				100
324-601	B-S-MBP-10-6	Dark blue	10^6	10
324-605				50
324-610				100

2. **Instant version for immediate release**

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



Art-No.	Product Code	Colour of cap	Population	Quantity
Instant version for immediate release				
324-551	B-S-MBP-I-10-5-SV4 Instant-MBP-SCBI	Light orange	10^5	10
324-555				50
324-550				100
324-651	B-S-MBP-I-10-6-SV4 Instant-MBP-SCBI	Dark orange	10^6	10
324-655				50
324-650				100

1.2. for formaldehyde (LTSF) sterilization processes according to EN ISO 11138-5

FORM

G. stearotherophilus available with population of 10^6 , paper carrier

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.	Product Code	Colour of cap	Population	Quantity
325-601	B-F-MBP-10-6	Yellow	10^6	10
325-605				50

1.3. for all hydrogen peroxide / plasma sterilization processes

VH202

G. stearotherophilus, available with population of 10^6 , free of cellulose carrier



Art-No.	Product Code	Colour of cap	Population	Quantity
327-601	B-V-MPB-10-6	White	10^6	10
327-605				50

1.4. for ethylene oxide sterilization processes according to EN ISO 11138-2

EO

B. atrophaeus, available with population of 10^6 , paper carrier



Art-No.	Product Code	Colour of cap	Population	Quantity
326-605	B-E-MBP-10-6	Red	10 ⁶	50
326-610				100
336-605	B-E-MBP-EP-10-6*			50
336-610				100

(*) Additional the following resistance determinations according to European Pharmacopeia (EP) are carried out:

Kill	25 min 54°C, 600 mg/l EO, 60% rel. humidity
Survival	50 min 30°C, 600 mg/l EO, 60% rel. humidity

1.5 Process Challenge Device for self-contained biological indicators

Bio-C-PCDs, 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.

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 class 2 indicator according to EN ISO 11140-1.

STEAM

VH202

FORM

EO

Each PCD contains 5 seal rings in addition for replacement in the screw cap.



Art-No.	Product code	PCD version	Penetration characteristics
300-010	B-PM-RCPCD-0	round	Very low requirements for air removal
300-011	B-PM-OCPCD-1	oval	Minimal requirements for air removal
300-016	B-PM-RCPCD-1	round	
300-012	B-PM-OCPCD-2	oval	Low requirements for air removal
300-017	B-PM-RCPCD-2	round	
300-013	B-PM-OCPCD-3	oval	Air removal less difficult than Hollow Load Test according to EN 867-5 (Draft EN ISO 11140-6)
300-018	B-PM-RCPCD-3	round	
300-014	B-PM-OCPCD-4	oval	Air removal equal to Hollow Load Test according to EN 867-5 (Draft EN ISO 11140-6)
300-019	B-PM-RCPCD-4	round	
300-015	B-PM-RCPCD-5	round	Air removal more difficult than Hollow Load Test according to EN 867-5 (Draft EN ISO 11140-6)

1.6 Accessories

1.6.1 Replacement parts for PCD



Art-No.	Product code	Quantity
300-005	Replacement screw cap (with M14x1 thread)	5
300-006	Replacement seal kit for all Bio-C-PCDs	5

1.6.2 Crusher for SCBIs

to activate all **gke** SCBIs. The **gke** incubator already includes a crusher.



Art-No.	Product code	Quantity
224-002	I-C	1

2. **gke Steri-Record® Stearo-Ampoules with *G. stearothermophilus* suspension and growth medium according to EN ISO 11138-1 + 3**

STEAM

to monitor extreme wet steam or liquid sterilization processes. The ampoule has a diameter of 11 mm and a height of 45 mm. It contains a 1.5 ml *Geob. Stearothermophilus* suspension with growth medium and pH-indicator, available with nominal populations of 10^5 and 10^6 . The $D_{121^\circ\text{C}}$ values are typically 1.5 – 2 min. However, other $D_{121^\circ\text{C}}$ -values can be delivered on request.



Art-No.	Product code	Population	Quantity
225-550	B-S-AMP-10-5	10^5	50
225-650	B-S-AMP-10-6	10^6	

3. ***gke* Steri-Record® Incubator and accessories**

3.1. Incubators

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 3.2 accessories) has to be ordered separately.

The plug contains a CE conformity for the low voltage directive.



Art-No.	Product code	Content	Temperature [°C]
Incubators including aluminium block for SCBIs			
610-119	I-37-AB-MBP	Incubator, to incubate <i>B. atrophaeus</i> biological indicators	37
610-120	I-57-AB-MBP	Incubator, to incubate <i>G. stearothermophilus</i> biological indicators	57
610-121	I-V-AB-MBP	Incubator with variable temperature selection	30-60
610-122	I-V-T-AB-MBP	Incubator with variable temperature selection and programming of the incubation time	
Incubators without aluminium block			
610-109	I-37	Incubator, to incubate <i>B. atrophaeus</i> biological indicators	37
610-110	I-57	Incubator, to incubate <i>G. stearothermophilus</i> biological indicators	57
610-111	I-V	Incubator with variable temperature selection	30-60
610-112	I-V-T	Incubator with variable temperature selection and programming of the incubation time	

3.2. Accessories

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



Art-No.	Product code	Content	Diameter
610-113	I-AB-MBP	Aluminium block for all <i>gke</i> <i>Steri-Record</i> ® Mini-Bio-Plus SCBIs	10 mm
610-114	I-AB-AMP	Aluminium block for all <i>gke</i> <i>Steri-Record</i> ® Stearo-Ampoules	11 mm
610-115	I-AB-CM	Aluminium block for all <i>gke</i> <i>Steri-Record</i> ® growth medium tubes	14.5 mm

4. **gke Steri-Record® Suspensions and inoculation kit**

The spore suspensions (10 ml) are available in 40% ethanol/water, packaged in glass bottles with rubber septum and comply with EN ISO 11138-1.

4.1. **For steam, formaldehyde and hydrogen peroxide sterilization processes** ***Geob. Stearothermophilus* (ATCC No. 7953)**

The suspension will be delivered with certificate which states nominal population and $D_{121^{\circ}\text{C}}$ -value for steam according to EN ISO 11138-3. *G. Stearothermophilus* is also used for formaldehyde and hydrogen peroxide sterilization processes. A standard for H_2O_2 does not yet exist. The D-value for formaldehyde and hydrogen peroxide* can be determined at extra cost.

STEAM

FORM

VH2O2



Art-No.	Product code	Population	Population per bottle
228-107	B-S-F-V-SUS-10-7	10^7	10^8
228-108	B-S-F-V-SUS-10-8	10^8	10^9

* D-value determination is carried out with individual process parameters

4.2. **For dry heat and ethylene oxide sterilization processes** ***B. atrophaeus* (ATCC No. 9372)**

The suspension is delivered with certificate which states nominal population and D-value for ethylene oxide and dry heat according to EN ISO 11138-2 + 4.

DRY

EO



Art-Nr.	Product code	Population	Population per 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}

4.3. **Direct Inoculation Kit with *Geob. Stearothermophilus* suspension**

to test complex instruments using steam, formaldehyde or hydrogen peroxide sterilization processes. The precision syringe can also be used for ethylene oxide and dry heat sterilization processes if a *B. atrophaeus* suspension is purchased.

STEAM

FORM

VH2O2



Art-No.	Product code	Content
228-110	Inoculation Kit	2x 1.5 ml <i>Geob. Stearothermophilus</i> 10^7 CFU/ml suspension in 40% ethanol with one precision syringe with a needle of 20 cm lengths

5. **gke Steri-Record®** Biological indicator spore strips

The biological indicators consist of bacteria spores inoculated on filter paper strips with the size of 6 x 38 mm and packaged in glassine envelopes. Packages of 100, 500 and 1.000 strips are available. 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). After the spore strips is aseptically removed from the glassine envelope and placed into the PCD like a chemical indicator. At the end of the sterilization process the PCD must not be opened but transferred into a microbiological laboratory where the strip is aseptically removed and incubated. For further information see separate product catalogue.

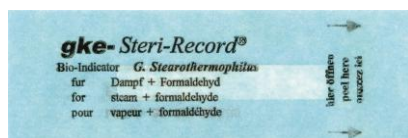
5.1 For steam and formaldehyde sterilization processes

Geob. stearothermophilus (ATCC No. 7953)

For Art.-No. 330-501 both resistance values D_{FA} and $D_{121^{\circ}C}$ are determined according to EN ISO 11138-3 + 5 and are specified in the certificate.

STEAM

FORM



Art-No.	Product code	Sterilization process	Population	Quantity
223-501	B-S-SS-10-5	Steam	10 ⁵	100
223-505				500
223-510				1.000
223-601	B-S-SS-10-6		10 ⁶	100
223-605				500
223-610				1.000
330-501	B-S-F-SS-10-5	Steam + formaldehyde	10 ⁵	100

5.2 For hydrogen peroxide sterilization processes

VH202

Geob. stearothermophilus (ATCC No. 7953)

Spore strips on PET plastic carrier. For resistance determination there is no standard available. The D-values are determined in a resistometer and are specified in the certificate.



Art.-No.	Product code	Population	Quantity
332-601*	B-V-SS-10-6	10^6	100
332-604**			40

(*) Individually packaged

(**) Packaged together for use in PCDs and open applications.

5.3 For ethylene oxide and dry heat sterilization processes

B. atrophaeus (ATCC-No. 9372) according to EN ISO 11138-2 + 4

DRY

EO



Art-No.	Product code	Population	Quantity
221-601	B-E-H-SS-10-6	10 ⁶	100
221-605			500
221-610			1.000
231-601	B-E-H-SS-EP-10-6***		100
231-605			500
231-610			1.000

(***) additional determinations according to EP.

6. Growth media in test tubes

Test tubes with CASO-Boullion (TSB) with pH-indicator and plastic screw cap.

The test tubes have optimized dimensions and volume to fit all kind of spore strips. If germs are growing the pH-indicator changes its colour and allows a quick evaluation of the result.

6.1 For steam and hydrogen peroxide sterilization processes

G. stearothermophilus

STEAM

VH2O2



Art-No.	Product code	Quantity
223-010	B-S-V-CM	10
223-100		100

6.2 For formaldehyde sterilization processes

G. stearothermophilus

The growth medium contains a neutralization agent for remaining formaldehyde, so that the pre-treatment with Na₂SO₃ is not required as described in EN ISO 11138-5.

FORM



Art-No.	Product code	Quantity
330-010	B-F-CM	10
330-100		100

6.3 For ethylene oxide and dry heat sterilization processes

B. atrophaeus

DRY

EO



Art-No.	Product code	Quantity
221-010	B-E-H-CM	10
221-100		100

Basic Principles in Kill Kinetics and Design of Sterilization Processes

Author: Dr. Ulrich Kaiser, *gke* GmbH

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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* ATCC 7953) 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 live 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.

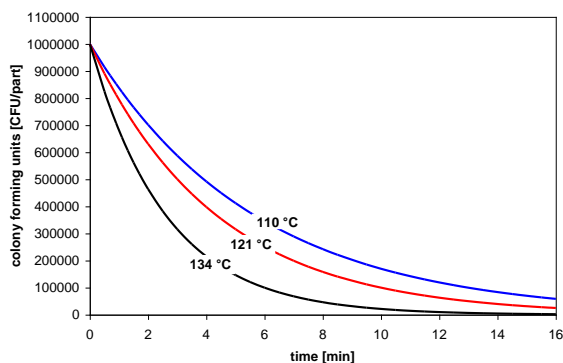


Diagram 1: Survival curve for steam sterilization at different temperatures

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.

$$\frac{dN}{dt} = -k' * N = \text{Reaction velocity} \quad (1)$$

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:

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

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:

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

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 [in 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.

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

D_T = Decimal Reduction Factor [min] or [Mrad] at a tested temperature [t]

k = Reaction kinetics constant of the decimal logarithm [min^{-1}]

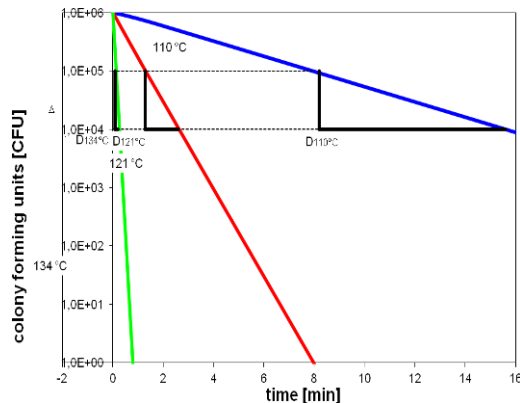


Diagram 2: Definition of the D-value at different temperatures

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

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

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:

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

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 are required. After sterilization the biological indicators are checked for growth. A minimum of 7 different sterilization time are to be tested.

- minimum 1 sterilization time where all biological indicators are growing
- minimum 4 sterilization times where at least some biological indicators are growing
- minimum 2 sterilization times where no growth of biological indicators 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

t_1 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 $t_1 - t_7$.

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

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

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

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

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

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

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

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

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 $\bar{\mu}$ may be calculated using the following equation:

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

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

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

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:

- One exposure in which the sample is not subjected to the sterilant (e.g. 0 time exposure)
- At least one exposure in which the viable population is reduced to 0,01 % of the original inoculums (4 \log_{10} reduction)
- A minimum of three exposures covering the intervals between exposure a) and exposure b) above.

Not less than four test samples shall be used for each exposure in each determination. 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.

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:

$$\text{Survival time} = (\log N_0 - 2) \times D \quad [\text{min}]$$

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

$$\text{Kill time} = (\log N_0 + 4) \times D \quad [\text{min}]$$

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

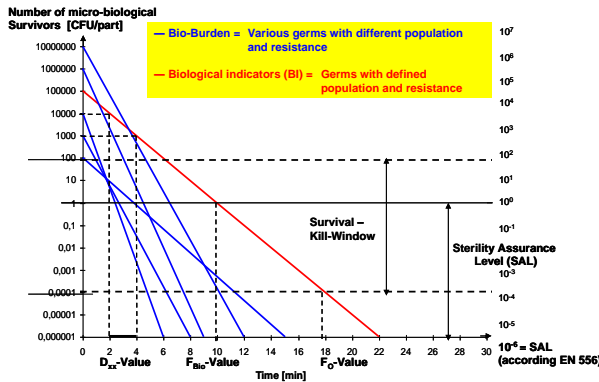


Diagram 3: Definition of: Bio-Burden, SAL, Bio-logical indicator, F_{Bio} -Value, F_0 -Value

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 maybe 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 maybe labelled sterile according EN 556-1 if the $SAL \leq 10^{-6}$ is reached for terminal sterilized products. For liquid fillings in part 2 of EN 556 an SAL of $\leq 10^{-3}$ is accepted since the production processes cannot achieve better results. If a sterility assurance level of $\leq 10^{-6}$ 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:

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

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}]
E_a	= 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 E_a 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, see diagram 4.

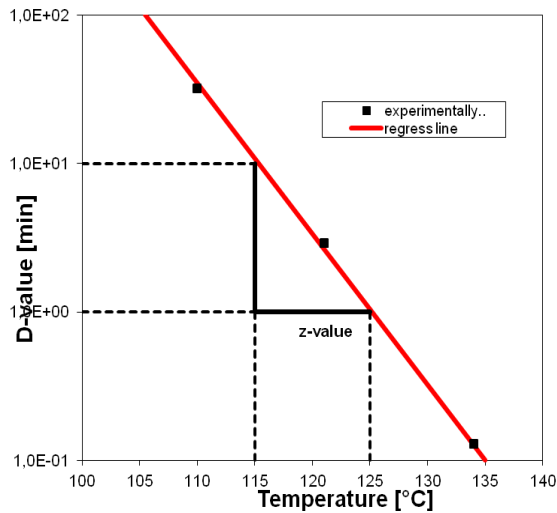


Diagram 4: Determination of the z-value

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

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

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-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 already germs are killed.

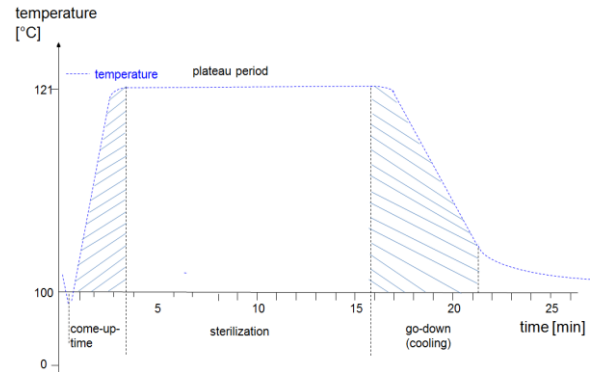


Diagram 5: F_0 -value integral of all times above 100°C

This inactivation has to be added to the plateau sterilization time. If the z-value is 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 one defined temperature, in radiation sterilization it is defined by a radiation dose.

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

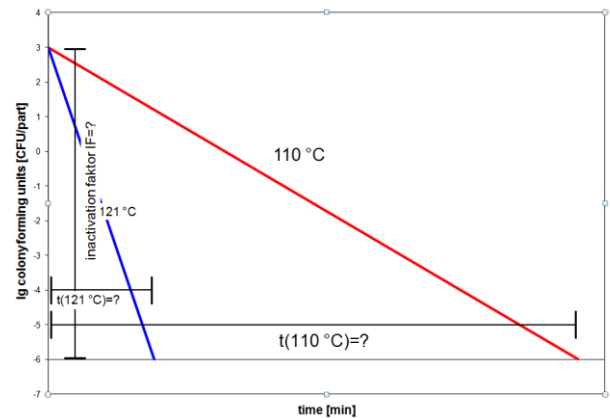


Diagram 6: Illustration of the F-value

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 a z = 10°C.

5. Design of sterilization processes

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-value $\leq 10^{-6}$ 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 value 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:

$$N_0 = 10^3 \text{ CFU}$$

Expected SAL: $N_F = 10^{-6} \text{ CFU} = \text{SAL} = 10^{-6}$

D_{121} -value = 1,5 min

z-value = 10°C

Exercise 1:

Calculate the inactivation factor necessary:

$$\text{IF} = \lg N_0 - \lg N_F$$

$$\text{IF} = \lg 10^3 - \lg 10^{-6} = 3 - (-6) = 9$$

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

Exercise 2:

Which sterilization time at 121°C is required:

$$F_0 = (\lg N_0 - \lg N_F) \cdot D_T$$

$$F_0 = (3+6) \cdot 1,5 \text{ min} = 13,5 \text{ min}$$

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 $F_{110^\circ\text{C}, z=10 \text{ K}}$?

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

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

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

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

$$\lg D_{110^\circ\text{C}} = \lg D_{121^\circ\text{C}} + \frac{11^\circ\text{C}}{z}$$

$$\lg D_{110^\circ\text{C}} = \lg 1,5 + 1,1 = 1,276$$

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

2. Calculation of the sterilization time using equation 6:

$$F_{110^\circ\text{C}, 10} = (\lg N_0 - \lg N_F) \cdot D_T$$

$$F_{110^\circ\text{C}, 10} = (3+6) \cdot 18,8 \text{ min} = 170 \text{ min}$$

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

Exercise 4:

Which temperature has to be used that the sterilization time should not be longer than 3 minutes? 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

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

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

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

$$T_1 = 127,56^\circ\text{C}$$

For a sterilization time of 3 minutes a temperature of 127,6°C is required. In diagram 6 all 3 processes are plotted.

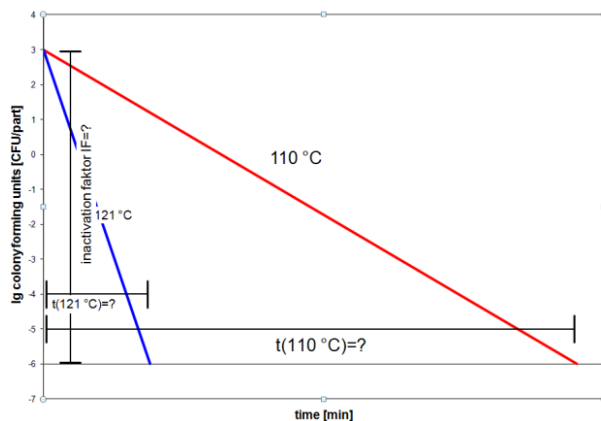


Diagram 7: Illustration of the examples

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

If the starting conditions like in health care 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 Pharmacopoeia (EP) and the US Pharmacopoeia (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_0 value at 134°C is that temperature equilibration times are added. Short sterilization times below 1 minute

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 the all other times have to be added to reach 134°C. It is not absolute 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
<i>Atrophaeus</i>	9372	ethylene oxide, dry heat
<i>Stearothermophilus</i>	7953	steam, formaldehyde, H ₂ O ₂
<i>Pumilus</i>	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:

$$F_{\text{Bio}} = D_{121^{\circ}\text{C}} \text{ value} \times \log (\text{population})$$

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-value of the biological indicator at the end of the sterilization process should reach 10^{-4} . Therefore the F_{Bio} -value can be calculated:

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

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

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 $\text{SAL} \leq 10^{-6}$ is achieved, the F_{Bio} -value of the biological indicator should be above the bioburden of the load (see diagram 8).

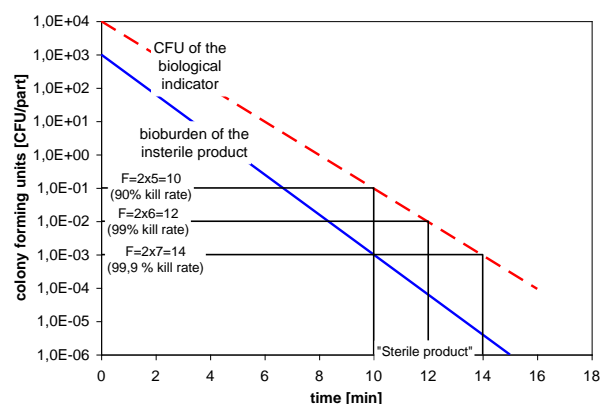


Diagram 8: Selection of biological indicators

6.4 Positioning of biological indicators

Biological indicators should be never 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 always be placed 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

Sym- bol	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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gke GmbH

Auf der Lind 10

D-65529 Waldems

Germany



+49 61 26 94 32 - 0



+49 61 26 94 32 - 10



info@gke.eu



www.gke.eu