F_0

a technical note

- What it means
- How to calculate it
- How to use it for adjustment, control and validation of moist-heat sterilization processes

FEDEGARI GROUP

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What it means
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How to use it for adjustment, control and validation of moist-heat sterilization processes

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The F_0 algorithm was first introduced in 1968 in the international practice of food industry, and proposed by FDA in 1976 for the pharmaceutical sterilization of Large Volume Parenterals: it is now officially included in most Pharmacopoeias.

Yet, F_0 is still regarded with some suspicion from a conceptual point of view, and frequently misinterpreted. It is always necessary to remember that F_0 has been invented in the industrial field of heat sterilization processes of water-containing products.

The purpose of this Fedegari Technical Note, firstly distributed in 1988 and perseveringly revised, is to clarify the nature of F_0 and its related parameters (D, z, PNSU/SAL), and to explain their use and limits for the setting, adjustment, control and validation of moist-heat sterilization processes.

THE AUTHORS

CONTENTS

1. ESSENTIALS OF MOIST-HEAT STERILIZATION KINETICS

Let us suppose to immerse in saturated (i.e. condensing) steam, at constant temperature, a system contaminated by a microbiological species (which we assume, for the sake of simplicity, to be pure and homogeneous): e.g. a vial containing an aqueous suspension of a certain sporogenous microorganism.
It has been experimentally shown that, under the above conditions, the reaction of thermal degradation of the microorganism at issue obeys the laws of chemical reactions.
Using N to indicate the number of microorganism present in the system at a given moment, the variation of this number as the function of a chosen time t of exposure to the selected sterilization temperature can be written as:

$$\frac{dN}{dt} = -K\,N$$

where K is a constant which is typical of the species and conditions of the chosen microorganism.

The degradation reaction, i.e. the sterilization reaction, therefore develops like a first order chemical reaction (i.e. like a chemical decomposition reaction) in which the reaction rate is proportional, in each moment, only to the amount of product still to be degraded (or decomposed).

This seems to be obvious for dry sterilization, but less rigorous for steam sterilization, in which the water vapor molecules also seem to take part in the reaction. Actually, this bimolecular reaction is of the first order, since the steam is present in high excess all the reaction long and its concentration may be regarded as constant.
The above expression can be developed as follows:

$$\frac{dN}{N} = -K\,dt \qquad (1)$$

$$\frac{dN}{N} = -K \qquad dt$$

and, by converting from base *e* or Naperian logarithms, which are less practical in this specific case, to base 10 logarithms, the following is obtained:

$$\log N = -k\,t + \text{constant}$$

where $k = \frac{K}{2.303}$ due to the shift from base e logarithms to base 10 ones.

At time zero, the following is true:

$$t = 0$$
$$N = N_0$$

therefore

$$\log N_0 = \text{constant}$$

from which

$$\log N = -k\,t + \log N_0 \qquad (2)$$

which leads to

$$\log\frac{N}{N_0} = -kt$$

and therefore

$$\frac{N}{N_0} = 10^{-kt} \qquad (3)$$

where:

- N_0 = initial number of microorganism
- t = elapsed exposure (= sterilization) time
- N = number of microorganism after the exposure time t
- k = reaction rate constant which depends on the species and conditions of the microorganism

Expression (3) shows that the number of microorganism decreases exponentially depending on the sterilization time. If this expression is converted into a chart, with log N as the function of t, Diagram 1 is obtained:

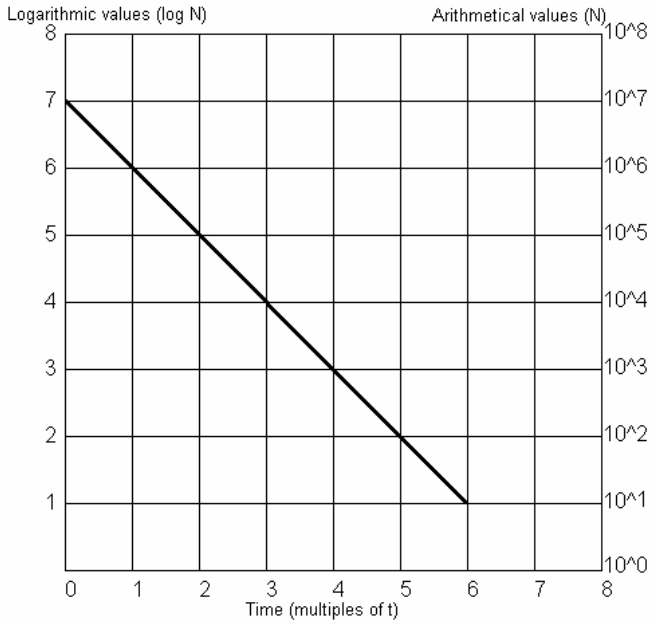


Diagram 1

Here we see that a constant percentage reduction of the concentration of viable microorganism occurs for each arbitrary time interval t. We can therefore draw a first conclusion:

The time required to reduce the microorganism concentration to any pre-set value is the function of its initial concentration.

The sterilization reaction is therefore neither an "all-or-nothing" process nor a "potential barrier" process as was once thought.

1.1. D-VALUE OR DECIMAL DECAY TIME

The D-value is defined as the decimal (or decadal) decay (or reduction) time: i.e. it is the time required, at a specified temperature T, to reduce the microbial population being considered by one logarithmic value, i.e. from 100% to 10% of the initial value.

2007, PDA has given to this parameter the name of Resistance value.

It is very easy to calculate the D-value on the base of the above expression (3): **it is the reciprocal of the reaction rate k**, since if $t = k^{-1}$, it is $N = 0.1N_0$.

At the temperature of 121°C, the D-values generally oscillate between 0.2 and 2 minutes: very often $D_{121} = 1$ is assumed in the absence of more specific experimental data. It is immediately evident that the result of sterilization at constant temperature can be very different depending on the D-value of the contaminating microbial species (or on the largest D-value, in case of mixed contamination). The following graph shows that a residual contamination of 10^{-6} is achieved in eight minutes, starting from an initial unit contamination of 10^2, at 121°C if D = 1. Sixteen minutes are required for the same result if D = 2 and 4 are sufficient if D = 0.5 (see Diagram 2).

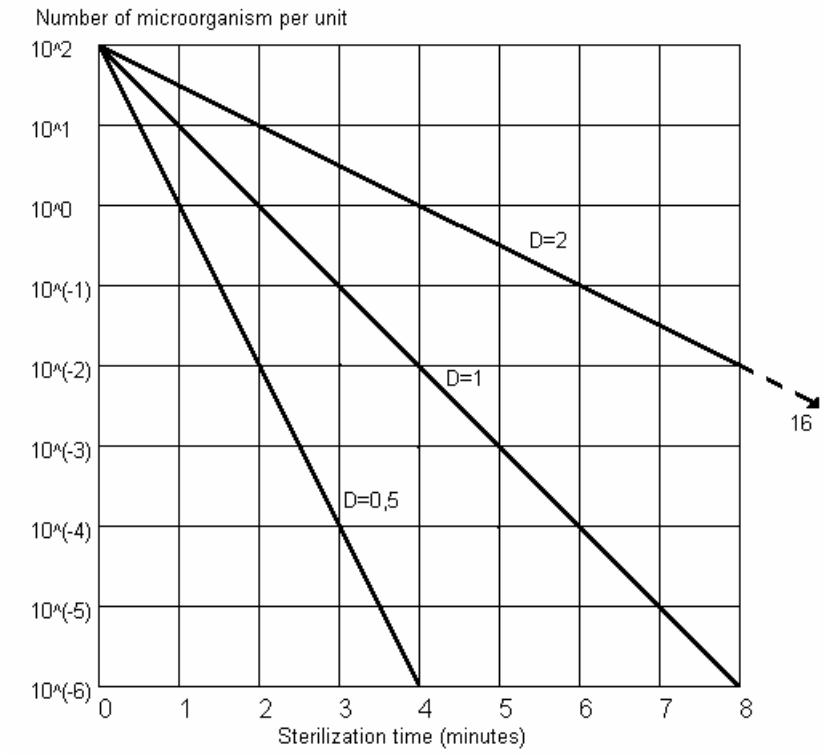


Diagram 2

1.2. STERILITY AS "PROBABLE EFFECT" OF EXPOSURE TIME

Let us now consider what happens within a batch of units (vials, bottles or others) with an initial constant unit contamination of 100 microorganisms = 10^2. If the D-value at 121°C is assumed = 1, after one minute at 121°C, the reduction = to 10^1 = 10 microorganisms is achieved; after another minute, only 10^0 = 1 microorganism is still surviving. After another minute the surviving microbial population would be 10^{-1} = 1/10 microorganism.

A contamination of 1/10 must not be understood to mean that each unit contains 1/10 of a microorganism, which is biologically meaningless (in this case the unit would probably be sterile...) but that there is a probability of having 1/10 of the units still contaminated within the batch of sterilized units.

In fact, three minutes would be the necessary time to reduce the microbial population to a single surviving microorganism if the initial population were ten times larger than the one at issue. This higher initial contamination could be regarded either as a ten times larger number of microorganism in the same unit, or as the initial contamination of a ten times larger unit. If the unit is not considered any longer as the single vial or bottle, but as the whole of all the items produced over a period of time, the initial number of microorganism present in each item has to be multiplied times the number of items produced, and the exposure time to achieve the reduction to the same number of viable microorganism left in the whole of the items produced, has to be correspondingly increased.

The following example will be helpful to focus the matter.

A new sterile product in ampoules has to be launched; the number of ampoules to be produced over all the life period of the product is expected to be 10^{10}. The maximum number of contaminated ampoule deemed to be acceptable is $10^{0} = 1$: this obviously means that the probability of having non sterile ampoules after the sterilization must not exceed 10^{-10}. Let us also suppose that the microbial population within each ampoule after the filling and the sealing does not exceed 10^{3} microorganisms (this is a rather conservative approach, indeed): these must be destroyed by mean of moist-heat terminal sterilization at 121°C. The applicable D-value is 1 minute.
The total number of microorganism to be destroyed during the life of the product will be:

$$10^{10+3} = 10^{13}$$

If this whole microbial population were exposed to moist-heat at 121°C over a period of thirteen minutes, it would be reduced to 10^{-13} times it initial number, i.e. to $10^{13-13} = 10^{0} = 1$. The exposure time of thirteen minutes would thus be sufficient (under all the other above hypotheses) to prevent the total number of contaminated ampoules from exceeding the value of one. From the point of view of each single ampoules, thirteen minutes of exposure would reduce the microbial population to the theoretical value of :

$$10^{3-13} = 10^{-10}$$

To interpret this numeric value as the probability of still having one contaminated ampoule in ten thousand million sterilized ampoules means that a single ampoule will still be contaminated out of a whole of 10^{10} (or ten ampoules out of a whole of 10^{11}).

This probability value is defined as PNSU (Probability of Non Sterile Unit).

In recent times the name PNSU as sterility evaluation criterion has been replaced, at least in Europe, by the SAL (Sterility Assurance Level). The new name has generated some controversy, since a level of assurance is commonly deemed to be good if high, but SAL has officially been defined by European Pharmacopoeia in such a way that its numerical value is the same of PNSU. The name SAP (Sterility Assurance Probability) had been proposed as well, but without any success.

The above discussion and example lead to the conclusion that the optimum exposure time of a sterilization process must take in due account not only the initial microbial population within the single item to be sterilized and the species and conditions of the contaminating microorganism, but also the total number of items expected to be sterilized over the life period of the product.

The survival lines so far examined are merely theoretical. Actually, the lines are not straight and the most common difference is that they are concave or convex, especially for high concentrations: i.e. they resemble the path of curves B and C with respect to the theoretical straight-line path A (see Diagram 3).

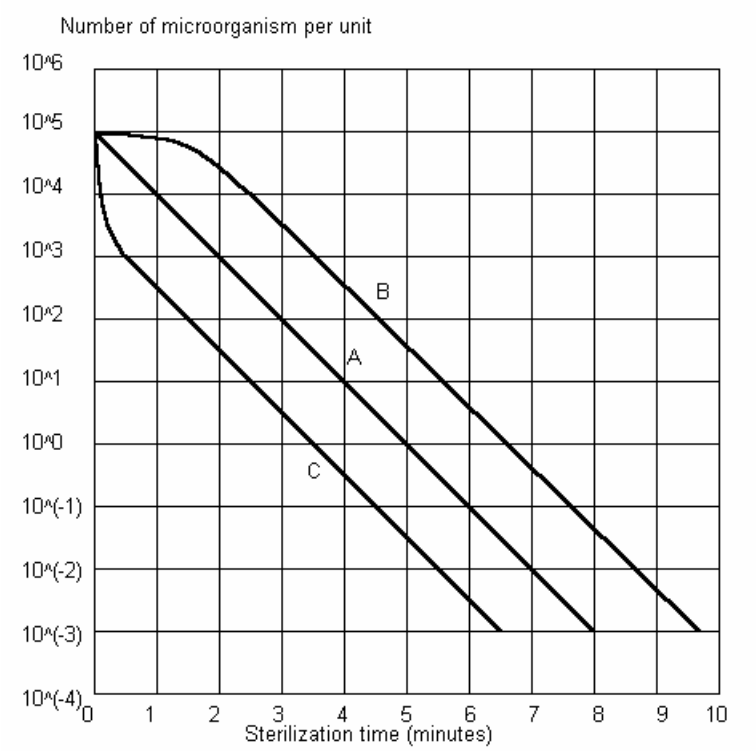


Diagram 3

1.3. z-VALUE OR TEMPERATURE COEFFICIENT

All the above considerations have been developed under the basic assumption that the temperature of the condensing steam is kept constant for all the duration of the exposure. It seems rather obvious that the D-value will change as the temperature changes. If the D-values obtained from experimental data for a given microbial species are plotted on a semi-logarithmic chart as the function of the temperature T, a path similar to Diagram 4 is obtained:

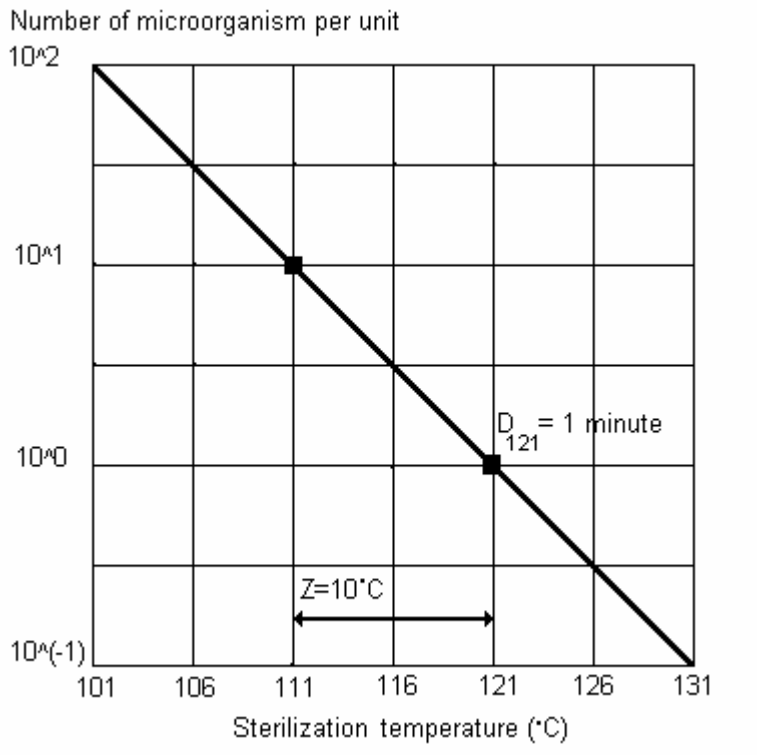


Diagram 4

In this case, it can be seen that D-value is 1 minute at 121°C (i.e. the average value which is very often assumed to be acceptable in the absence of more precise experimental data). It can also be seen that, in the example of Diagram 4, D-value varies by a factor of 10 if the temperature varies by 10°C.

The z-value is defined as the temperature coefficient of microbial destruction, i.e. as the number of degrees of temperature which causes a 10-fold variation of D (or, more generally, of the sterilization rate).

The z-values generally oscillate between 6 and 13 for steam sterilization in the range 100 to 130°C; z-value is often assumed to be equal to 10 in the absence of more precise experimental data.

The fact that D-value varies by 10 times for a variation of 10°C, when z = 10, must not lead to the false assumption that D varies by one time (i.e. doubles) for an increase of 1°C; obviously this is not true.
It is actually a matter of finding the number which yields 10 when raised to the tenth power. This number is 1.2598...

Therefore a variation of 1°C entails a variation of D-value of 26%.

This is quite a large percentage which illustrate the dramatic effects which are generated when the sterilization temperature is also only a few degrees lower than the expected value, perhaps only in some point of the load.

It is also useful to remember that the effect of temperature variation decreases considerably both as the temperature raises and if the sterilization method is changed: z-value drops to approximately one half (and even less) for dry sterilization at approximately 200°C. Under these conditions, z-value is about 20 instead of about 10. Therefore, the small temperature differences which can be so dramatic in steam sterilization are much less effective in dry sterilization.

Table 1 lists "average" D-values and z-values for some "typical" microorganisms; in fact the actual D-values and z-values depend to a large extent on the medium which contains the microorganisms and on their history.

AVERAGE VALUE OF D AND z FOR SOME TYPICAL MICROORGANISMS		
Microorganism	D_{121} (minutes)	z (°C)
Clostridium botulinum	0.2	10
Geobacillus stearothermophilus	2.0	6
Bacillus subtilis	0.5	10
Bacillus megaterium	0.04	7
Clostridium sporogenes	0.8 - 1.4	13
Clostridium histolyticum	0.01	10

Table 1

Actually, at 121°C no microorganism has exactly D = 1' and z = 10 °C. However, the combined use of these two parameters in calculating F_0 and PNSU provides ample margins of safety for the microorganisms which are commonly dealt with.

1.4. F_0 OR EQUIVALENT EXPOSURE TIME AT 121°C

As seen above, D-value is a function of the exposure temperature T in saturated (i.e. condensing) steam conditions for each different microorganism:

$$D = D(T)$$

On the basis of the definition of coefficient z it has also to be:

$$D(T - z) = D(T) * 10$$

With the obvious condition that $D = D_0$ if $T = T_0$, the mathematical function which satisfies the above relationship is (see further explanation in the Note at the end of this paragraph):

$$D = D_0 * 10^{\frac{T_0 - T}{z}} \qquad (4)$$

where D_0 is the value of D at the temperature T_0 and for a given microorganism.

The basic assumption which leads to the above formula is obviously that the z-value is the same on both sides of the reference temperature T_0. No doubt this is not true form a rigorous point of view, but it has proven to be both a helpful and a safe enough abstraction.

Let us now calculate the time interval required to obtain at a constant temperature T_0 the same reduction of a microbial population obtained at the actual exposure temperature T, continuously variable over a certain time interval t.
It has obviously to be:

$$\int_0^{t_0} \frac{dN_{T_0}}{N} = \int_0^{t} \frac{dN_T}{N}$$

and recalling expression (1) and the definition of D-value:

$$\int_0^{t_0} \frac{dt_0}{D_0} = \int_0^{t} \frac{dt}{D}$$

D-value is variable with the actual exposure temperature and is given by expression (4), but D_0 is a constant, so we may write:

$$t_0 = \int_0^{t} 10^{\frac{T-T_0}{z}} dt \qquad (5)$$

It is thus possible to calculate the lethal effect of the exposure of a microbial population to a variable temperature T by relating it to a hypothetical sterilization performed at a constant temperature T_0 for the time t_0 .
If the constant reference temperature is assumed equal to 121.11°C (originally 250 F) and the z-value equal to 10, the equivalent time given by expression (5) is named F_0:

$$F_0 = \int_0^{t} 10^{\frac{T-121.11}{10}} dt \qquad (6)$$

F_0 is the *equivalent* exposure time at 121.11°C of the *actual* exposure time at a variable temperature, calculated for an ideal microorganism with a temperature coefficient of destruction equal to 10 °C.

Firstly introduced by the National Canners Association in 1968 (a), F_0 became a topic in pharmaceutical production since the FDA used it extensively in the historical (even if never come into force and finally repealed) *Proposed Rules* of June 1st, 1976 (b), with the following definition (section 212.3):

"F_o means the equivalent amount of time, in minutes at 121°C or 250 F, which has been delivered to a product by the sterilization process".

For the practical calculation of F_0, "a z-value of 10°C or 18 F is assumed; the term z-value means the slope of the thermal death time curve and may be expressed as the number of degrees... required to bring about a tenfold change in the death rate".
In most cases, the exact value of 121.11 °C is replaced by an approximated 121 °C. Furthermore, the knowledge of the temperature values as the continuous function of elapsing time is generally not available, and F_0 is calculated as follows:

$$F_0 = \Delta t \sum 10^{\frac{T-121}{z}} \qquad (7)$$

where:

- Δt = time interval between two next measurements of T
- T = temperature of the sterilized product at time t
- z = temperature coefficient, assumed to be equal to 10°C

If we assume a sterilization lasting 15 minutes, constantly at 121°C, we obtain:

$$F_0 = 15 * 10^{\frac{121-121}{10}} = 15 * 10^0 = 15 * 1 = 15'$$

indeed according to the definition of F_0.

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

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

Therefore, a 15 minutes sterilization at 111°C is equivalent, in terms of lethal effect, to 1.5 minutes at 121°C; this can be easily expected if z = 10.
Similarly, if we assume a 15 minutes sterilization constantly at 124°C, we have:

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

It has to be emphasized that the mathematical equivalence between different levels of temperature keeps a biological value if and only if the exposure in all conditions is actually to moist-heat, i.e. if there is actual presence of saturated steam on the surface or inside the object to be sterilized.

MATHEMATICAL NOTE. The Laplace transform of a given function f(x) is the function L[f(x)] = F(y) defined as:

$$L\left[f(x)\right] = F(y) = \int_0^{+\infty} e^{-yx} * f(x)dy$$

The following is easy to verify: if F(y) is the Laplace transform of the function f(x), then $e^{-yz} * F(y)$ is the Laplace transform of the function f(x-z).

Now let us consider the equation $D(T - z) = D(T) * 10$ and the Laplace transforms of both members of it.

$$F(y) = L\left[D(T)\right]$$
$$e^{-yz} * F(y) = L\left[D(T - z)\right]$$

Then we can write:

$$e^{-yz} * F(y) = 10 * F(y)$$

The obvious solution of this equation:

$$y = -\frac{ln10}{z}$$

is the value of the pole of the Laplace anti transform of the function D(T).

$$L\left[D(T)\right] = \frac{c}{y + \frac{ln10}{z}}$$

where c is constant.

By transforming the above equation we obtain:

$$D(T) = c * e^{-\frac{ln10 * T}{z}} = c * 10^{-\frac{T}{z}}$$

The value of c can be calculated with the condition $D = D_0$ if $T = T_0$.

The final solution is then: $D = D_0 * 10^{\frac{T_0 - T}{z}}$

1.5. LETHAL RATES

Due to its exponential expression, the calculation of F_0 is not immediate. Tables have therefore been developed which list the so-called *Lethal Rates*, i.e. the equivalence coefficients allowing to compare the exposure at the temperature T to the exposure for the same time at 121°C. Lethal Rates may also be regarded the F_0-values for single unit of time.

Tables 2 and 3 show two examples of F_0 calculation. In Table 2, it is assumed z = 10 °C, and therefore F_0-values are calculated by its rigorous definition at 121.11 °C (250°F). In Table 3, z-values are assumed as variable between 7 and 12 and different equivalent times at 121°C are calculated on such a basis. It is interesting to notice how much the variation of z-value considerably influences the Lethal Rates when T varies.

It should also be noted from Table 3 that the absolute change of Lethal Rates for the same change of temperature is bigger if z-value decreases, than if it rises. This depends on the position of z-value as denominator of the fraction which is the exponent of the expression of F_0.

In both senses, the effect of temperature changes is much greater as the z-value becomes smaller.

TABLE OF LETHAL RATES

in condition of saturated steam for a reference temperature of 121.11°C with z = 10°C, and for temperature values between 90°C and 130°C, with intervals of 0.1°C

T°C	+0.0	+0.1	+0.2	+0.3	+0.4	+0.5	+0.6	+0.7	+0.8	+0.9
	LETHAL RATE									
90	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001
91	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001
92	.001	.001	.001	.001	.001	.001	.001	.001	.001	.002
93	.002	.002	.002	.002	.002	.002	.002	.002	.002	.002
94	.002	.002	.002	.002	.002	.002	.002	.002	.002	.002
95	.002	.003	.003	.003	.003	.003	.003	.003	.003	.003
96	.003	.003	.003	.003	.003	.003	.004	.004	.004	.004
97	.004	.004	.004	.004	.004	.004	.004	.005	.005	.005
98	.005	.005	.005	.005	.005	.005	.006	.006	.006	.006
99	.006	.006	.006	.007	.007	.007	.007	.007	.007	.008
100	.008	.008	.008	.008	.008	.009	.009	.009	.009	.010
101	.010	.010	.010	.010	.011	.011	.011	.011	.012	.012
102	.012	.013	.013	.013	.013	.014	.014	.014	.015	.015
103	.015	.016	.016	.017	.017	.017	.018	.018	.019	.019
104	.019	.020	.020	.021	.021	.022	.022	.023	.023	.024
105	.024	.025	.026	.026	.027	.027	.028	.029	.029	.030
106	.031	.032	.032	.033	.034	.035	.035	.036	.037	.038
107	.039	.040	.041	.042	.043	.044	.045	.046	.047	.048
108	.049	.050	.051	.052	.054	.055	.056	.057	.059	.060
109	.062	.063	.064	.066	.067	.069	.071	.072	.074	.076
110	.077	.079	.081	.083	.085	.087	.089	.091	.093	.095
111	.097	.100	.102	.104	.107	.109	.112	.115	.117	.120
112	.123	.126	.128	.131	.135	.138	.141	.144	.148	.151
113	.154	.158	.162	.166	.169	.173	.177	.182	.186	.190
114	.194	.199	.204	.208	.213	.218	.223	.299	.234	.239
115	.245	.251	.256	.262	.268	.275	.281	.288	.294	.301
116	.308	.315	.323	.330	.338	.346	.354	.362	.371	.379
117	.388	.397	.406	.416	.426	.435	.446	.456	.467	.477
118	.489	.500	.512	.523	.536	.548	.561	.574	.587	.601
119	.615	.629	.644	.659	.674	.690	.706	.723	.739	.757
120	.774	.792	.811	.830	.849	.869	.889	.910	.931	.953
121	.975	.997	1.021	1.044	1.069	1.093	1.119	1.145	1.172	1.199
122	1.227	1.256	1.285	1.315	1.346	1.377	1.409	1.442	1.475	1.510
123	1.545	1.581	1.618	1.655	1.694	1.733	1.774	1.815	1.857	1.901
124	1.945	1.990	2.037	2.084	2.133	2.182	2.233	2.285	2.338	2.393
125	2.448	2.506	2.564	2.624	2.685	2.747	2.811	2.877	2.994	3.012
126	3.082	3.154	3.228	3.303	3.380	3.459	3.539	3.622	3.706	3.792
127	3.881	3.971	4.063	4.158	4.255	4.354	4.456	4.559	4.666	4.774
128	4.885	4.999	5.116	5.235	5.357	5.482	5.608	5.740	5.874	6.010
129	6.150	6.294	6.440	6.590	6.744	6.901	7.062	7.226	7.394	7.567
130	7.743	7.293	8.108	8.297	8.490	8.688	8.890	9.097	9.309	9.526

Table 2

TABLE OF LETHAL RATES

in condition of saturated steam for a reference temperature of 121°C and z-values of 7°C to 12°C; and for temperature values between 100°C and 130°C, with intervals of 0.5°C

TEMPERATURE (°C)	z-VALUES (°C)					
	7	8	9	10	11	12
	LETHAL RATE					
100	.001	.002	.005	.008	.012	.018
101	.001	.003	.006	.010	.015	.022
102	.002	.004	.008	.013	.019	.026
103	.003	.006	.010	.016	.023	.032
104	.004	.007	.013	.020	.028	.038
105	.005	.010	.017	.025	.035	.046
106	.007	.013	.022	.032	.043	.056
107	.010	.018	.028	.040	.053	.068
108	.014	.024	.036	.050	.066	.083
109	.019	.032	.046	.063	.081	.100
110	.026	.042	.060	.079	.100	.121
111	.037	.056	.077	.100	.123	.147
112	.052	.075	.100	.126	.152	.178
113	.072	.100	.129	.158	.187	.215
114	.100	.133	.167	.200	.231	.261
114.5	.118	.154	.190	.224	.257	.287
115	.139	.178	.215	.251	.285	.316
115.5	.164	.205	.245	.282	.316	.348
116	.193	.237	.278	.316	.351	.383
116.5	.228	.274	.316	.355	.390	.422
117	.268	.316	.359	.398	.433	.464
117.5	.316	.365	.408	.447	.481	.511
118	.373	.422	.464	.501	.534	.562
118.5	.439	.489	.527	.562	.593	.619
119	.518	.562	.599	.631	.658	.681
119.5	.611	.649	.681	.708	.731	.750
120	.720	.750	.774	.794	.811	.825
120.5	.848	.886	.880	.891	.901	.909
121	1.00	1.00	1.00	1.00	1.00	1.00
121.5	1.11	1.16	1.14	1.12	1.11	1.10
122	1.39	1.33	1.29	1.23	1.22	1.21
122.5	1.64	1.54	1.47	1.14	1.37	1.33
123	1.93	1.78	1.67	1.59	1.52	1.47
123.5	2.28	2.05	1.90	1.78	1.69	1.62
124	2.68	2.37	2.15	2.00	1.87	1.78
125	4.39	3.16	2.78	2.82	2.31	2.15
126	5.18	4.22	3.59	3.16	2.85	2.61
127	7.20	5.62	4.64	3.98	3.51	3.16
128	10.0	7.50	6.00	5.01	4.33	3.83
129	13.9	10.0	7.74	6.31	5.34	4.64
130	19.3	13.3	10.0	7.94	6.58	5.62

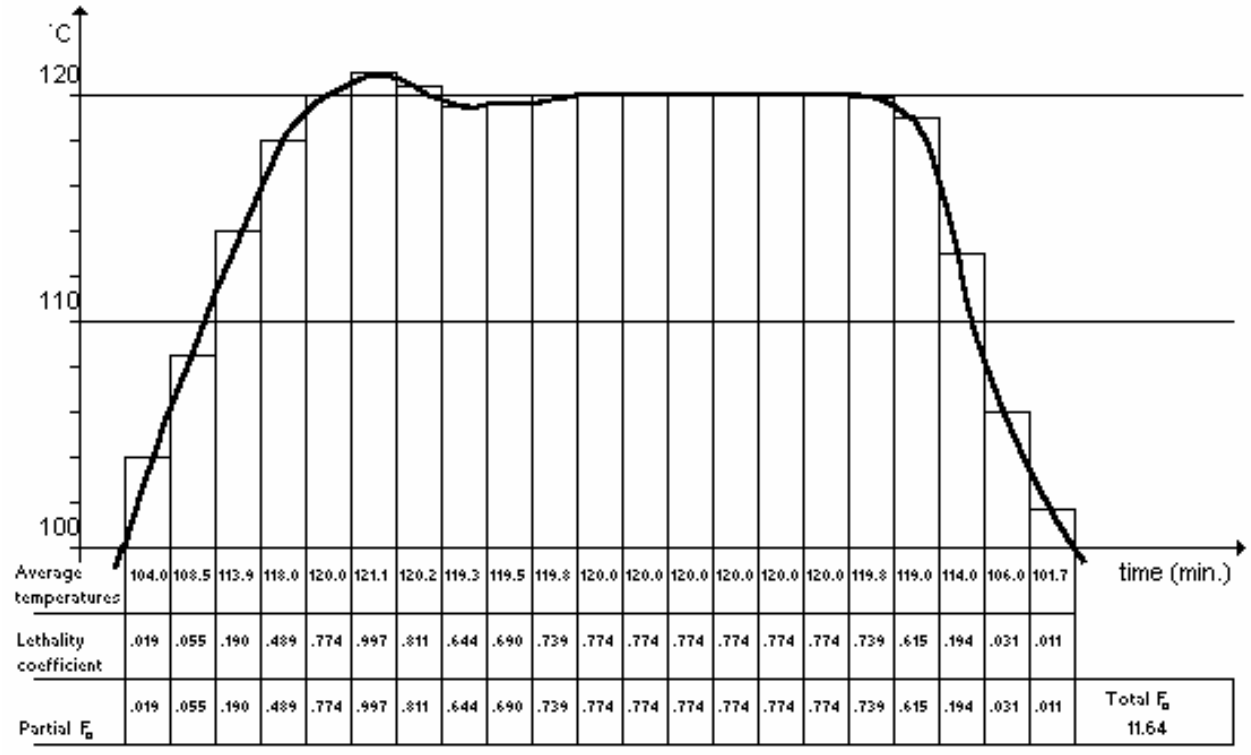
Table 3

1.6. EXAMPLE OF "POST-CALCULATION" OF F_0

As mentioned above, it is usual for the sterilization temperature not to remain exactly at the set value all the exposure time long; furthermore, the heating and cooling phases also entail a certain lethal dose of **moist-heat** and *may* be considered in calculation.

The graph in Table 4 is an example of graphic calculation of F_0 performed after the process on the basis of the recording of the sterilization temperature inside a container filled with solution. The calculation was performed by taking one minute intervals ($\Delta_t = 1$), using the Lethal Rates of Table 1 and including the lethal doses of the heating and cooling phases (above 100°C, when both the numerical values of Lethal Rates are meaningful *and* the moist-heat conditions may be supposed as already attained — this condition is of an utmost importance and has always to be verified).

Determining F_0 after the process is completed is useful, but the real-time calculation of F_0 during the process is much more interesting. This calculation is easily performed with electronic systems. In this case, it is possible to control sterilization no longer in terms of sterilization time but rather in terms of F_0 related to a container which has been identified, during validation, as the one which receives the smallest lethal dose of the entire load. **Due to the essential requirement of moist-heat conditions for a meaningful calculation of F_0-values, the control of exposure time should never be based on F_0-targets, but in the cases of terminal sterilization of aqueous preparations.**



Average temperatures	104.0	108.5	113.9	118.0	120.0	121.1	120.2	119.3	119.5	119.8	120.0	120.0	120.0	120.0	120.0	120.0	119.8	119.0	114.0	106.0	101.7
Lethality coefficient	.019	.055	.190	.489	.774	.997	.811	.644	.690	.739	.774	.774	.774	.774	.774	.774	.739	.615	.194	.031	.011
Partial F_0	.019	.055	.190	.489	.774	.997	.811	.644	.690	.739	.774	.774	.774	.774	.774	.774	.739	.615	.194	.031	.011

Total F_0 11.64

Table 4

1.7. SYMBOLS AND DEFINITIONS USED IN STERILIZATION TECHNOLOGY

Table 5 summarizes the symbols and associated descriptions of the terms most frequently used in moist-heat sterilization technology.

SYMBOL	PHYSICAL DIMENSION	DEFINITION	DESCRIPTION
D_{T_o}	Time	D-value (Decimal decay time)	The time required, at a reference temperature T_0 in moist-heat conditions, to reduce the number of microorganisms of a given species to 10% (1 logarithmic reduction)
D_T	Time	D-value at T°C	The time required, at the temperature of T °C in moist-heat conditions, to reduce the number of microorganisms of a given species to 10% (1 logarithmic reduction)
$F_{(T,z)}$	Time	Equivalent exposure time	Equivalent exposure time to moist-heat conditions related to a specific temperature T and to a specific value of z
F_0	Time	"Reference" exposure time, "F zero", or “F nought”	Equivalent exposure time in moist-heat conditions related to the temperature of 121.11°C (approximately 121°C) and to z = 10
N_0	None	Initial biological burden	Number of viable microorganisms contained in a unit before sterilization
N_0	None	Surviving biological burden	Number of microorganisms contained in a unit, surviving a sterilization of U minutes at a given temperature
z	Temperature difference (°C)	z-value (Temperature coefficient)	Number of degrees of temperature variation which causes a 10-fold variation in the value of D_{121}
L	None	Lethal Rate	Ratio of microbial reduction rates at T (the actual exposure temperature to moist-heat conditions) and at T_{ref} (the reference temperature, generally 121°C) for a given value of z (generally 10°C)
PNSU or SAL	None	Probability of Non Sterile Unit	Number which expresses the probability of finding 1 non-sterile unit in a certain number of sterilized units (batch)

Table 5

2. DEFINITION OF "STERILE" AND "STERILIZATION"

Sterile
Free from viable microorganisms

Sterilization
Any physical or chemical process which destroys all life forms, with special regard to microorganisms (including bacteria and sporogenous forms), and inactivates viruses.

Therefore the terms "sterile" and "sterilization", in a strictly biological sense, describe the absence and, respectively, the destruction of all viable microorganisms. In other words, they are absolute terms: an object or system is either "sterile" or "non-sterile". The destruction of a microbial population subjected to a sterilization process follows a logarithmic progression: only a treatment of infinite duration can provide the absolute certainty that the entire microbial population has been destroyed, and that the system is sterile.

By making the conditions of the sterilization treatment more drastic (i.e. increasing the exposure time and/or the temperature) usually entails a decay of the qualities of the product and certainly increases the process costs. It is, therefore, agreed that the product is acceptable as sterile when the probability of finding a non-sterile unit in a sterilized batch entails a risk which is lower than the other risks associated with the use of the product itself.

More properly, in the pharmaceutical industry, in order to define a unit as sterile one must be able to certify, on a statistical basis related to the conditions of preparation and sterilization of that specific product and of that specific batch, that less than one unit in a million is exposed to the risk of not being sterile. The probability of finding a non-sterile unit (PNSU = Probability of Non Sterile Unit, or SAL) must therefore be smaller (as mathematical value) than 10^{-6}.

3. REAL TIME CALCULATION OF F_0 WITH A COMPUTERIZED AUTOCLAVE

Electronic technology allows the use of a process controller for the integrated management of a sterilization autoclave. If the process controller is sufficiently sophisticated, besides the usual control, monitoring and alarm functions, it can also calculate F_0 in real time and therefore allow, if biologically appropriate, to control the process on the basis of this algorithm. A typical computerized autoclave control system, for example, operates as follows.

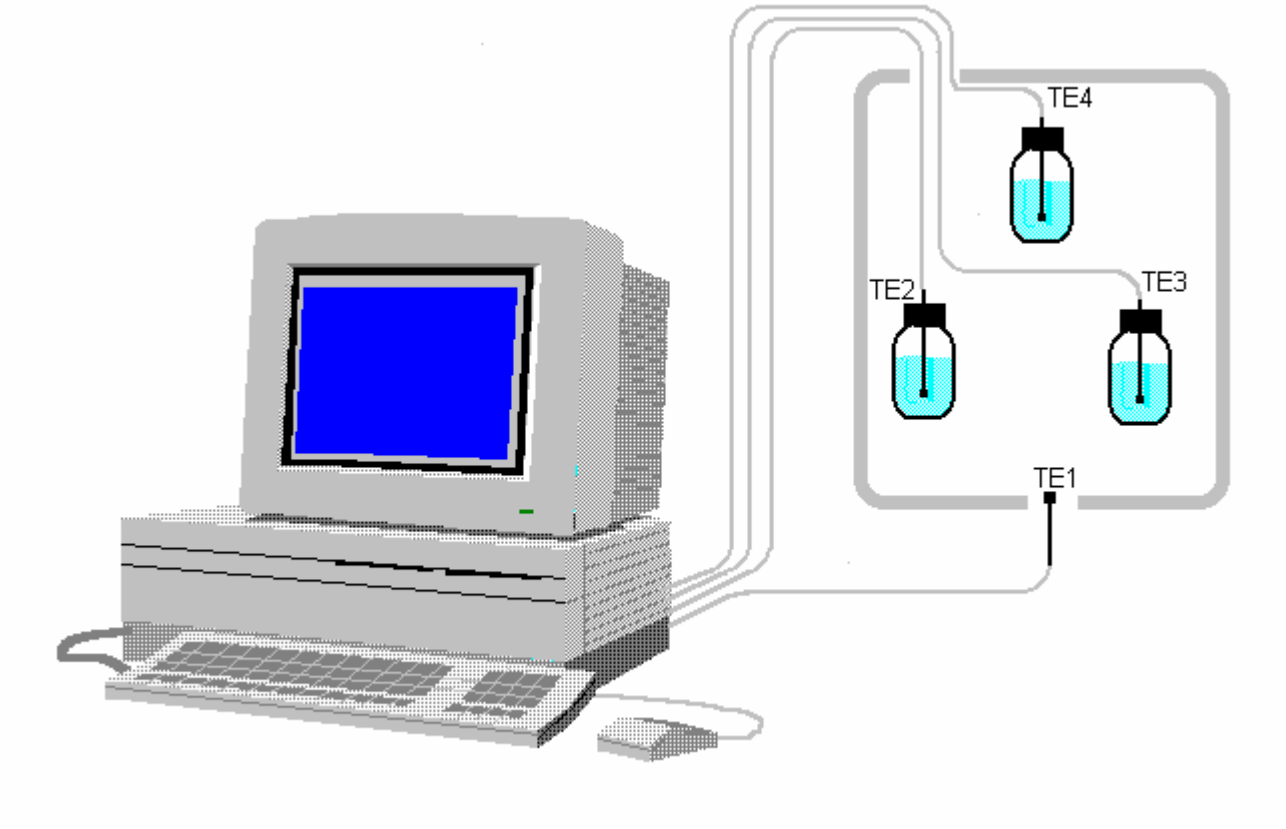


Figure 1

The autoclave is generally provided with multiple temperature probes in its chamber. These probes control the process: one is inserted in the sterilizer drain line, while the others are flexible and can be inserted in containers of the load to be sterilized and are immersed in the solution contained therein. The operator can choose to control the sterilization process according to three alternative modes.

3.1. "TRADITIONAL" CONTROL BASED ON EXPOSURE TIME

The programmer pre-sets four parameters:

1. the sterilization set temperature, e.g. 121°C
2. the sterilization temperature range around this value, e.g. 120.5°C + 1°C, so that the acceptable oscillation range will be 120.5°C to 121.5°C

3. the duration of the sterilization phase, e.g. 20 minutes
4. the acceptable time of excursions from the lower limit of the sterilization temperature oscillation range, e.g. 10 minutes

In these conditions, the sterilization phase begins when the "coldest" heat probe, among those enabled by the programmer to control the process, has entered the acceptable range (see Figure 2). If all the oscillations of all the heat probes remain in the acceptable oscillation range, the sterilization phase end 20 minutes after the "coldest" heat probe has entered the range. However, if one or more heat probes get colder than the lower limit of acceptable oscillation, the computer reacts as follows.

The duration of the "excursions" (regardless of which heat probes recorded them) are individually smaller than the parameter pre-set in step 4 (10 minutes in the example): the sterilization time count remains held during the "exits", and therefore the duration of the sterilization phase is increased by the value of the sum of all the exits of the same probe (see Figure 3).

An "excursion" is greater than the parameter set at item 4, for example 12 minutes: as soon as the excursion exceeds 10 minutes, the sterilization phase restarts from the beginning and the sterilization time count restarts only when the temperature returns within the range of tolerance. Alarms as "Sterilization temperature lack" and "Sterilization time suspended" or "Sterilization time reset" monitor the above anomalies.

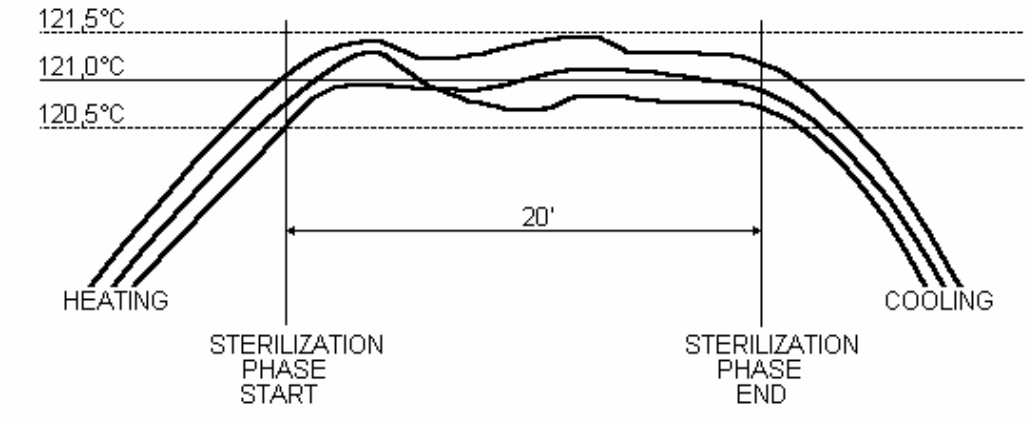


Figure 2

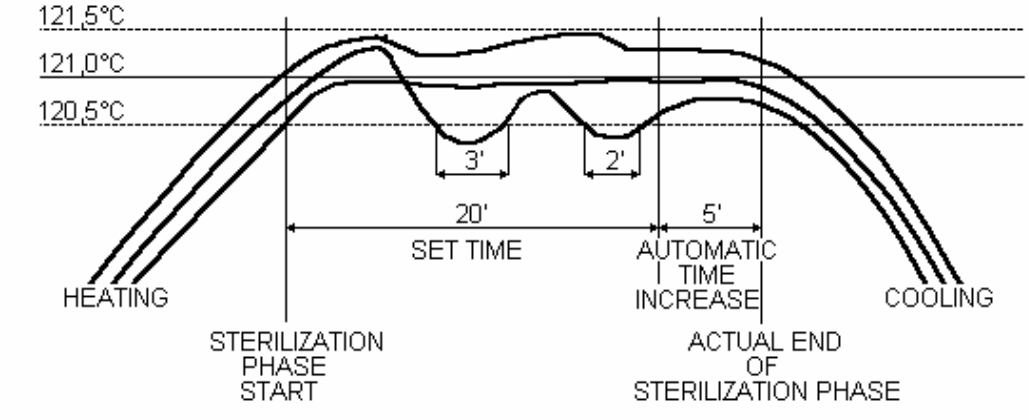


Figure 3

3.2. F_0-BASED CONTROL

The responsible person for the sterilization process sets the following parameters:

1. the sterilization set temperature, e.g. 121°C
2. the sterilization temperature range around this value, e.g. 120.5°C + 1°C, so that the acceptable oscillation range will be 120.5°C to 121.5°C
3. the target value of F_0 which, when summed up by the coldest probe, causes the end of the sterilization phase, e.g. F_0 = 15' (F_0 is adjustable between 1 and very high values)
4. the F_0 calculation start temperature, which usually can be pre-set from 90°C upward (the previous attainment of moist-heat conditions has always to be ascertained and monitored). If the calculation start temperature is set to a value 0.5°C lower than the sterilization temperature (as in Example 1, see below), only the lethal doses provided during the sterilization phase are taken into account. If it is set to 100°C (as in Example 2, see below), the lethal doses provided during heating are taken into account for terminating the sterilization phase, whereas the lethal doses provided during cooling (down to the pre-set value) are also taken into account for calculation. **It is always necessary to remember that moist-heat conditions have to be attained and preserved to give F_0-values a biological meaning: for this reason, it is a sound practice not to calculate F_0-values for temperatures lower that the minimum sterilization temperature (120.5°C in our examples) when hard/porous goods are sterilized**.
5. the value of the temperature coefficient z, which is variable between 5°C and 20°C but is normally set to 10°C (to obtain a properly said F_0 value).

The calculation of F_0 is performed independently for each probe on a very small time base: e.g. 1 second or less. Therefore, every second or less, and separately for every temperature probe used for monitoring the process, the computer takes the temperature of entry and exit from the time base, averages them, inputs this average temperature into the formula of F_0, calculates partial F_0 and adds it to the previously accumulated F_0 for that probe. Every time interval selected by the programmer, these values are recorded and printed in digital terms. The values accumulated by the coldest and hottest probes are displayed on the screen and are refreshed every 1 or 2 seconds. When they reach the pre-set target value, the sterilization phase ends.

Let us examine some examples of F_0-based control which will clarify the above description. For the sake of simplicity they refer to a single probe.

Example 1 (Figure 4)
The calculation of F_0 starts when the sterilization begins, i.e. when the calculation start temperature corresponds to the minimum sterilization temperature, i.e. the lowest bound of the acceptable sterilization temperature band. The phase ends when the "coldest-in-average" probe has accumulated the target value of F_0 (12' in this case). The calculation of F_0 ends almost immediately after the sterilization phase has terminated and the moist heat conditions have ceased (e.g. by pulling drying vacuum).

Example 2 (Figure 5)
The calculation of F_0 starts already when the probe exceeds the pre-set value during the heating phase (100°C in this example, because the monitoring probes are inserted in an aqueous product). When the sterilization phase is entered, the probe has already accumulated an F_0 of 1.1'. The sterilization phase ends when the probe has accumulated the target F_0 value (15' in this case). However, the calculation of F_0 continues until the probe leaves the pre-set value of 100°C. It can thus be seen that an additional lethal dose F_0 = 0.9' is provided *during the cooling phase to the aqueous product*.

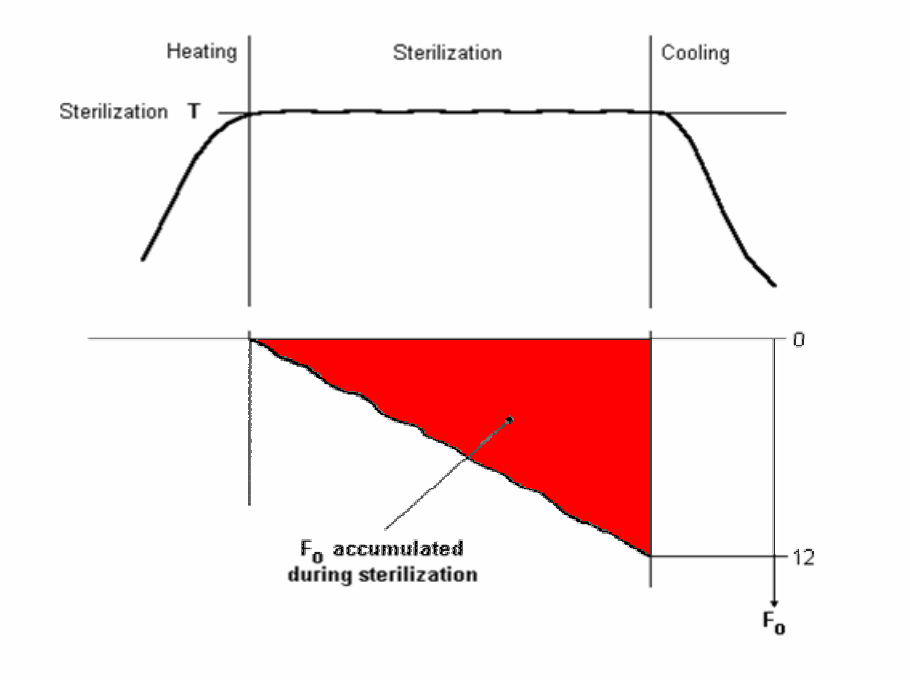


Figure 4

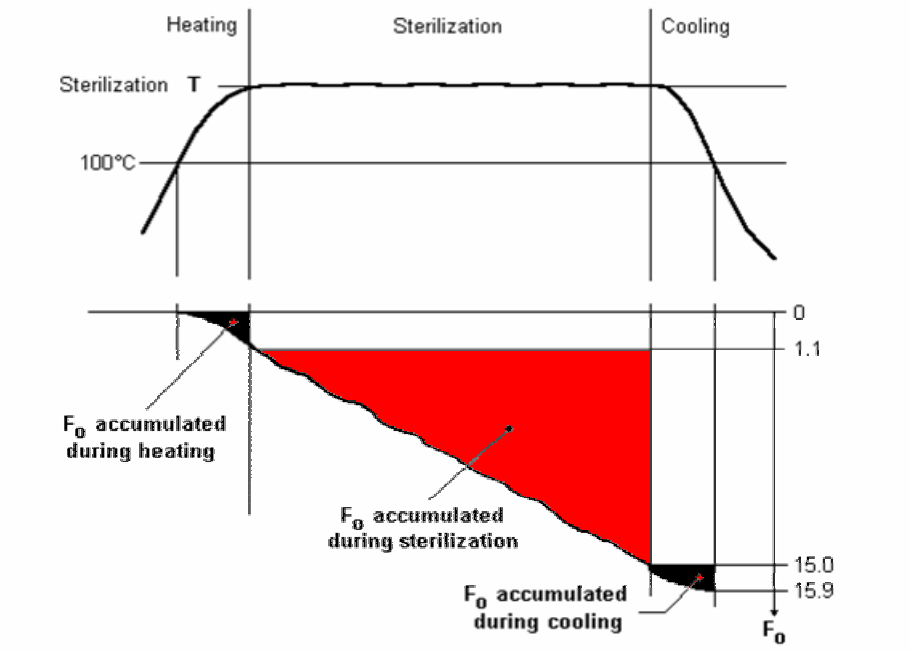


Figure 5

The calculation of the lethal doses provided during heating and cooling is necessary when highly heat-sensitive preparations are terminally sterilized. The ability to select the z-values allows the calculation of lethal doses with respect to the heat-sensitivity characteristics of a specific and critical contaminating microorganism. This possibility must be considered as a refinement in calculation allowed by the capabilities of the computer.

Obviously, when a process is controlled according to F_0, "excursions" from the acceptable temperature oscillation range no longer cause the reactions specified in items a) and b) of paragraph 3.1. Actually, if the temperature drops, the lethal dose accumulated during that period is automatically reduced in the calculation of F_0. The reverse is true if the temperature rises. However "excursions" from the acceptable temperature range (whether above or below it) still generate the alarm "Sterilization temperature lack" as in the case of paragraph 3.1, whereas suspension or reset of sterilization time are no longer applicable.

The F_0-based management of the sterilization process allows highly rational control of the procedure even in case of power loss or blackout. In such conditions, the process controller, which is battery buffered, continues to operate but naturally no longer receives signals from the autoclave; the autoclave itself is equally unable to execute the command signals sent by the process controller. In case of power failure, all the autoclaves valves and blocking devices are naturally moved to their resting position, which corresponds to the maximum safety condition.

Example 3 (Figure 6)
Assume now the power failure occurs during the sterilization. The process controller is capable of detecting the times at which the power failure starts and ends, and the temperature at which each heat probe enters and exits the power failure period. In practice, the conditions of Figure 6 occur; as in the previous examples, Figure 6 relates to a single probe for the sake of simplicity.

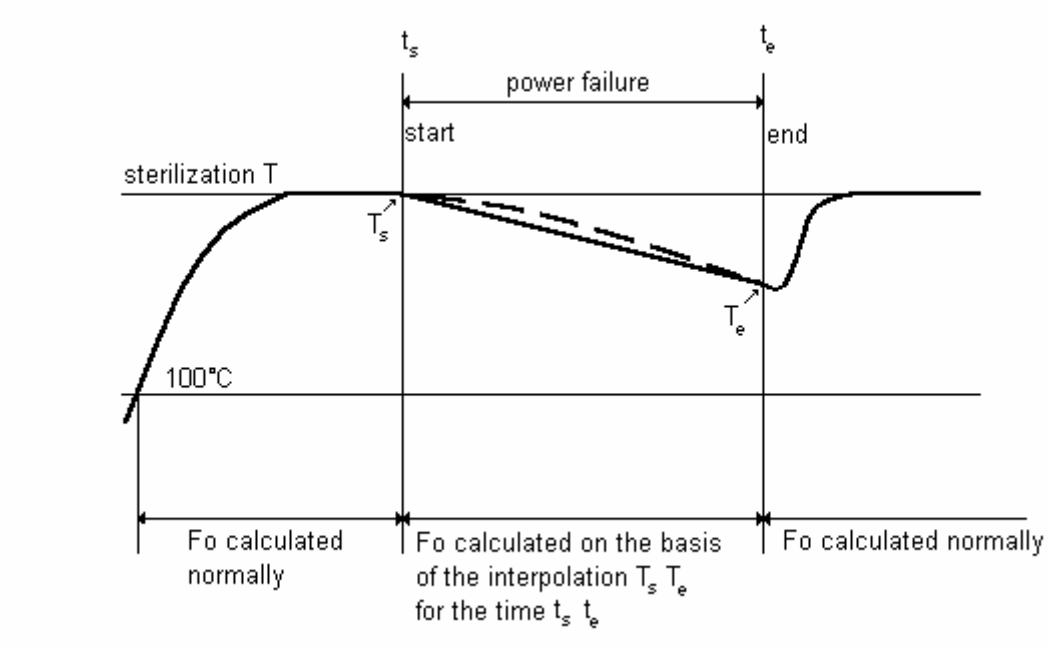


Figure 6

The controller has naturally been unable to determine the trend of the temperature during the time interval T_s-T_e When power returns, it therefore calculates F_0 for this time interval on the basis of the linear interpolation between the temperatures T_s-T_e. Such a calculation is conservative with respect to the actual trend of the temperature (indicated in broken lines). Even if not immediately intuitive, the shape of the actual trend can easily be demonstrated with experimental investigations.

Example 4 (Figure 7)
If the power failure has lasted long enough as to entail the exit of the temperature from the F_0 calculation start value (e.g. 100°C), the reaction of the computer when the power failure ends is schematically indicated in Figure 7 and can be summarized as follows: linear interpolation between T_s and T_e; calculation of F_0 during power failure as linear interpolation between the temperatures T_s-100°C for the time interval t_e -t_s; at the end of the blackout, the regular calculation of F_0 resumes only when the temperature again exceeds 100°C.

Obviously, F_0-based control of sterilization is extremely useful in all sterilization processes. It is practically indispensable when it is necessary to sterilize highly heat-sensitive products for which the "survival probability" approach has been adopted during validation.

The heat probes enabled for calculation must naturally be inserted in the solution of a few representative units arranged in the point (or, more realistically, in the region) of the load which has been determined as "coldest" during validation.

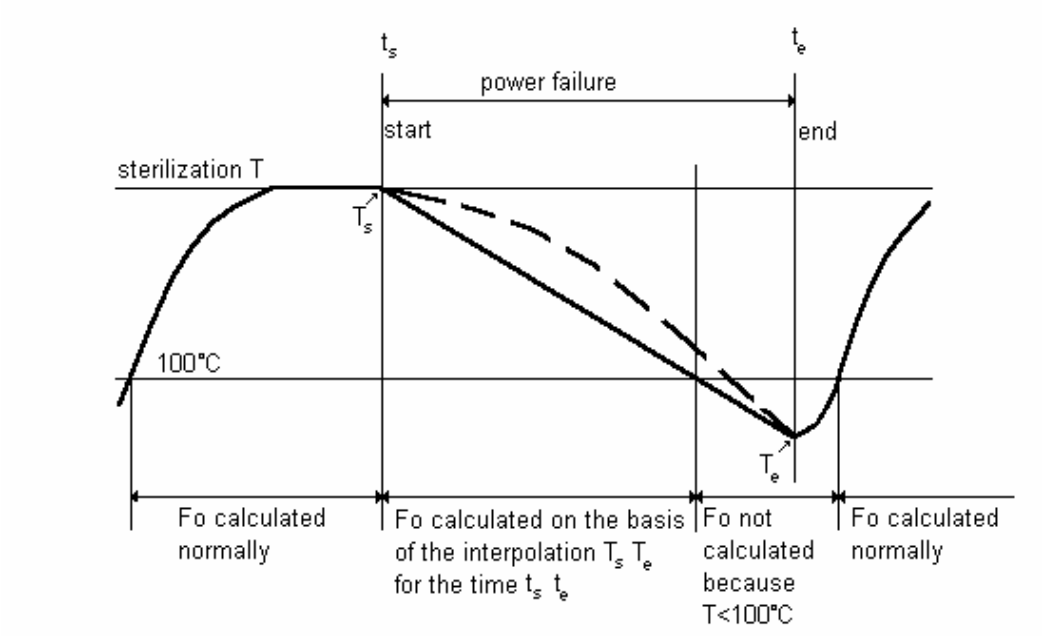


Figure 7

3.3. STERILIZATION TIME-BASED CONTROL WITH CALCULATION/PRINTOUT OF F_o VALUES

Sterilization is controlled exactly as specified in paragraph 3.1.
However, the programmer also pre-sets the parameters of items 6 and 7 of paragraph 3.2. This phase is therefore ended when an "effective" sterilization time is reached, but the calculation of F_0 is simultaneously performed and printed (for each enabled probe) as specified in paragraph 3.2. This calculation is merely for verification, but is nonetheless important, since it allows the determination of lethal doses provided in the points monitored by the enabled heat probes.

The calculation is extremely useful when the sterilization process is validated with the "overkill" (i.e. "superabundant lethal dose") approach, in which, as it known, it is necessary to prove that a lethal dose equal to specified minimum has been provided during the sterilization phase to the coldest point of the load.

It is evident that if a couple of flexible probes enabled for F_0 calculation (and appropriately set for this purpose) are introduced in representative containers arranged in the coldest points of the load, they will provide F_0 values which can be accepted as unequivocal evidence of the execution of sterilization in the spirit of the previously performed validation.

4. SUMMARY OF PRECEDING CONCEPTS IN LAYMAN'S TERMS

The following simplified summary may be used to explain these concepts in an easily understood manner to those who may be less trained, but who would nevertheless benefit from grasping the essence of the work they are performing.

NOTE: the term "Unit" defines a physically delimited system within which microorganism can "homogenate" and proliferate.

A bottle or a vial, together with their contents, are a unit. It is more difficult but equally necessary to extend the concept of unit to a container which contains for example a filtering system or a certain mass of clothing.

1. Up to some tens of years ago, steam sterilization was thought to be a "potential-barrier", i.e. "all-or-nothing" phenomenon. This would mean that once a certain temperature is reached and maintained for a certain time, all the microorganisms contained in a unit die within that time, regardless of their number. The risks of such an assumption are in any case evident.

2. Nowadays, it has been shown that steam sterilization instead proceeds like a first order chemical reaction (“destruction reaction”) and, therefore, at a specific rate which is higher as the temperature rises and is a function of the number of microorganisms present in the unit.

3. This rate can be expressed by means of the Decimal Decay Time, indicated by the D-value.

4. The D-value is the time, in minutes, required to reduce the number of microorganisms present in the unit by 90%.

5. The D-value varies according to the kind of microorganism (and to its "history"), the medium in which it is immersed and, as mentioned, the sterilization temperature.

6. At the temperature of 121°C **in moist-heat conditions**, the D-value is generally between 0.5 and 2 minutes: for microorganisms commonly dealt with, it is often assumed, as an average, that D = 1 minute.

7. This means that at the end of each minute at 121°C **in moist-heat conditions** the number of microorganisms reduces to one tenth of the number at the beginning of that minute.

8. Therefore, if a unit is kept at 121°C **in moist-heat conditions** for 3 minutes, the number of microorganisms contained therein is reduced to one thousandth (1/10 x 1/10 x 1/10 = 1/1000) of the initial number.

9. If the initial bacterial load of a batch of units being sterilized is on the average 1000 (i.e. 1000 microorganisms per vial or bottle), after 3 minutes of treatment **in moist-heat conditions** at 121°C it is reduced on the average to 1.

10. After a further minute of sterilization (4 minutes altogether) this reasoning leads one to the conclusion that the load has dropped to 1/10, i.e. 0.1. However, this must not be understood to mean that at this point each unit contains one tenth of a microorganism (in which case the units would be sterile...) but must be taken to mean that there is a probability that 1/10 of the units are still contaminated.

11. After 9 minutes of treatment at 121°C **in moist-heat conditions**, the bacterial load of the batch at issue is reduced, on the average, to 1/1,000,000. The probability of still having a contaminated unit in that batch is therefore 1 in 1,000,000.

12. This is the minimum assurance of sterilization which must be achieved in the pharmaceutical field, though a greater assurance, for example 10^{-9}, i.e. 1 in 1 billion, is often sought.

13. This assurance is expressed as PNSU (or SAL): Probability of Non Sterile Unit. PNSU =10^{-6} or SAL =10^{-6} means that the probability of finding a non-sterile unit in a batch is 1 in 1 million.

14. In order to achieve a given PNSU or SAL it is necessary to meet several conditions:

    to statistically know the initial bacterial load (or “bioburden”) of the batch (which is anything but easy to determine);
    to be certain that even the coldest point inside the units of the batch has received a lethal moist-heat dose sufficient to obtain the required PNSU;
    if the sterilization is not performed at 121°C, to be capable of relating to 121°C (by calculation) the effectiveness of sterilization in order to correctly apply the previously defined concept of D.

15. F_0 is defined as:

    the equivalent exposure time at 121.11°C of the actual exposure time at a variable temperature, calculated for an ideal microorganism with a temperature coefficient of destruction equal to 10 °C.

    (The definition proposed by FDA in 1976 was: *“the equivalent amount of time, in minutes at 121°C or 250 F, which has been delivered to a product by the sterilization process”.)*

    NOTE: The exact temperature equivalent to 250°F is 121.11°C.

16. The "overkill", i.e. "over-sterilization", approach is generally used when a sterilization process for heat-resistant products is validated.

Essentially, with this approach it is necessary to provide an F_0 which is safety and according to some suggestions not lower than 15' exclusively during the sterilization phase (i.e. ignoring the lethal heat doses provided during heating and cooling, this resulting in a good safety margin with respect to the minimum value) to the unit placed in the coldest point of the load.

17. In practice, it is conceptually easy and relatively trouble-free to relate by calculation the sterilization time to 121°C or to F_0 after the process. On the contrary, this is difficult to do in "real time", i.e. while sterilization is in progress, since the calculation must be performed so quickly that the use of a computer is unavoidable.

18. If F_0 = 15' is to be achieved, the required exposure time is shorter than 15 minutes if the sterilization temperature is higher than 121°C and longer than 12 minutes if the sterilization temperature is lower than 121°C, **provided that moist-heat conditions are attained and preserved in any case**.

19. For most of the microorganisms with which we commonly deal, it is assumed that every 10°C of shift from the temperature of 121°C entails a tenfold change in the sterilization rate, **provided that moist-heat conditions are preserved in any case**.

20. Therefore, if we work at 111°C, in order to achieve an F_0 of 15', it is necessary to sterilize for 12 x 10 = 120 minutes, whereas if we work at 131°C then 12/10 = 1.2 minutes, i.e. 72 seconds, are theoretically sufficient, , **provided that moist-heat conditions are preserved in any case**.

21. If we want to determine the extent by which the sterilization rate in moist-heat conditions varies for temperature variations of 1°C we must find the number which yields 10 when raised to the tenth power: this number is 1.26. This means that a 1°C variation in the sterilization temperature causes an increase (or reduction) of the sterilization rate by a factor of 1.26, i.e. 26%.

22. Similarly, it can be shown that a temperature variation of 0.1°C in moist-heat conditions causes a rate variation with a ratio of 1.02, i.e. approximately 2%.

23. It is therefore evident that even small temperature variations around 121°C cause highly significant and hardly negligible variations in the sterilization rate.
    For example, sterilizing at 119°C in fact means increasing (approximately) the exposure time by 1.24 x 1.24 = 1.5376 times to relate it to 121°C.
    Therefore, for example, if F_0 = 15' is to be achieved, it is necessary to sterilize for about 23' instead of 15'.

24. The following mathematical expression allows the calculation of F_0 and is provided for information:

$$F_0 = \Delta t \sum 10^{\frac{T-121}{10}}$$

where T is the actual temperature in moist-heat conditions at the time interval being considered, and the number at the denominator of the exponent is the number of degrees C which causes a 10-fold variation of the sterilization rate (z-coefficient).

25. A Lethal Rate table (see Table 6) has been compiled which allows to pass, by means of a simple multiplication, from any sterilization time at a certain temperature at a certain temperature to F_0 for temperatures between 90 and 130.9°C with intervals of 0.1°C.

26. Let us analyze Table 6. Choose the temperature, in whole Centigrade degrees, in the left column and the tenths of degree to be added in the top row. The intersection of the two values yields the required rate. For example, the rate framed with thin lines is for 120.0°C, the double framed rate is for 120.2°C and the thick-framed rate is for 121.8°C. The rate for 121.1°C is very close to 1 (it would be exactly 1 for 121.11°C).

27. Therefore, if we refer to the factor for 120.0°C we can say that any sterilization time at 120.0°C must be multiplied by 0.774 to make it equal to the time at 121.1°C, i.e. to express it as F_0.
    Thus:
    1 minute at 120.0°C = 1' x 0.774 = 0.77 minutes at 121.1°C
    15 minutes at 120.0°C = 15' x 0.774 = 11.61 minutes at 121.1°C
    20 minutes at 120.0°C = 20' x 0.774 = 15.87 minutes at 121.1°C

    NOTE: The decimals of the time values are tenths and hundredths of a minute, not seconds.

28. If a calculation of F_0 after the process is to be performed on the basis of a chart of the temperature taken inside a unit subjected to sterilization, it is possible to operate as described in Table 4 (paragraph 1.6).

29. Figure 8 is an illustration of the concepts of F_0, D and PNSU (or SAL).

30. **Never forget that in moist-heat sterilization technology, any temperature equivalence is always conditioned by the actual presence of saturated, i.e. condensing steam on the surface or inside the product to be sterilized.**

TABLE OF LETHAL RATES

in condition of saturated steam for a reference temperature of 121.11°C = 250°F with z = 10°C, and for temperature values between 90°C and 130°C, with intervals of 0.1°C

TEMP.°C	+0.0	+0.1	+0.2	+0.3	+0.4	+0.5	+0.6	+0.7	+0.8	+0.9
	LETHAL RATE									
90	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001
91	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001
92	.001	.001	.001	.001	.001	.001	.001	.001	.001	.002
93	.002	.002	.002	.002	.002	.002	.002	.002	.002	.002
94	.002	.002	.002	.002	.002	.002	.002	.002	.002	.002
95	.002	.003	.003	.003	.003	.003	.003	.003	.003	.003
96	.003	.003	.003	.003	.003	.003	.004	.004	.004	.004
97	.004	.004	.004	.004	.004	.004	.004	.005	.005	.005
98	.005	.005	.005	.005	.005	.005	.006	.006	.006	.006
99	.006	.006	.006	.007	.007	.007	.007	.007	.007	.008
100	.008	.008	.008	.008	.008	.009	.009	.009	.009	.010
101	.010	.010	.010	.010	.011	.011	.011	.011	.012	.012
102	.012	.013	.013	.013	.013	.014	.014	.014	.015	.015
103	.015	.016	.016	.017	.017	.017	.018	.018	.019	.019
104	.019	.020	.020	.021	.021	.022	.022	.023	.023	.024
105	.024	.025	.026	.026	.027	.027	.028	.029	.029	.030
106	.031	.032	.032	.033	.034	.035	.035	.036	.037	.038
107	.039	.040	.041	.042	.043	.044	.045	.046	.047	.048
108	.049	.050	.051	.052	.054	.055	.056	.057	.059	.060
109	.062	.063	.064	.066	.067	.069	.071	.072	.074	.076
110	.077	.079	.081	.083	.085	.087	.089	.091	.093	.095
111	.097	.100	.102	.104	.107	.109	.112	.115	.117	.120
112	.123	.126	.128	.131	.135	.138	.141	.144	.148	.151
113	.154	.158	.162	.166	.169	.173	.177	.182	.186	.190
114	.194	.199	.204	.208	.213	.218	.223	.229	.234	.239
115	.245	.251	.256	.262	.268	.275	.281	.288	.294	.301
116	.308	.315	.323	.330	.338	.346	.354	.362	.371	.379
117	.388	.397	.406	.416	.426	.435	.446	.456	.467	.477
118	.489	.500	.512	.523	.536	.548	.561	.574	.587	.601
119	.615	.629	.644	.659	.674	.690	.706	.723	.739	.757
120	.774	.792	.811	.830	.849	.869	.889	.910	.931	.953
121	.975	.997	1.021	1.044	1.069	1.093	1.119	1.145	1.172	1.199
122	1.227	1.256	1.285	1.315	1.346	1.377	1.409	1.442	1.475	1.510
123	1.545	1.581	1.618	1.655	1.694	1.733	1.774	1.815	1.857	1.901
124	1.945	1.990	2.037	2.084	2.133	2.182	2.233	2.285	2.338	2.393
125	2.448	2.506	2.564	2.624	2.685	2.747	2.811	2.877	2.944	3.012
126	3.082	3.154	3.228	3.303	3.380	3.459	3.539	3.622	3.706	3.792
127	3.881	3.971	4.063	4.158	4.255	4.354	4.456	4.559	4.666	4.774
128	4.885	4.999	5.116	5.235	5.357	5.482	5.608	5.740	5.874	6.010
129	6.150	6.294	6.440	6.590	6.744	6.901	7.062	7.226	7.394	7.567
130	7.743	7.293	8.108	8.297	8.490	8.688	8.890	9.097	9.309	9.526

Table 6

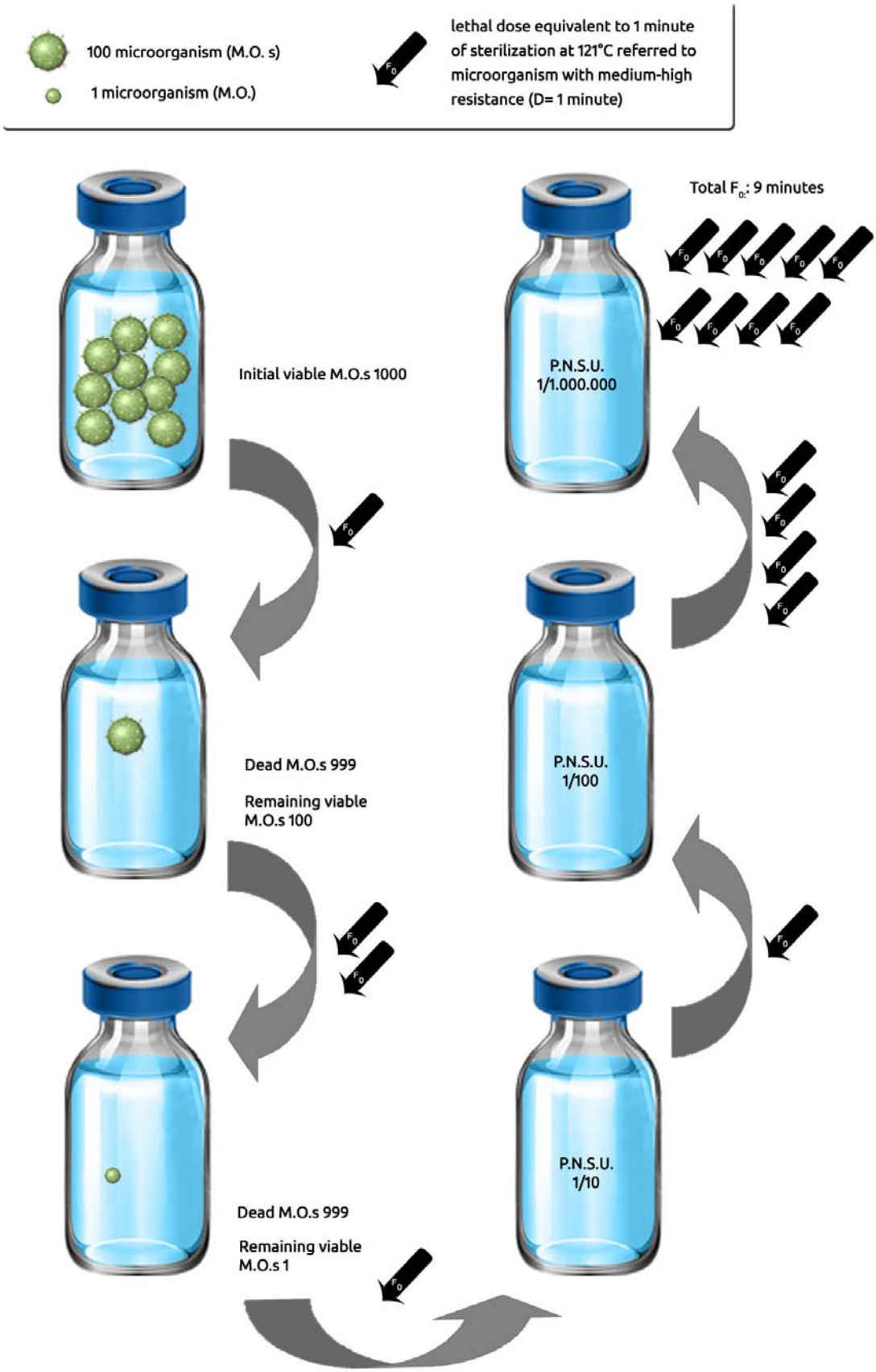

100 microorganism (M.O. s)
1 microorganism (M.O.)
lethal dose equivalent to 1 minute of sterilization at 121°C referred to microorganism with medium-high resistance (D= 1 minute)
Total F_0: 9 minutes
Initial viable M.O.s 1000
P.N.S.U. 1/1.000.000
Dead M.O.s 999
Remaining viable M.O.s 100
P.N.S.U. 1/100
Dead M.O.s 999
Remaining viable M.O.s 1
P.N.S.U. 1/10

Figure 8

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