

F_0

Technical Note

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What it means

How to calculate it

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

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The F_0 algorithm was introduced several years ago in the international practice of pharmaceutical sterilization and is also officially included in the latest edition of the Italian Pharmacopoeia.

Yet F_0 is still scarcely used and sometimes is even regarded with some suspicion from a conceptual point of view. On the contrary, F_0 is extremely useful for adjusting, controlling and validating moist-heat sterilization processes.

The purpose of this technical note is to clarify the nature of F_0 and of related parameters (D , z , PNSU), and to explain their use for the setting, adjustment, control and validation of moist-heat sterilization processes.

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1. ESSENTIALS OF STEAM STERILIZATION KINETICS

Let us suppose to immerse in pressurized saturated steam, at constant temperature, a system contaminated by a micro biological 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 micro-organism.

It has been experimentally shown that, under the above conditions, the reaction of thermal degradation of the micro-organism at issue obeys the laws of chemical reactions.

Using N to indicate the number of micro-organism 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:

$$\frac{dN}{dt} = -KN$$

where K is a constant which is typical of the species and conditions of the chosen micro-organism.

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 vapour 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:

$$\frac{dN}{N} = -Kdt \quad (1)$$

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

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

$$\log N = -kt + \text{constant}$$

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:

$$\begin{aligned} t &= 0 \\ N &= N_0 \end{aligned}$$

therefore

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

from which

$$\log N = -kt + \log N_0 \quad (2)$$

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which leads to

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

and therefore

$$\frac{N}{N_0} = 10^{-kt} \tag{3}$$

where:

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

Expression (3) shows that the number of micro-organism 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:

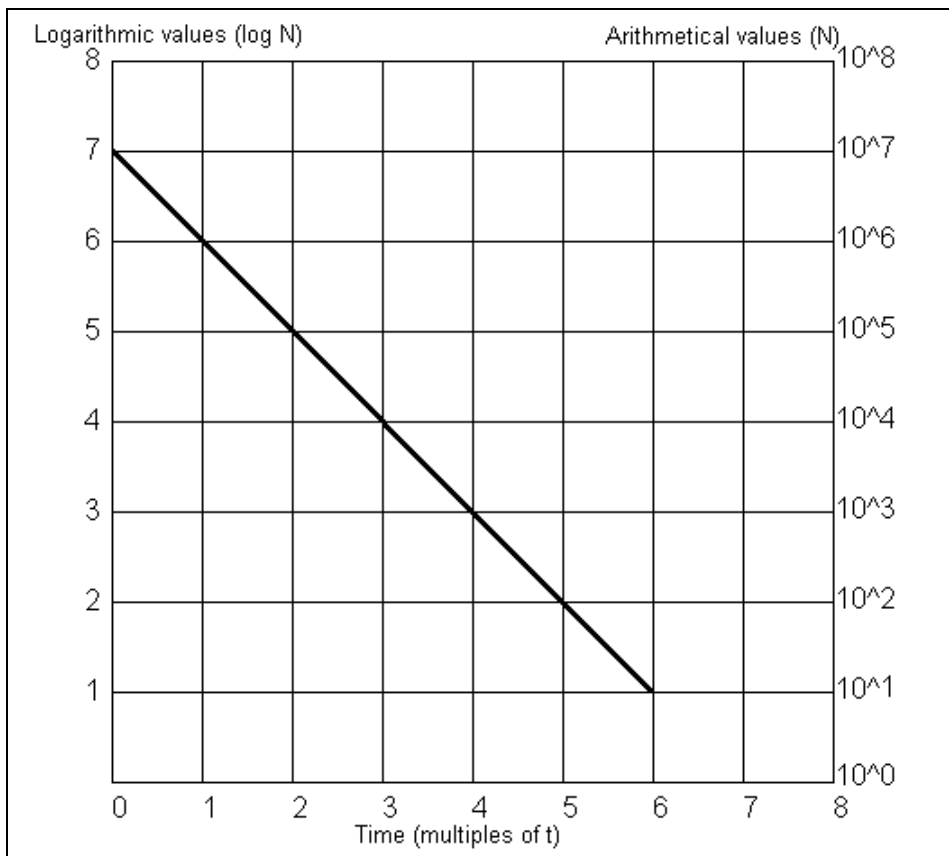


Diagram 1

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

The time required to reduce the micro-organism 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.

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).

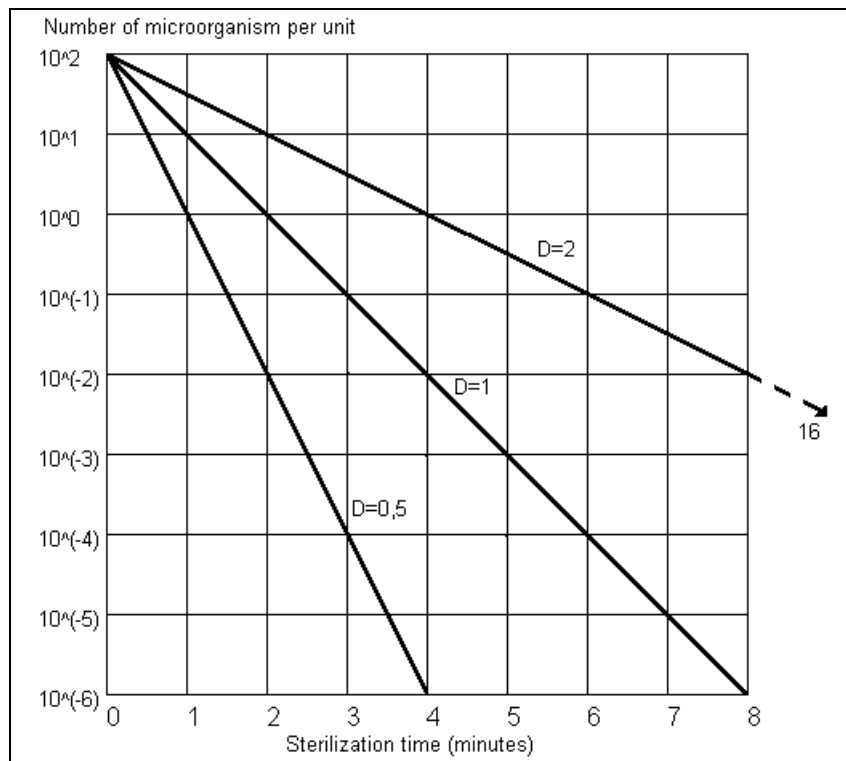


Diagram 2

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 micro-organisms = 10^2 . If the D-value at 121°C is assumed = 1, after one minute at 121°C, the reduction = to $10^1 = 10$ micro-organisms is

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achieved; after another minute, only $10^0 = 1$ micro-organism is still surviving. After another minute the surviving microbial population would be $10^{-1} = 1/10$ micro-organism.

A contamination of $1/10$ must not be understood to mean that each unit contains $1/10$ of a micro-organism, 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 micro-organism 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 micro-organism 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 micro-organism 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 micro-organism 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 micro-organisms: 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 micro-organism 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 its 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 PNSU as sterility evaluation criterion is being replaced by the SAL (Sterility Assurance Level). The name itself could generate some misunderstanding since a level of assurance is commonly deemed to be good if high, but SAL seems to have been

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defined in such a way that its numerical value is the same of PNSU. This notwithstanding, it is sometimes calculated as the reciprocal value of PNSU. The SAP (Sterility Assurance Probability) criterion has been proposed as well: SAP seems for the moment to have been granted the same definition of PNSU, even if it would be better understandable if its value approached the unity after a satisfactory sterilization.

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 micro-organism, 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 strictly 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).

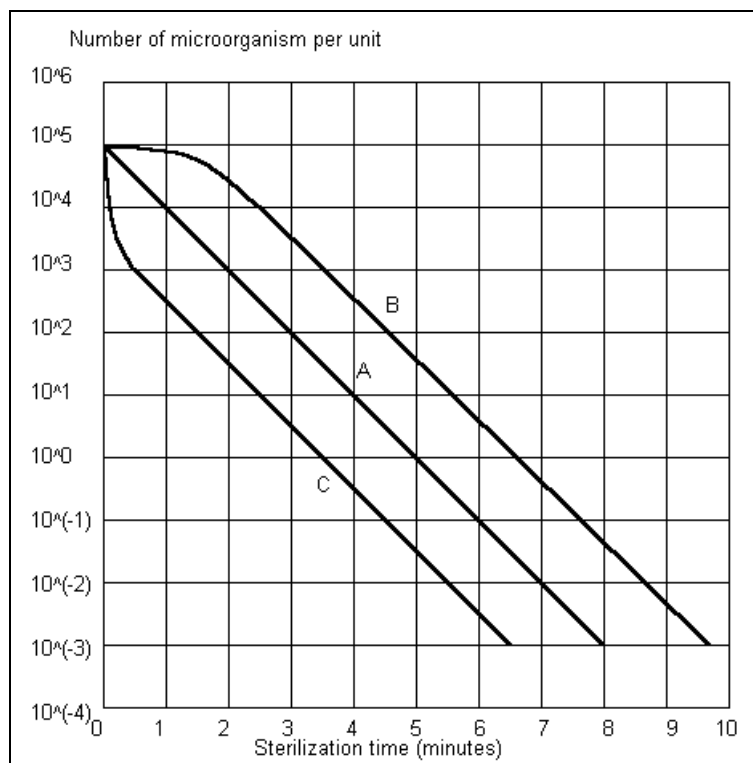


Diagram 3

1.3. z-VALUE OR TEMPERATURE COEFFICIENT

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

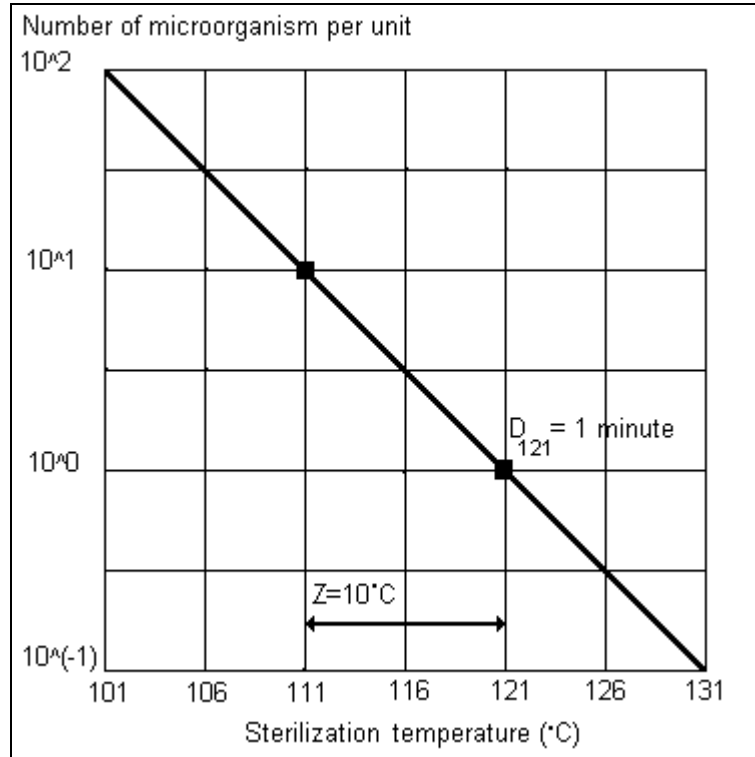


Diagram 4

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 exact experimental data). It can also be seen that 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 1.24.

Therefore a variation of 1°C entails a variation of D-value of 24%.

As can be seen, this is quite a considerable value 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 as the temperature raises and drops to approximately one half (and even less) for dry sterilization at approximately 200°C. Under these condition 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" micro-organism; in fact the actual D-values and z-values depend to a large extent on the medium which contains the micro-organisms and on their history.

AVERAGE VALUE OF D AND z FOR SOME TYPICAL MICRO-ORGANISMS		
Micro-organism	D ₁₂₁ (minutes)	z (°C)
Clostridium botulinum	0.2	10
Bacillus stearothermophilus	2.0	6
Bacillus subtilis	0.5	10
Bacillus megaterium	0.04	7
Bacillus cereus	0.007	10
Clostridium sporogenes	0.8 - 1.4	13
Clostridium histolyticum	0.01	10

Table 1

Actually, at 121°C no micro-organism has exactly D = 1 and z = 10. However, the combined use of these two parameters in calculating F₀ and PNSU provides ample margins of safety as regards the micro-organisms which are commonly dealt with.

1.4. F₀ OR EQUIVALENT EXPOSURE TIME AT 121°C

As seen above, D is thus a different function of the exposure temperature T for each different micro-organism:

$$D = D(T)$$

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 = D₀ if T = T₀, the mathematical function which satisfies the above relationship is (see further explanation in the note at the end of this paragraph):

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

where D₀ is the value of D at the temperature T₀ and for a given micro-organism.

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 T₀. No doubt this is rigorously not true, but it has proven to be both a helpful and a safe abstraction.

Let us now calculate the time interval required to obtain at a constant temperature T₀ 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:

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

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 an 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 :

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

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

Firstly introduced by the National Canners Association in 1968 (a), F_0 has to become a topic in pharmaceutical production since the FDA used it extensively in the "Proposed rules" of June 1st, 1976 (b), with the following meaning (section 212.3):

" F_0 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 calculation of it, "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 practice, the knowledge of the temperature values as the continuous function of elapsing time is not available, and F_0 is calculated as follows:

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

where:

- Δt = time interval between to following 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:

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

indeed according to the definition of F_0 .

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

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

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:

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

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

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

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.

$$F(y) = L[D(T)]$$

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

Then we can write:

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

The obvious solution of this equation:

$$y = -\frac{\ln 10}{z}$$

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

$$L[D(T)] = \frac{c}{y + \frac{\ln 10}{z}}$$

where c is constant.

By transforming the above equation we obtain:

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

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. LETHALITY RATES OR LETHALITY FACTORS

The calculation of F_0 , with its exponential expression, is not immediate. Tables have therefore been developed which list the so-called Lethality Rates, i.e. the coefficients required to pass from a certain time at the temperature T to the equivalent time at 121°C, i.e. to F_0 .

Tables 2 and 3 are two examples. The first has $z = 10$, and therefore allows to obtain F_0 by definition. The second table has z -value variable and allows to obtain equivalent times at 121°C as before, but with values of z which can be chosen between 7 and 12. It is interesting to notice that the variation of z considerably influences the Lethality Rates when T varies.

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It should also be noted that when T rises the Lethality Rates rise more when z-value decreases than when 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 other words, the effect of temperature variations is greater as the z-value becomes smaller. This fact will become better apparent from an inspection of the table provided in Table 3.

TABLE OF LETHALITY RATES

for a reference temperature of 121.11°C and z = 10°C;
obtainable starting from the temperature T comprised 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
LETHALITY 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 LETHALITY RATES

for a reference temperature of 121°C and z variable between 7°C and 12°C;
obtainable starting from the temperature T comprised between 100°C and 130°C with intervals of 0.5°C

TEMPERATURE (°C)	VALUES OF z (°C)					
	7	8	9	10	11	12
	LETHALITY 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, it is usual for the sterilization temperature not to remain exactly at the pre-set value all the exposure time long; furthermore, the heating and cooling phases also entail a certain lethal dose (which has practical significance only for temperatures above 100°C) and may (but need not) be considered in calculation.

The graph provided 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 Lethality Rates of Table 1 and including the lethal doses of the heating and cooling phases (above 100°C).

Determining F_0 after the process is completed certainly is meaningful, 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.

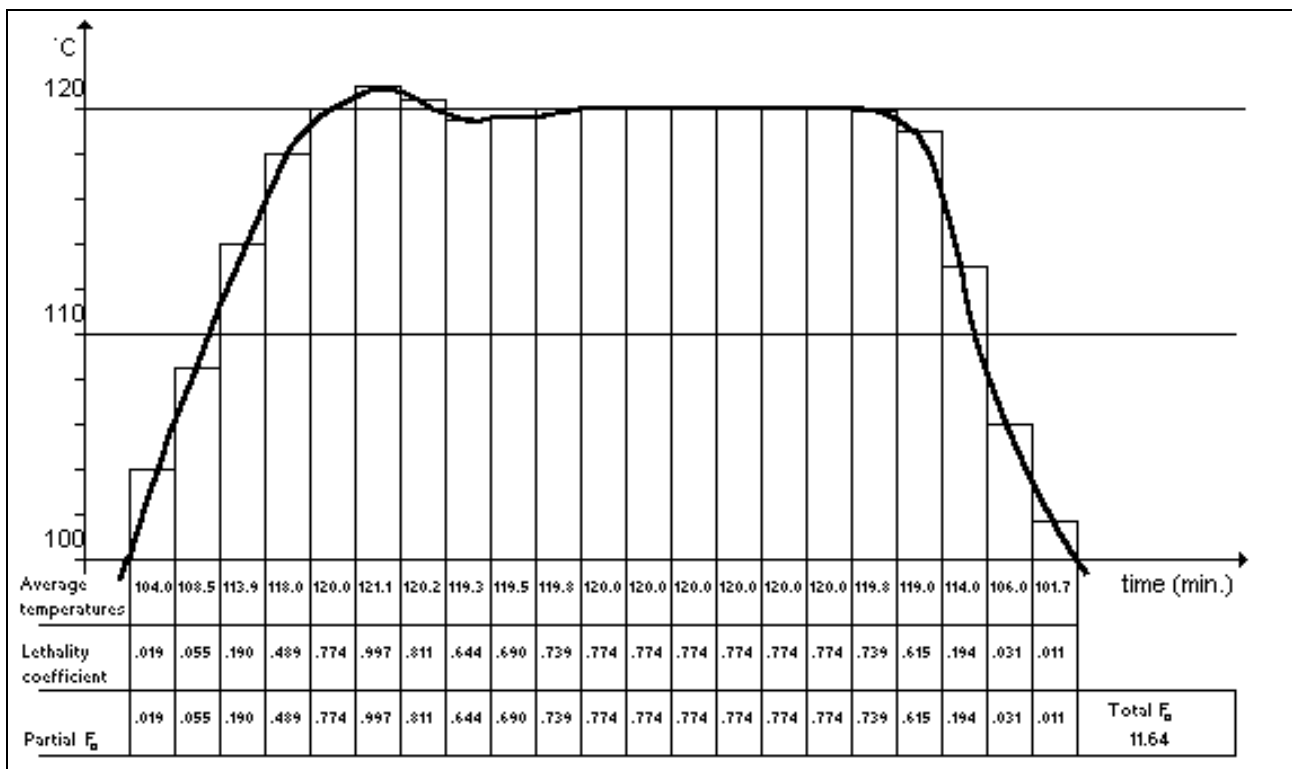


Table 4

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	Time	D-value (Decimal decay time)	The time required, at a given temperature T, to reduce the number of micro-organisms 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, to reduce the number of micro-organisms of a given species to 10% (1 logarithmic reduction)
$F(z,T)$	Time	Equivalent exposure time	Equivalent exposure time related to the specific temperature T and to the specific value of z indicated
F_0	Time	"Reference" exposure time, "F zero"	Equivalent exposure time related to the temperature of 121°C and to z = 10
N_0	None	Initial biological load	Number of viable micro-organisms contained in a unit before sterilization
N_U	None	Surviving biological load	Number of micro-organisms 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 $D_{121,1}$
L	None	Lethal Ratio	Lethality ratio between T (the temperature being considered) and T_{ref} (the reference temperature, generally 121°C) for a given value of z (generally 10)
$PNSU$	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 micro-organisms

Sterilization

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

Therefore the terms "sterile" and "sterilization", in a strictly biological sense, describe the absence or destruction of all viable micro-organisms. 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. Therefore only a treatment of infinite duration provides the absolute certainty that the entire microbial population has been destroyed and that the system is sterile.

Making the characteristics of the sterilization treatment more drastic (i.e. increasing time and/or temperature) usually entails a decay of the qualities of the product and certainly increases 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 we 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) must therefore be lower than 10^{-6} .

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3. REAL TIME CALCULATION OF F_0 WITH A COMPUTERIZED AUTOCLAVE

Electronic technology allows the use of a computer for the integrated management of a sterilization autoclave. If the computer is sufficiently sophisticated, besides the usual control, monitoring and alarm functions, it can also calculate F_0 in real time and therefore control the process according to this algorithm.

A typical computerized autoclave control system, for example, operates as follows.

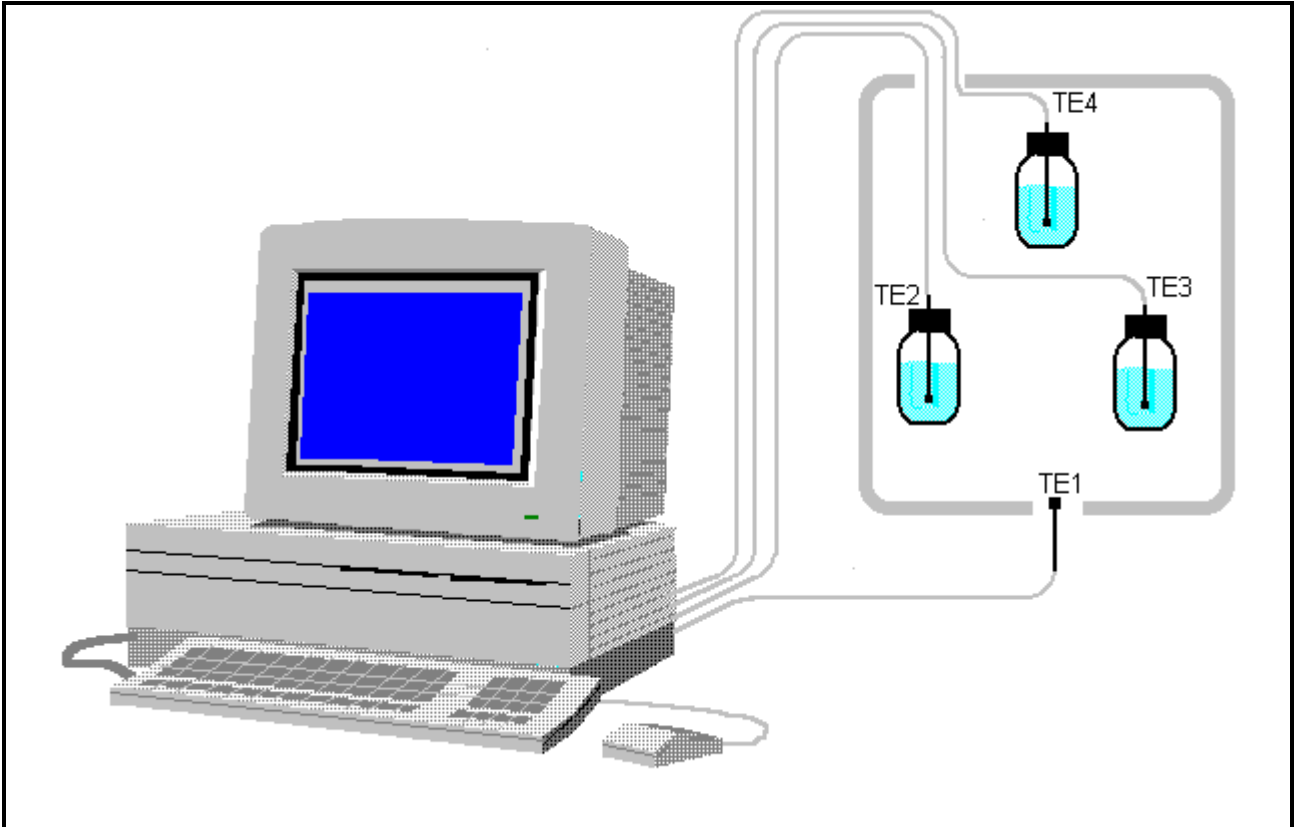


Figure 1

The autoclave is generally provided with multiple heat 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 temperature, e.g. 121°C
2. the acceptable oscillation of this temperature around this value, e.g. $\pm 0.5^\circ\text{C}$, 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

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- 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.

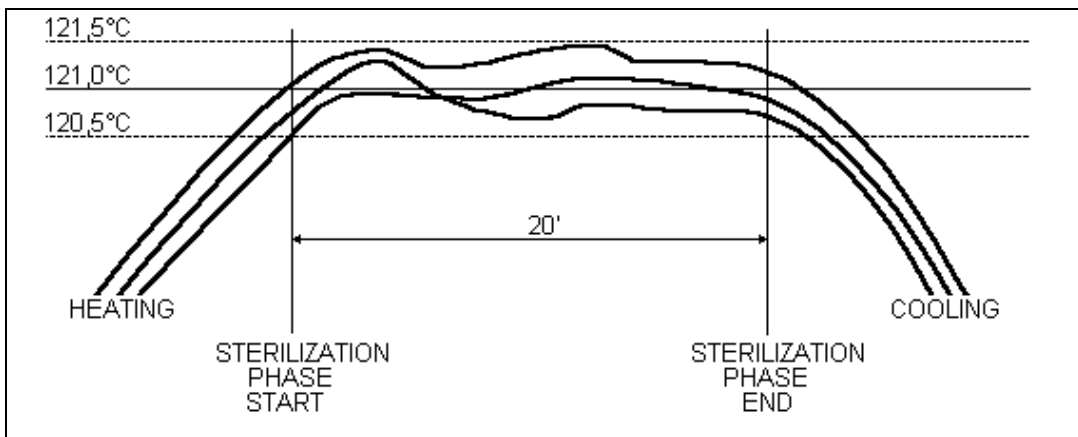


Figure 2

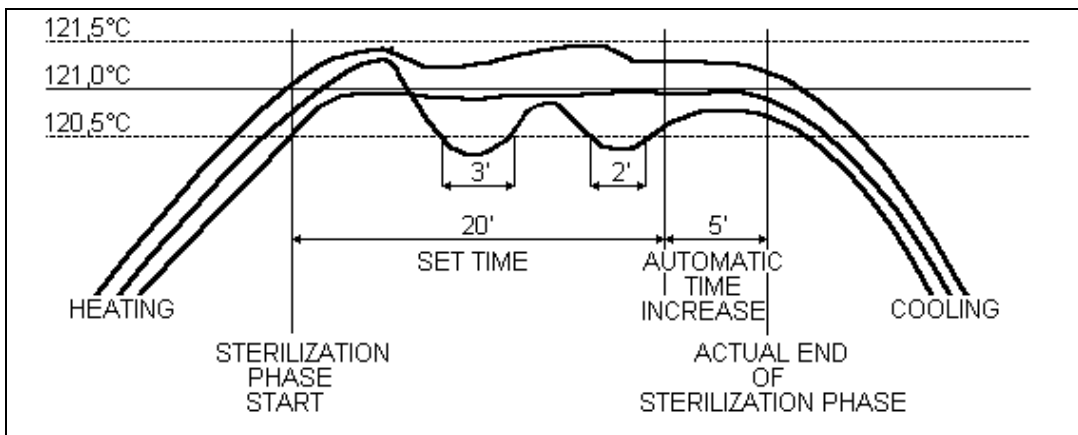


Figure 3

3.2. F₀-BASED CONTROL

The programmer sets the following parameters:

1. the sterilization temperature, e.g. 121°C
2. the acceptable oscillation of this temperature around this value, e.g. $\pm 0.5^{\circ}\text{C}$, so that the acceptable oscillation range will be 120.5 to 121.5°C
3. the value of F₀ which, when totalled by the coldest probe, causes the end of the sterilization phase, e.g. F₀ = 15 (F₀ is adjustable between 1 and 999)
4. the F₀ calculation start temperature, which can be pre-set from 90°C upward: if it 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
5. the value of the temperature coefficient z, which is variable between 5 and 20 but is normally set to 10 (to obtain a properly said F₀ value).

The calculation of F₀ is performed independently for each probe on a very small time base: e.g. 2 seconds. Therefore, every two seconds and for every probe, the computer takes the temperature of entry and exit from the time base, averages them, inputs this average temperature into the formula of F₀, calculates partial F₀ and adds it to the previously accumulated F₀ for that probe. Every 20 seconds (or a longer or shorter interval, depending on programming) these values are recorded by the printer of the computer in digital terms. The values accumulated by the coldest and hottest probes are displayed on the screen and are refreshed every 2 seconds. When they reach the pre-set value, the sterilization phase ends.

Let us examine some examples of F₀-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 F₀ starts when the sterilization begins, i.e. when the calculation start temperature corresponds to the lower temperature of the acceptable oscillation range. The phase ends when the probe (assumed to be the least favoured) has accumulated the pre-set value of F₀ (12 in this case). The calculation of F₀ ends when the sterilization phase terminates.

Example 2 (Figure 5)

The calculation of F₀ starts when the probe exceeds the pre-set value (100°C in this case) during the heating phase. When the sterilization phase is entered, the probe has already accumulated an F₀ of 1.1 minutes. The sterilization phase ends when the probe has accumulated the pre-set F₀ value (15 in this case). However, the calculation of F₀ continues until the probe leaves the pre-set value of 100°C. It can thus be seen that an additional lethal dose F₀ = 0.9 minutes is provided during the cooling phase.

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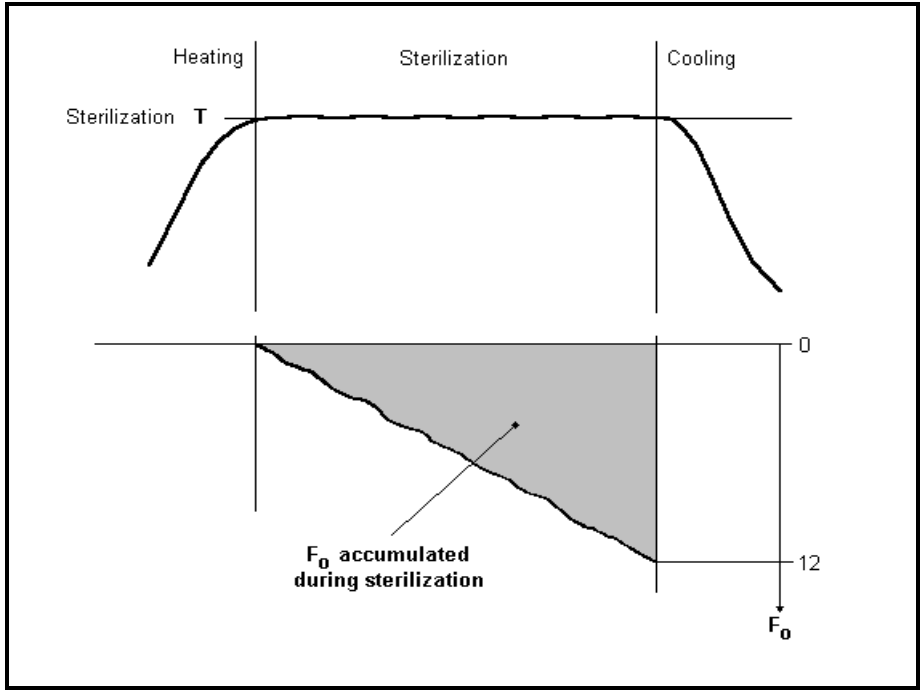


Figure 4

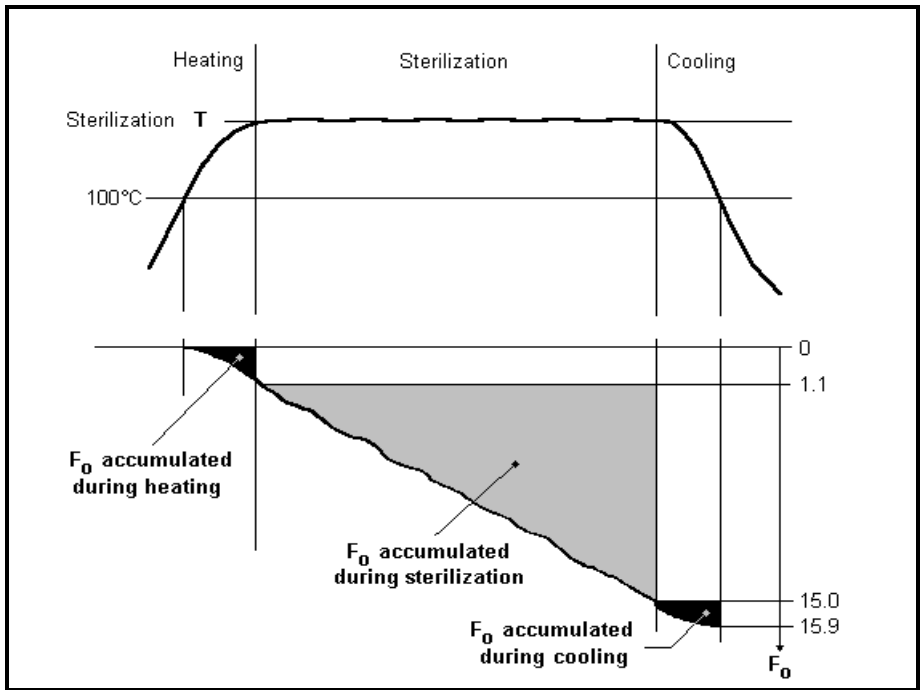


Figure 5

The calculation of the lethal doses provided during heating and cooling is necessary when highly heat-sensitive products are sterilized.

The ability to select the value of z allows the calculation of lethal doses with respect to the heat-sensitivity characteristics of a specific and critical contaminating micro-organism. 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 computer, which is battery buffered, continues to operate but naturally no longer receives signal from the autoclave; the autoclave itself is equally unable to execute the command signals sent by the computer. 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 computer 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.

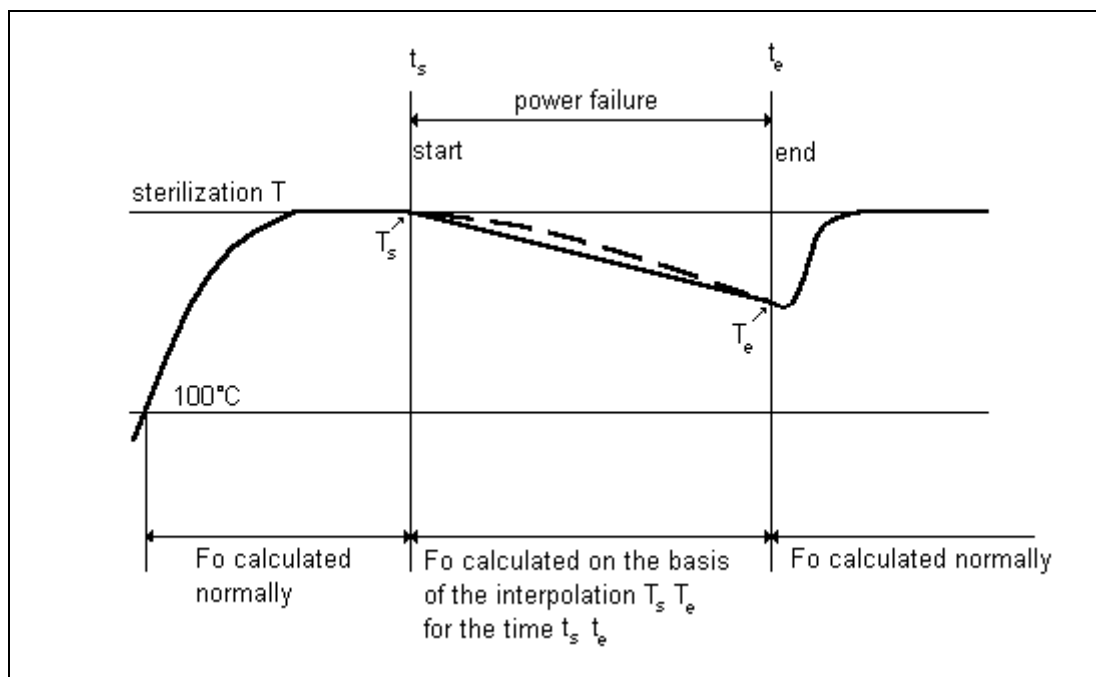


Figure 6

The computer 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 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 a 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 $T_s-100^\circ\text{C}$ for the time interval t_1-t_{100} ; 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.

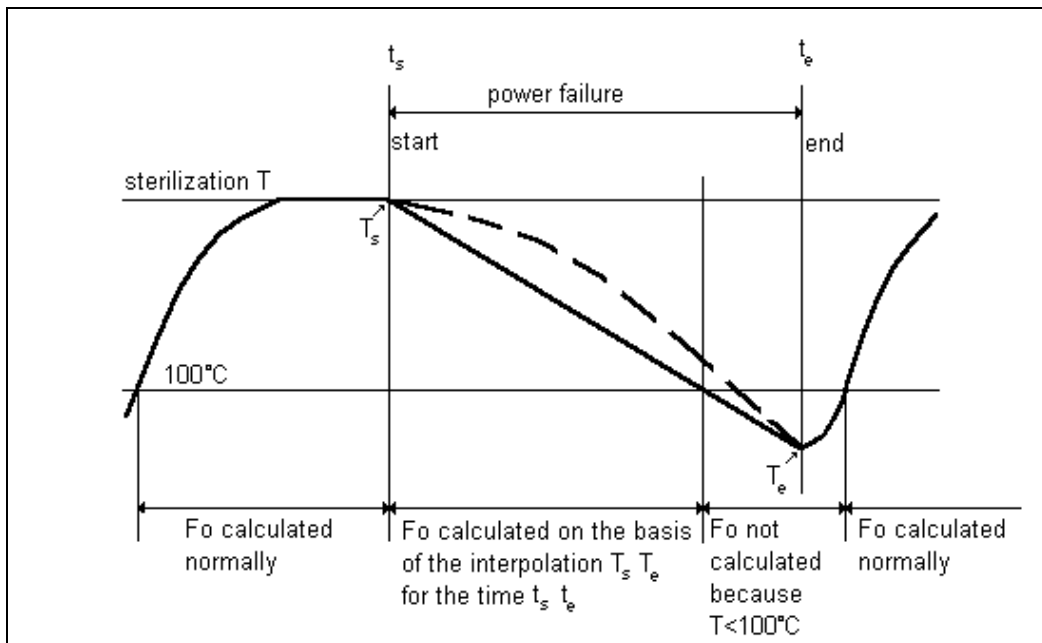


Figure 7

3.3. STERILIZATION TIME-BASED CONTROL WITH CALCULATION/PRINTOUT OF F_0 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 at least $F_0 = 12$ has been provided during the sterilization phase to the coldest point of the load.

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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.

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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 micro-organism can "homogenate" and proliferate.

A bottle or 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 just a few 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 micro-organisms contained in a unit die within that time, regardless of their number. The risks of such an assumption are evident.
2. Nowadays, it has been shown that steam sterilization instead proceeds like a first order chemical reaction and therefore at a specific rate which is higher as the temperature rises and is a function of the number of micro-organisms 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 micro-organisms present in the unit by 90%.
5. The D-value varies according to the kind of micro-organism (and to its "history"), the medium in which it is immersed and, as mentioned, the sterilization temperature.
6. At the temperature of 121°C, the D-value is generally between 0.5 and 2 minutes: for micro-organisms commonly dealt with we can assume, as an average, that $D = 1$ minute.
7. This means that at the end of each minute at 121°C the number of micro-organisms reduces to one tenth of the number at the beginning of that minute.
8. Therefore, if a unit is kept at 121°C for 3 minutes, the number of micro-organisms contained therein is reduced to one thousandth ($1/10 \times 1/10 \times 1/10 = 1/1000$) 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 micro-organisms per vial or bottle), after 3 minutes of treatment 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

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be understood to mean that at this point each unit contains one tenth of a micro-organism (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, 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: Probability of Non Sterile Unit. $PNSU = 10^{-6}$ 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 it is necessary to meet several conditions:
 - to statistically know the initial bacterial load 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 heat dose sufficient to obtain the required PNSU
 - if the sterilization at 121°C is not performed, 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 time during which sterilization is actually performed at 121°C, or
 - the time during which sterilization is performed at another sterilization temperature, related by calculation to 121°C so as to be equivalent in terms of lethal heat dose, i.e. of microbial destruction effectiveness

NOTE: The exact reference temperature of F_0 is 121.11°C, as this value corresponds to 250°F

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 routinely not lower than 12 (and indeed has a good safety margin with respect to this minimum value) exclusively during the sterilization phase (i.e. ignoring the lethal heat doses provided during heating and cooling) 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.

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18. If $F_0 = 12$ is to be achieved, the required exposure time is less than 12 minutes if the sterilization temperature is greater than 121°C and greater than 12 minutes if the sterilization temperature is lower than 121°C .
19. Let us now try to quantify that "less" and "greater" than 12 minutes. For most of the micro-organisms with which we commonly deal, it is assumed that every 10°C of shift from the temperature of 121°C entail a tenfold change in the sterilization rate.
20. Therefore, if we work at 111°C , in order to achieve an F_0 equal to 12 it is necessary to sterilize for $12 \times 10 = 120$ minutes, whereas if we work at 131°C then $12/10 = 1.2$ minutes, i.e. 72 seconds, are sufficient.
21. If we want to determine the extent by which the sterilization rate varies for temperature variations of 1°C we must find the number which yields 10 when raised to the tenth power: this number is 1.24. This means that a 1°C variation in the sterilization temperature causes an increase (or reduction) of the sterilization rate by a factor of 1.24, i.e. 24%.
22. Similarly, it can be shown that a temperature variation of 0.1°C causes a rate variation with a ratio of $1 \rightarrow 1.02$, 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 $1.24 \times 1.24 = 1.5376$ times to relate it to 121°C . Therefore, for example, if $F_0 = 12$ is to be achieved, it is necessary to sterilize for about 19' instead of 12'.
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 at the time 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 Lethality 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 analyse 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 naturally approximated to 1 (it would be exactly 1 for 121.11°C).

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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 .

Therefore:

1 minute at 120.0°C = 1' x 0.774 = 0.77 minutes at 121.1°C

15 minutes at 120.0° = 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.

TABLE OF LETHALITY RATES

for a reference temperature of 121.111°C = 250°F and z = 10°C;
obtainable starting from the temperature T comprised 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
LETHALITY 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

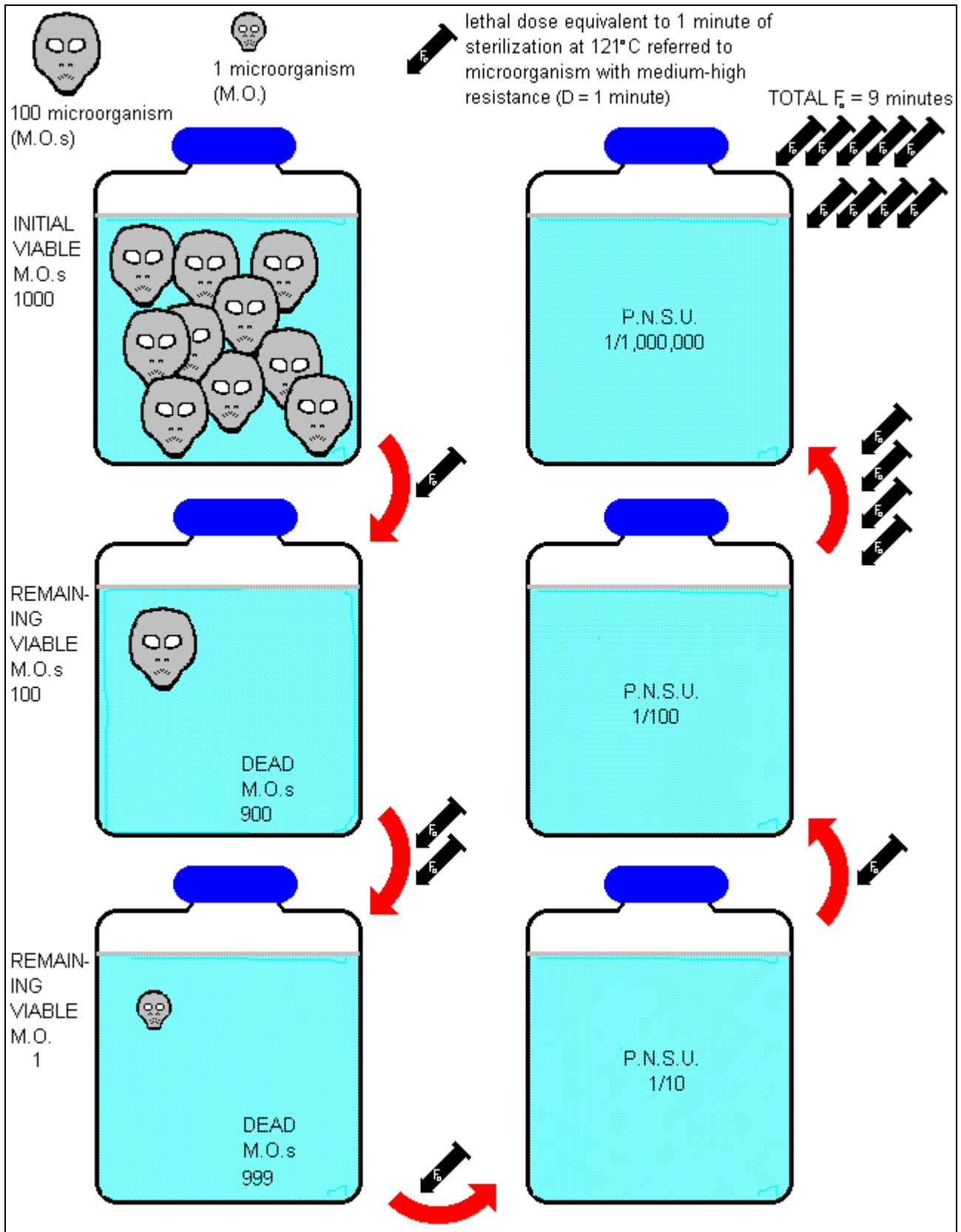


Figure 8

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