# The *F* concept: use and misuse in sterilization practice

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# Temperature for moist-heat sterilization - USP

USP 43, <1229.1>:

“The process lethality at temperatures other than 121° can be calculated to determine lethality equivalent to that provided at 121°.

Moist heat sterilization process efficacy is not intrinsically linked to a target temperature of 121°, which is simply the Celsius conversion of 250°F, and other temperatures can be used.”

# Temperature for moist-heat sterilization - EP

EP 10.0, 5.1.2, 3-1-1:

"Sterilisation processes can be operated at temperatures lower than the standard 121 °C (for longer exposure times) or at higher temperatures (for shorter exposure times). The *z*-value (the temperature difference that leads to a 10-fold change of the *D*-value of the biological indicator) is used to compare the efficacy of 2 cycles operated at different temperatures. **For a *z*-value determination, the D-value must be determined at 3 or more temperatures. The intended process temperature should be within the range of the 3 temperatures."**

# Equivalent sterilization time *F*

Thus, no doubt that no fixed sterilization temperature is indicated by rules or standards (in Europe, only a lowest limit of 110 °C), and the following question becomes essential:

*Which is the lethal effect of the exposure of a microbial population to a variable temperature* T *under moist-heat conditions in comparison with a hypothetical sterilization performed at a constant temperature* $T_0$ *under the same moist-heat conditions for the same time?*

The relationship between the biological effects of moist-heat at different temperatures depends on:

- the variable temperature difference from a constant one $T_{ref}$ assumed as reference
- the reference temperature $T_{ref}$ itself
- the not intrinsically constant temperature coefficient *z*-value

Mathematics provides the theoretical solution of this problem by an algorithm called *F*

# *F* physical

The mathematical algorithm *F* is more properly named the *physical F* and is often indicated as $F_{phys}$

PDA Technical report No.1 rev. 2007, Glossary of Terms:

"***$F_{Physical}$***: A term used to describe the delivered lethality *calculated based on the physical parameters of the cycle*. The $F_{Physical}$-value is the integration of the lethal rate (*L*) over time. The lethal rate is calculated for a reference temperature ($T_{ref}$) and *z*-value using the equation: $L = 10^{(T-Tref)/z}$."

According to USP 43, <1229.2>, $F_{phys}$ can be defined:

**"the equivalent sterilization time relative to a base temperature"**

# *F* biological

$F_{phys}$ is completely different from the *biological F*, that expresses the effectiveness of sterilization from the point of view of microorganisms and is often indicated as $F_{bio}$

PDA Technical Report No.1 rev. 2007, Glossary of Terms:

"***$F_{Biological}$*: A term used to describe the delivered lethality, *measured in terms of actual kill of microorganisms on or in a BI challenge system*. The $F_{Biological}$-value is calculated as $D_T$ x LR, where $D_T$ is the *D*-value of the BI system at the reference temperature (*T*) and *LR* is the actual logarithmic reduction (log $N_0$ – log $N_F$) of the BI population achieved during the cycle."

In the same glossary: "***Biological Indicator Challenge System (BI)*: A test system containing viable microorganisms of a pure, specified strain providing a defined resistance to a specified sterilization process."

The concept of $F_{bio}$ as the expression of delivered lethality measured by biological indicators is present in EP, but not in USP

# The *F* algorithm - Mathematical digression

*"The z-value is the change in temperature in degrees Celsius required to alter the D-value by a factor of 10 (the z-value relates the resistance of a micro-organism to changes in temperature)"* (EP 10.3, 5.1.5)

This ***definition*** may be expressed by a mathematical equation:

$$D_{(T-z)} = 10 \cdot D_T$$

The mathematical ***function*** that satisfies this equation with reference to a known D-value $D_R$ at a "reference" temperature $T_R$ is the solution of a problem of variational calculus. The solution is:

$$D = D_R \cdot 10^{(T_R-T)/z}$$

Let's now remember that the exposure time after which a microbial population N is reduced to a fraction of the initial population $N_0$ is:

$$t = D \log_{10} (N_0/N)$$

So, the same microbial reduction effect as after a time $t_R$ at temperature $T_R$ will be reached at different temperatures after a time:

$$t = D \cdot t_R / D_R$$

If we consider infinitesimal intervals of exposure time $dt_R$ at a reference temperature $T_R$ equivalent to infinitesimal intervals of exposure time dt at the continuously variable temperature T is thus:

$$\mathbf{dt_R = dt \cdot 10^{(T-T_R)/z}}$$

# The *F* algorithm – Practical formula

In practical terms of finite time intervals, the mathematical expression of the equivalent exposure time *F* at a variable *T* with respect to the exposure time at a fixed $T_{ref}$ becomes:

$$F = \Delta t \cdot \Sigma 10^{(T - T_{ref}) / z}$$

where:

$\Delta t$ = constant time interval between two subsequent temperature measurements

$T$ = mean value of the variable sterilization temperature during each time interval, °C

$T_{ref}$ = fixed reference temperature for F calculation, °C

$z$ = temperature coefficient of the D-value, °C

The shorter the time intervals between two next measurements, the more accurate the calculation of the equivalent exposure time. In moist-heat sterilization practice, intervals of a second or half a second are widely used

At a first glance, *D*-values could seem not involved in the calculation of equivalent time, but in fact, at least two *D*-values (experimental data) are necessary to calculate the z-value, according for instance to EP 10.3, 5.1.5:

$$z = (T_2 - T_1) / (\log_{10} D_1 - \log_{10} D_2) = (T_2 - T_1) / (\log_{10} D_1 / D_2)$$

A graphical method to evaluate z-values by plotting at least two D-values in a semilogarithmic D-T chart, is given by PDA TR#01, rev. 2007, par. 3.1.2

# The $F_0$ "physical": equivalent exposure time

If the reference temperature is assumed equal to 121 °C (originally 250 °F = 121.11 °C, in the past the most common temperature for moist-heat sterilization) and *z*-value equal to 10 °C (originally 18 °F), the physical equivalent time is named $F_0$ ("F zero" or "F naught"). With the same symbols as in the previous slides, the formula becomes:

$$F_0 = \Delta t \cdot \Sigma 10^{(T - 121) / 10} \qquad (1)$$

"Cycle efficacy for steam sterilization often is measured using $F_0$, which is defined as the equivalent exposure time at 121 °C. $F_0$ is a means for quantifying steam sterilization effectiveness by determining the equivalent sterilization time in minutes relative to a base temperature of 121 °C and a *z*-value of 10 °C … The $F_0$ calculation should begin at 100° and should continue through the end of the dwell period provided that saturated steam conditions are maintained." (USP 43 <1229.1>, relevant to sterilization by direct contact)

The reduction of the reference temperature from 121.11 °C to 121 °C is now universally adopted. A curious exception is Def. 3.17 of EN ISO 17665-1. This reduction causes an overevaluation of almost 2.57% in the resulting $F_0$, which may be regarded as negligible in practice

# $F_0$ "physical" in Pharmacopoeias

USP 43 provides equations of "physical" equivalent time (as in the previous slide or similar ones) for "direct contact" moist-heat sterilization (<1229.1>), "aqueous liquids" sterilization (<1229.2>), dry-heat sterilization (1229.8), and dry-heat depyrogenation (<1228.1>)

EP 10.3, 5.1.5 simply states that "the total *F* of a process … can be calculated by integration of lethal rates with respect to time at discrete temperature intervals above the minimum temperature" of 110 °C, with z = 10 °C for moist-heat sterilization

To our knowledge, neither EP, nor other European rules or standards provide an equation for what is called "calculated effectiveness from physical parameters ($F_{phys}$)". Apparently, Europeans are expected to know the equation of *Fphys* or "lethal rates" from scientific literature

# $F_0$ "physical": predictive use

The $F_0$-equation is commonly used also to predict the exposure duration at a constant process temperature other than the reference one and capable to deliver the same lethality dose expressed as expected $F_0$-target. For this purpose, equation (1) is used in the form:

$$t_T = F_0 \cdot 10^{(121 - T) / 10} \qquad (2)$$

where:

$t_T$ = predicted exposure time to constant temperature *T* under moist-heat conditions for delivering a lethal dose $F_0$, minutes

T = constant process temperature, °C

$F_0$ = expected lethal dose expressed as equivalent time at 121 °C, minutes

Some official documents express minimum sterilization requirements as a combination of minimum equivalent lethal dose $F_0$ and minimum temperature to be attained during the sterilization process. This is the case of EP 10.0, 5.1.1 and the correlated EMA 2019 Guideline, 4.1.1, that demands $F_0 \geq 8'$ at $T \geq 110$ °C for a properly said sterilization. EMA 2019 Guideline, 4.1.1 Table 1 also specifies as "overkill" a process with attained $F_0 \geq 12'$ at $T \geq 121$ °C.

Any lighter treatment is called in Europe "Post-aseptic processing terminal heat treatment"

# $F$ or $F_0$ "biological"
## Evaluation of the actually delivered lethality

The biological expression of delivered lethality is given by the equation (PDA TR#01 rev. 2007, 4.4.1.6):

$$F_T = D_T \cdot (\text{Log } N_0 - \text{Log } N_F) \qquad (3)$$

where:

$F_T$ = delivered lethality referred to reference temperature T, as biologically evaluated by the measured inactivation of a BI whose D is known at T, minutes

$D_T$ = *D*-value of the BI at the reference temperature T, minutes

$N_0$ = initial population of BI, units

$N_F$ = final population of BI, units

**It may be worth to note that *z*-value is not involved the calculation of $F_{bio}$**

Only if a $D_{121}$ is used, this biological $F_T$ should properly assume the name the $F_{0bio}$, as apparently in EMA 2019 Sterilisation Guideline, Table 1 (dated March 6, 2019). This document is strictly related with European rules, but EP 10.0, 5.1.1 (dated July 2019) uses the symbol $F_{bio}$ and defines it by an equation $F_{bio} = D_{121} \cdot (\text{Log } N_o - \text{Log } N)$ that is already present in EN ISO 17665-1:2006, D.4.2 for the calculation of the minimum $N_0$ of BIs to be used in "full cycle approach". To make things plainer,, update EP 10.3, 5.1.5 (June 2020) changes this $F_{bio}$ to a mere $F$ but writes it as $F_0$ in the equation $F_0 = D_{121} \cdot (\text{Log } N_o - \text{Log } N)$ for reference to moist-heat processes in contrast with dry-heat ones…

# $F$ or $F_0$ "biological": predictive use

Regardless to this perhaps unimportant lack of uniformity in symbology, it is recognized (PDA TR#01 rev. 2007, 4.4.1.6) that the $F_{bio}$ equation may be practically used for the actual calculation of delivered lethality *only if the final condition of the BIs is not of complete inactivation;* this limitation, in fact, commands the use of "reduced cycles" for validation

On the contrary, "the BI inactivation requirements of the qualification sterilization cycle are for BIs to be negative; this requires a large $F_{PHY}$. At this $F_{PHY}$-delivered condition, it will not be possible to measure an $F_{BIO}$ since this BI condition is outside the measurable quantal area."

In this case, equation (3):

$$F_T = D_T \cdot (\text{Log } N_0 - \text{Log } N_F)$$

can be used "to determine the lethality ($F_T$) requirement to kill the BI to a probability of non sterility (PNSU)" of a still detectable level (let's say $10^{-2}$), which will provide information of the effectiveness of the measured physical parameters

# $F$ or $F_0$ "biological"

## Calculation of the lethality to be delivered for an effective process

If the value of $N_F = 10^{-6}$ is used in the $F_{bio}$ equation and both $N_0$ and $D_{121}$-values are supposedly known for the actual product, the $F_{bio}$ equation may be used to define the sterilization cycle.

"Regardless of the number and heat resistance of the actual bioburden organisms in the load" (PDA TR#01 rev. 2007,4.1.1.1),

$D_{121} = 1.0$'

and

$N_0 = 10^6$

are assumed for the so-called "overkill design approach", resulting in an $F_{0bio}$-requirement of 12'. Temperature other than 121 °C may be considered with the additional assumption of z = 10 °C for the mathematical calculation of the delivered lethality $F_{0phys}$

"This approach assumes both a higher bioburden population and resistance than would be expected … Because worst case assumptions are made for the bioburden population and resistance with this design approach, there is little scientific necessity for routine bioburden monitoring of the load items." (PDA TR#01 rev. 2007, 4.1.1.1)

# $F$ or $F_0$ "biological"

## Selection of the challenging BIs

$F_{bio}$-equation "can be rearranged to determine the minimum starting population of the BI necessary to qualify the delivery of the desired biological lethality … Note that this is a separate exercise from using the model to determine the desired delivered lethality for product safety" (PDA TR#01 rev. 2007, 5.2.1.1):

$$\text{Log } N_0 = \text{Log } N_F + F/D$$

where:

$N_0$ = initial population of BI ("biological challenge"), units

F = "desired lethality determined during process design"

D = *D*-value of the BI at the same reference temperature at which the desired lethality is referred, minutes

$N_F$ = "the population of the biological challenge after exposure. For calculation purposes, if the biological challenge is killed, then it can be assumed that there is less than one surviving microorganisms, which is depicted as $N_F = 10^0$ in this equation"

Very clear examples of PDA's explain the use of this method.

The $F_{bio}$ equation is also quoted, unfortunately in a rather confusing way but with an explicit reference to PDA now revised Validation Monograph, in EN ISO 17665-1:2006, D.4.2 to calculate, with an additional safety margin of "0,5 x log to the base 10" (i.e., 3.1623 times more), the minimum population of a biological indicator to be used in the "full cycle approach" for "qualifying a sterilization process" of the "overkill" type. This means a desired lethality of 12 equivalent minutes at 121 °C (to be inserted as *F* in the above equation)

# Physical and biological *F*-values (I)

$F_{phys}$ can be easily calculated by a process controller and used for control while the sterilization process is going on. On the contrary, $F_{bio}$ can not be calculated ongoing, as it involves biological laboratory measurements to be done after the sterilization process

According to EP 10.0, 5.1.1, "calculated effectiveness from physical parameters ($F_{phys}$) is correlated with biological effectiveness ($F_{bio}$). $F_{bio}$ expresses the lethality, in minutes, provided by the process in terms of destruction of the biological indicators used." "In cycle validation … adequate biological effectiveness is verified by exposure of biological indicators" in the "positions in the load that are the most difficult to sterilise."

**"The $F_{bio}$ determined for the most-difficult-to-sterilise position is used *to define the parameters necessary to achieve reliably the required SAL* equal to or less than $10^{-6}$ for the required cycle."**

As a "rule of thumb", $F_{bio}$ might be intended for compliance in validation; $F_{phys}$ in the most unfavorite position is expected to routinely exceed $F_{bio}$

# Physical and biological *F*-values (II)

"Validation of $F_{0Phys}$ and $F_{0Bio}$" is required also by EMA 2019 Sterilisation Guideline, Table 1, which is related to EP. Unfortunately, no practical instructions are provided for complying with this requirement.

*"Evaluating the $F_{physical}$ and $F_{biological}$ Agreement"* is the title of the already quoted Paragraph 4.4.1.6 of PDA TR#01 rev. 2007, (see Slide No. 13) which provides very useful information, but also summarizes the matter with these challenging words:

While there is no standard approach to designing studies to evaluate the agreement of $F_{BIO}$ and $F_{PHY}$, several approaches have been detailed in literature. (39, 40) It is important to note that while this evaluation provides a higher degree of process understanding, many successful cycles have been developed and qualified without this evaluation. One of the goals of this technical report is to promote this cycle development objective and to stimulate additional exploration into appropriate methods for its evaluation.


39. Evans, K., Pflug, I.J., Carrying Out Biological Qualification, The Control Operation of Moist-Heat (Steam Sterilization) Processes for Producing Sterile Pharmaceuticals and Medical Devices, *PDA Journal of Pharmaceutical Science and Technology*, 54 (2) (2000) pp 117–135

40. Pflug, I.J., Chapter 17B, Microbial Control in Pharmaceuticals and Medical Devices using Moist Heat (Steam Autoclave), *Microbiology and Engineering of Sterilization Processes*, 12th Edition, Parenteral Drug Association (2007)

# *F*-values: a comparison *phys* vs *bio*

$$F_{phys} = \Delta t \cdot \Sigma 10^{(T - T_{ref}) / z}$$

- independent of initial and final bioburden
- no biological counts directly involved
- dependent on the trend of a *D*-value, expressed by *z*-value
- meaningful only if the sterilization conditions (in our case, condensing steam or liquid water in contact with microorganisms at the measured temperature) are attained and continuously maintained
- evaluable *while* a process runs

$$F_{bio} = D_{T_{ref}} \cdot (\text{Log } N_0 - \text{Log } N_F)$$

- independent of actual temperature, time, *z*-value
- no physical measurements directly involved
- dependent on a *D*-value at a reference temperature
- meaningful independently of the actual compliance of a process with required sterilization conditions
- evaluable only *after* the completion of a process

# Cautions for physical $F_0$

$F_0$ "physical", or $F_{0phys}$, is obtained from a mathematical equation for an expected equivalent exposure time at 121 °C with the assumption z = 10 °C.
$F_{0phys}$ assumes a biological meaning only under proper sterilizing conditions, i.e., if there is steady contact of the microorganisms with liquid water, that means inside a water solution or in presence of condensing steam on the contact surface) at the temperature used for calculation: if these conditions are not in compliance, the calculation of $F_{0phys}$ becomes biologically meaningless

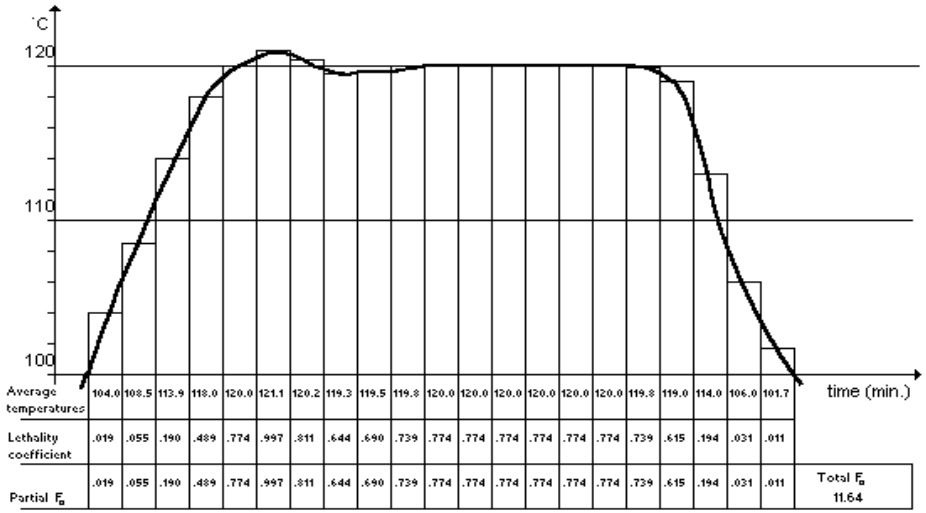
The range of validity of *z*-value should always be remembered as well. To use a *z*-value to calculate *D*-values, and consequently $F_{0phys}$-values, would be improper beyond the experimental range of validity of *z*-value itself (remember EP 10.0, 5.1.2, 3-1-1 quoted in Slide no. 3)

This restriction is consistent both with the minimum temperature of 110 °C required for $F_0$ calculation by European rules and standards (exceptions shall be justified), and a requirement of EP 10.0, 5.1.2, 3-1-2 on the "establishment of a validation cycle": "A reduced cycle is chosen such that the temperature is not more than 1 *z*-value below the reference sterilization process temperature."

# Some correct uses of $F_0$ (I)

- Calculation of lethality accumulated by *aqueous liquids* at temperatures different from 121 °C, provided that 10 °C are acceptable as *z*-value in the range of calculation.

  This refers to the so-called “liquid loads”.

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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

Here above, lethal rates (here named “lethality coefficients”) are calculated with the old $T_{ref}$ = 121.11 °C. With the actual $T_{ref}$ = 121.00 °C, lethal rates and $F_0$-values would result almost 2.57% bigger.

# Some correct uses of $F_0$ (II)

- Actual exposure monitoring by the equation $F_{0phys}$ the sterilization target for *liquid loads*

  This allows to directly take in account the lethality accumulated during the heating phase, and, possibly, to validate a reduced target for the exposure phase thanks to the certain, even if small, accumulation of lethality during the cooling phase. The slower are the heating and cooling phases, the more sensible the gain

- Evaluation by the equation $F_{0phys}$ of the lethality delivered by actual contact steam to solids at temperatures different from 121 °C by exposure to temperature, provided that 10 °C are acceptable as *z*-value in the range of calculation

  This refers to the so-called “porous/hard loads”.
  Only time intervals are to consider, while the presence of saturated, i.e., condensing steam on all the items of the solid load may be reasonably expected. Therefore, the calculation of $F_0$ shall not be started till the removal of air from the chamber and load surfaces has been completed and shall be immediately stopped as soon as the condition of condensing steam fails, typically at the end of the exposure phase, when drying by vacuum, or cooling with air circulation begins. At best, for porous/hard loads, the calculation of $F_0$ should occur only during the exposure phase, provided that the validated sterilization conditions are complied with

# Some correct uses of $F_0$ (III)

- Standard definition of general requirements for minimum lethality as expected equivalent time at 121 °C

  For instance, the European requirement: "All steam sterilisation processes require a minimum lethality of $F_0 \geq 8$ minutes and a minimum process hold temperature of 110 °C." (EMA 2019 Sterilisation Guideline, 4.1.1)

  Or the European definition: "**Overkill sterilisation**: A process with a lethality of $F_{0BIO} > 12$ minutes." (EMA 2019 Sterilisation Guideline, 6-Definitions)

- Prediction by the equation of $F_{0phys}$ of the exposure time theoretically required to have the same effectiveness of a given sterilization time at 121 °C (see Slide no. 11)

  A European "overkill cycle", defined by $F_{0bio} = 12'$ could theoretically be performed, for instance, at 124 °C with an exposure time of 6 minutes (at least in Europe, lower temperatures than 121 °C are not allowed for overkill cycles): in fact,
  $t = 12 \cdot 10^{(121-124)/10} = 12 \cdot 10^{-0.3} = 12 \cdot 0.5 = 6'$

  A required minimum lethality of $F_{0bio} = 8'$ could be delivered by an exposure of a solid to moist steam, or by a dwell of an aqueous liquid at T = 116 °C for
  $t = 8 \cdot 10^{(121-116)/10} = 8 \cdot 10^{0.5} = 8 \cdot 3.16 = 25.3'$

# Some correct uses of $F_0$ (IV)

- Evaluation of the uniformity of the lethality accumulated by a liquid load or delivered to a porous/hard load.

  This can be regarded as investigational and/or "validational" use of the $F_0$ as equivalent time. Rather frequently process specifications include monitoring of the maximum discrepancy of $F_0$ among the load (most and less "favorited" positions)

  Due to the above conditions for the use of $F_0$ with porous/hard loads, in this case the thermal uniformity can in fact be evaluated only during the exposure phase

Cautions shall be always respected in the selection and use of BIs for the measurement of the $F_{bio}$ delivered by cycles for porous/hard loads

# Some frequent misuses of $F_0$ (I)

All misuses of $F_0$ derive from forgetting that the $F_{0phys}$ has been introduced, originally by the food industry, for loads that always contain enough water to guarantee the continuous compliance with the basic requirement for most-heat sterilization, i.e., the contact of liquid water with the microorganisms to inactivate.

Therefore, the calculation of $F_{0phys}$ for porous/hard loads after the end of exposure phase is a big mistake. In fact, the saturation conditions required for the condensation of the steam in contact with the load fail immediately if a drying vacuum phase follows and become at least uncertain if a cooling phase begins, that demands the removal of the excess steam by air fed to the autoclave chamber.

# Some frequent misuses of $F_0$ (II)

For the above reasons, it is suggestable to restrict the calculation of $F_{0phys}$ in the cycles for porous/hard loads to the exposure phase itself and to monitor the phase by time. Monitoring by $F_{0phys}$ the sterilization target for porous/hard loads is another typical mistake.

In addition, with porous/hard loads the time between the completion of air removal and the start of the exposure is generally so short, that the contribution of it would make no practical difference for the overall cycle duration.

# Thank you

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