H & W TECHNOLOGY, LLC

Biological Indicators: Differentiation of Validation Results and Application in Steam Sterilization
Subtitle: Resistometers vs. Reality

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Abstract

Sterilization Indicators are generally validated in resistometers, providing a common baseline of measurement of the resistance of the indicator to the sterilization process. Testing in resistometers does not take into account the actual process experienced by the indicators in actual sterilization, causing something of a disconnect between the validated parameters of the indicators and their actual use. The proposed emphasis of the article is to examine this disconnect and provide mathematical models of the differential behavior between idealized testing (resistometers) and actual use in steam sterilization, and how one might interpret the data provided by the indicators to account for and deal with this differential.

Introduction

The observed performance of sterilization indicators appears to differ in actual cycles which include loads to be sterilized and the cycles in which they are validated, i.e., resistometer cycles. Indicators may show complete processing at a small fraction of the exposure time in actual use. This is especially true for biological indicators and for class 5 or integrator chemical indicators, but can and does also happen for class 6 cycle emulator chemical indicators. The difference between the conditions under which the indicators are validated and the conditions of their actual use are the source of this behavior.

Indicators are validated in resistometers, which are specialized sterilizers that execute cycles with negligible come-up and come-down times. This type of cycle limits exposure to the sterilant to a period that is essentially the exposure period and nothing more.

Real cycles expose the indicators (and goods to be sterilized) to sterilant for an appreciable time or dose prior to the beginning of the exposure period.

This paper explores how to mathematically model the two periods, come up and exposure, separating their contributions to exposure of the indicator. This modeling sets the required exposure basis on a real half cycle, rather than at total kill for a biological indicator of normal resistance or at a stated value for a chemical indicator that is insufficient to provide the appropriate degree of sterility assurance for the process. While this effect is present in all sterilization processes, steam sterilization is used as the example here, since its mathematical model is well accepted.

Terminology and Cycle Phases

When a sterilant is delivered to a load in any amount, the microbial death that takes place is labeled “lethality”.

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Lethality increases with time of exposure or dose of sterilant. While this paper focuses on steam sterilization, to provide a general approach that is applicable to all processes, at least philosophically, we will use the term "dose" instead of time, exposure, or any of the other terms that signify the amount of exposure of the item to be sterilized to sterilant. In steam sterilization, the dose is traditionally expressed as time.

Accumulated lethality for steam sterilization is modeled mathematically by the term F, which calculates the lethality as a function of dose expressed as time above 100°C and the characteristic temperature response of the death kinetics of biological indicator used (Z value).

The D-value is the time or dose (of sterilant) required to achieve inactivation of 90 % of a population of a test organism under stated dose conditions (ISO/TS 11139, definition 2.11). In other words, the dose of sterilant required to reduce the population of a biological indicator by a factor of 10 (one log reduction).

The Z-value is the change in exposure temperature of a thermal sterilization process, which corresponds to a tenfold change in D-value (AAMI/ISO 11138-1, definition 3.21, ANSI/AAMI/ISO 11138-3:2006, Annex B). In other words, the behavior of the resistance of the biological indicator to different processing temperatures.

Any steam sterilization cycle has multiple parts, listed below:

- Air Removal
- Come Up
- Exposure
- Post-exposure processing

The cycle phases are shown schematically in Figure 1, which is fractionated cycle.

The phases that concern us here are the Air Removal and Come up, lumped together as "Pre-Exposure" and Exposure. The reason to lump Air Removal and Come Up together is that, for steam sterilization, both contribute to pre-exposure lethality. In other sterilization processes, they both may or may not contribute. In steam sterilization, steam is introduced in the air removal phase and may cause some lethality, depending upon the details of the air removal. It is the point of this paper to provide a means for the reader to separate the contributions of Pre-Exposure lethality and Exposure phase lethality, and make informed decisions about how to use indicators to respond to this information.

Contributions to Lethality of Pre-Exposure and Exposure Phases

Each biological indicator has a population of bacterial spores on it. Each spore dies individually in a sterilization process. The large number of spores in a biological indicator allows us to apply statistics to the process and get the usual measures of effectiveness of sterilization, like D-value and survival/kill times. Since a spore may die when exposed to a very small dose of sterilant, lethality can begin to accumulate very early in the process,

and long before exposure conditions are achieved. This is shown in figure 2, where the portions of the cycle that might contribute to lethality are highlighted in yellow. According to conventional models of lethality in steam sterilization as used in F, any temperature 100°C or greater will cause some spore death.

To answer this question more definitively, we can calculate the lethality accumulated in each recorded interval of the process. The F formula is used to do this. This formula is:

$$F = \sum_{t} 10^{D(T-121.1)/z}$$

The formula calculates the total effective number of minutes of exposure at 121.1°C, for the given Z-value. If Z is set to 10, the term becomes Fo. The term inside the summation symbol, Σ, is the equivalent lethality accumulated in one minute of exposure at 121.1°C, and is reported with units of minutes. t is the amount of time during which the system is exposed to sterilization conditions. T is the temperature in °C. Each increment of the sum is over the lethality accumulated during one minute of exposure, i.e. for the average temperature over that minute. If the data collection time increment is something other than one minute, the increment must be multiplied by the fraction of a minute that is the interval. For one-second intervals, for example, each increment is multiplied by 1/60. If two minutes is the increment, you multiply the increment by two. Once this is done, the incremental data is summed to obtain the total lethality of the cycle.

For the cycle shown in figure 1, accumulated Fo is shown by the black line. For the periods of the cycle in which the temperature (the blue line) was relatively close to 100°C, there is little lethality accumulated, and the black line plateaus. When the temperature rises, the black line rises as well, since lethality accumulates at a much greater rate with higher temperatures.

Applying these idealized calculations to the data, the lethality prior to the beginning of the exposure phase was 40.75 minutes, while the lethality in the exposure phase was 69.52. Biological indicators typically require an accumulated lethality of approximately 15-18 minutes to provide total kill of all the spores in them, this level of inactivation was done more than twice **before the sterilization cycle even entered the exposure phase.**

This is not a problem with the indicators. Biological indicators and chemical indicators show the sum total of the lethality of the process to which they are exposed. If there is sufficient lethality delivered prior to entering the exposure phase, the indicator will show a completed cycle long before the cycle is complete. In any case, the indicator is likely to show some kill. This is typical in hospital steam sterilizer cycles. The real question is what differentiates an indicator's label claims from real use conditions, since these two things seem to be very different.

Resistometers

Resistometers are used to validate the resistance of indicators to a sterilization process. They are sterilizers that are, "test equipment designed to create defined reference

combinations of the physical and/or chemical variables of a sterilization process" (AAMI/ISO 11138-1, 2006). In other words, they are sterilizers that create very accurately-controlled process conditions and change from one process state to another very rapidly. The reason that they must change from state to state rapidly is to avoid any contribution of the pre-exposure and post-exposure conditions to the lethality of the process. Since Fo and other measures of lethality are mathematical models, it is not appropriate to use them to remove the effects of other cycle phases on the apparent resistance of the indicator being tested, since models are not reality, and may deviate from reality. Since steam is not saturated in the pre-exposure phase, and Fo implicitly assumes saturated steam, its use in this application is an approximation.

A steam resistometer cycle is shown in figure 2. In this cycle, the time spent with steam in the chamber, above 100°C prior to the exposure phase is three seconds. This corresponds to an accumulated Fo prior to exposure of 0.021 minutes, or the equivalent of 1.3 seconds exposure at 121.1°C. The results for a 134°C cycle in the same machine are 5.2 seconds equivalent 121.1°C exposure in come-up. These are very small amounts and can be neglected in practice, since a 121.1°C biological indicator that complies with ISO 11138-3 has to show a positive result (failed cycle) at no less than 16.5 minutes exposure at 121.1°C.

Resistometer testing tells us the amount of exposure at the actual exposure conditions required to kill the biological indicator or to cause the chemical indicator to show an endpoint response.

Reality

The fact that Fo is a model means that it can only tell you what would happen in the pre-exposure phase if the steam were saturated, if there were no residual air in the load, if there were no condensation, if the load was at the temperature of the chamber temperature sensor, and the Z-value of the indicator were 10, to state but a few limiting factors for this model. It provides a first-order approximation to the actual lethality experienced by the load in pre-exposure, but unfortunately, it is a best case, not a worst case.

In reality, the amount of lethality delivered during the pre-exposure phase is less than the calculation shows. This is because the steam in the pre-exposure phase is not saturated. It therefore delivers less energy to the load, and less energy results in less lethality. Nonetheless, the amount of lethality prior to the beginning of the exposure phase is significant, and is the cause of many observations that the indicator "turned" or showed a successful cycle in failed cycles before the cycle went into exposure.

This effect can be measured by testing BI's of known characteristics in the actual (or prototypical) load in the pre-exposure phase without continuing into exposure. This is done by setting an exposure time of zero, or, if the sterilizer controls do not execute a come-up if set for zero exposure time, you can set a minimum exposure time (whatever the controls will allow you to set as a minimum exposure time; optimally one second). In a real cycle, the BI's will likely show a low enumerated count or possibly go into fraction-negative range. A rigorous mathematical treatment of these two regimes will not

be given here, as there are already numerous excellent references reported and summarized in ANSI/AAMI/ISO 11138-1:2006 Annexes C, D, and E for determination of D-value and survival-kill response.

For a well-characterized load, i.e., a typical industrial load, the lethality results from the pre-exposure testing should be reasonably repeatable.

The total exposure then is the lethality accumulated in the pre-exposure phase and in the actual exposure phase.

The question is, why would you do this?

Rationale

In a bioburden methodology, this testing is only needed to select chemical integrators for routine cycle control, not for initial validation or revalidation. The CI's stated value should be the sum of the pre-exposure lethality and the lethality experienced during the exposure phase, if indicators with that stated value are available. Shorter stated values do not provide a loop-closing indication of cycle execution under the same condition as done in the validation, but if that is all that is available, they are the "best available technology" and can give a better sense of security of correct cycle conditions. Variations in steam quality, while not frequent in a well-controlled sterilization system, can and do happen, and are not apparent from normal parametric cycle records.

In an overkill methodology, a validation in which real BI's were shown to be inactivated to a 10^{0} to 10^{-1} SAL and the "half-cycle" exposure phase that did that work doubled, may not be really providing a full 10^{-6} SAL. To be able to state that the validated SAL has been achieved, it is essential that the "half cycle" that is doubled to create a full cycle include the contribution of the pre-exposure phase. The lethality of the half cycle is the sum of the pre-exposure and exposure lethality. The second half of the exposure phase in the full cycle should contribute just as much lethality as the first "half". Since the load has already had all residual air removed and is at temperature, the second half cycle in the full cycle is essentially the same as a resistometer cycle; it starts at exposure temperature and pressure. The required lethality, or total Fo, in this phase can be calculated as equal to the Fo of the pre-exposure phase plus the first half as validated. This is the equivalent time at the actual exposure temperature as calculated using the Fo equation using the nominal exposure setpoint as the temperature setpoint. This value will inevitably be greater than the first half of the exposure phase. By how much depends upon the detail of the products, their primary packaging, and the details of the pre-exposure phase.

Conclusion

The use of biological indicators is an effective means of validation and verification of sterilization cycles. The interpretation of those validation results requires modeling of the effects of all portions of the cycle that contribute to lethality, and not just assumption that the half cycle as validated includes only the first half of the exposure period. The safety factor of the second half of the exposure phase may be less than is expected and may call

into question an assumed 10^{-6} SAL. For properly-validated industrial processes, this is not expected to be an issue. In hospital sterilizers, this may be an issue that decreases the assumed margin of safety.

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Annex

Figure 1: steam cycle, fractionated

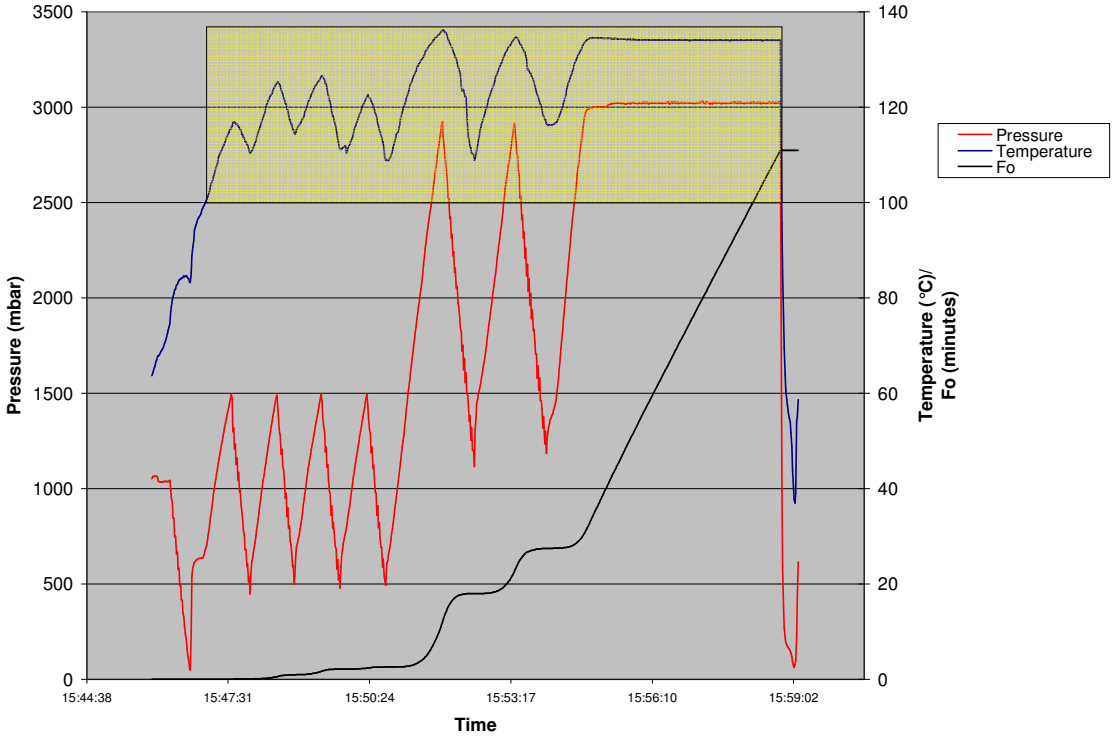
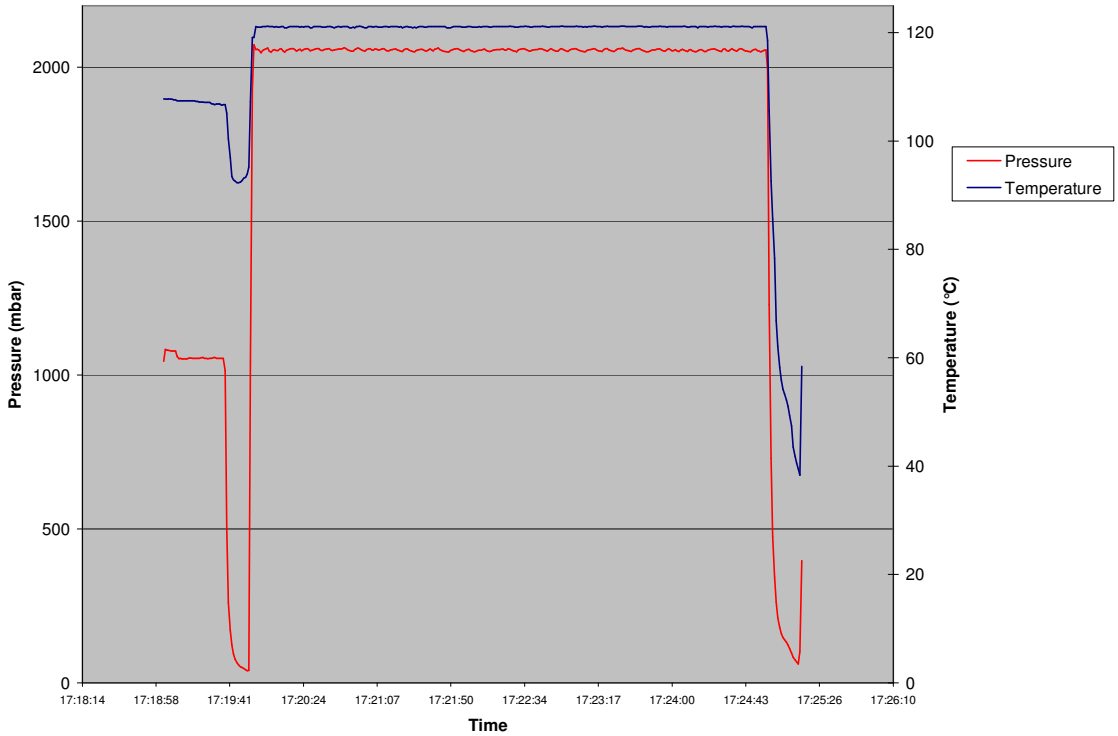


Figure 2: Steam Resistometer Cycle



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Figures 1 and 2 recorded on an H & W Technology ILS-20E process emulator resistometer.

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