Dead or alive

Thermal and biological inactivation both are the important aspect for successful moist heat sterilization studies in pharmaceutical and medical device business. Firms invest lots of quality time for investigating failure in biological inactivation during initial or periodic qualification. Inspectors are always curious to discuss these failures during regulatory inspection. This article will help you to understand statistical interpretation of microbial lethality although microbes do not really understand the mathematics.

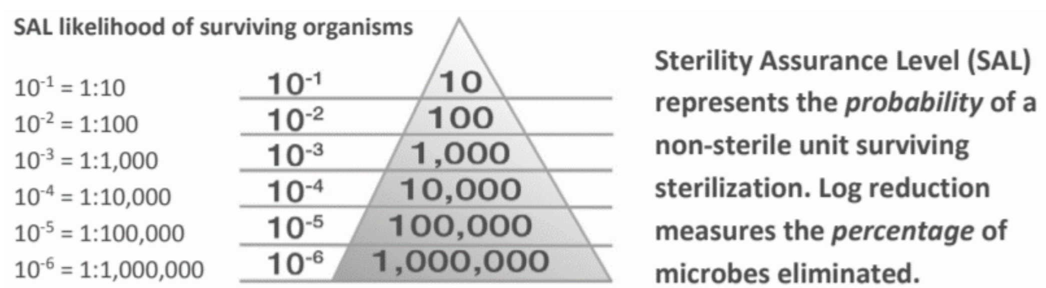
ISO 11135-1 states that complete kill must be obtained in the half-cycle. Those who are not familiar with BIs may think that complete kill is obtained within 6 SLRs. The logic being that the "overkill method" is centered around achieving 12 SLRs, which is equivalent to a Sterility Assurance Level (SAL) of 10^{-6}; therefore, half of 12 is 6. So, it is automatically assumed that in 6 SLRs (the half-cycle) all spores are killed, but this is NOT true.

Complete kill occurs at 8 SLRs

Theoretically, for a BI with a 1 x 10^{6} spore population (at 6 SLRs of the population), there would be one surviving spore per BI. However, there is not going to be exactly one spore per BI—some are going to contain one while others may contain zero, two or three spores. Statistically, this works out to 63% of the BIs being positive.

- At the log of the population + 1 (or 7 SLRs), statistically 10% of the exposed BIs would be positive.
- At the log of the population + 2 (or 8 SLRs), statistically 1% of the exposed BIs would be positive.

If the target is to have complete kill at the half-cycle, you should expose the units until the time point at which 8 SLR is achieved. Exposing the BIs to this time period (8 SLR) is approximate because you do not know at which time point prior to the cycle time the BIs were actually killed. For this reason, we suggest you run a cycle in which there is fractional kill and then slowly increase the cycle until complete kill is achieved. However, total kill at 8 SLR is sufficient if the legwork in developing a cycle has been completed for which the internal or embedded BI is killed in the half-cycle.



To assess the effectiveness of any sterilization process, scientists use a unit of measure called sterility assurance level, or SAL.

Log reduction does not equal SAL

While logarithmic calculations are used to express the above reduction of probability, it's important to understand that SAL is not the same measurement as log reduction. While SAL measures the probability of organisms surviving the sterilization process, log reduction measurements show the amount or percentage of live microbes eliminated after sterilization.

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Sterility Assurance Level (SAL)	Log reduction
For example, if a sterilization method has an SAL of 10^{-3}, which means there is a 1 in 1,000 chance of an organism surviving the sterilization process.	For example, a 3-log reduction means that the number of microbes has been lowered by 10^{-3}, or 1,000-fold. So, if a surface begins with 1,000 microbes on it, a 3-log reduction would reduce the number of microorganisms to 1

Spore log reduction (SLR)

Total kill of all BI's will appear approximately within an 8-log reduction, which is approximately at the theoretical kill time.

Equation	F_{bio} (Minute)	Average of viable spore that remain in each BI	Percentage of expose BI that will be grow positive*	Spore Log reduction (SLR)	SAL
(log 1.0 x 10^6– 2) x 2.0 min	8.0	100	99.999999%	4 SLR (This is the calculated survival time listed on the COA.)	-
(log 1.0 x 10^6 – 1) x 2.0 min	10.0	10	99.9%	5 SLR	-
(log 1.0 x 10^6 – 0) x 2.0 min	12.0	1	63%	6 SLR	-
(log 1.0 x 10^6 + 1) x 2.0 min	14.0	0.1	10%	7 SLR	10^{-1}
(log 1.0 x 10^6 + 2) x 2.0 min	16.0	0.01	1%	8 SLR (This is the theoretical kill time, which is close to the empirical kill time or where we first see total kill of all BIs in Biological Indicator Evaluator Resistometer vessel or F0 kill time.)	10^{-2}
(log 1.0 x 10^6 + 3) x 2.0 min	18.0	0.001	0.1%	9 SLR	10^{-3}
(log 1.0 x 10^6 + 4) x 2.0 min	20.0	0.0001	0.01%	10 SLR (This is the calculated kill time listed on the COA.)	10^{-4}
(log 1.0 x 10^6 + 5) x 2.0 min	22.0	0.00001	0.001%	11 SLR	10^{-5}
(log 1.0 x 10^6 + 6) x 2.0 min	24.0	0.000001	0.0001%	12 SLR (this is the sterility assurance level or SAL or 10^{-6})	10^{-6}

*Percentages calculated using the Halverson-Ziegler equation: MPN = In (n/r).

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CASE STUDY

Calculating Biological F_o (or F_{bio}) using biological indicator (BI) : *Geobacillus Stearothermophillus:*

Details of the Biological Indicator:		
Type of Indicator.	:	BI Ampoule
Biological indicator	:	*Geobacillus Stearothermophillus*
Spore Population.	:	2.4×10^6
D_{121} Value (Minutes)	:	1.9 minutes
Spore Enumeration (In house)	:	2.367×10^6
Survival time	:	(log 2.4×10^6– 2) x 1.9 min = 8.3 min
Kill time	:	(log 2.4×10^6 + 4) x 1.9 min = 19.7 min

The biological F_0 value for the specific biological indicator can be calculated as follows,

F_{bio} = D121 (log A - log B)
Where,
D_{121} = D value of the biological indicator at 121°C.
A = Biological indicator concentration or spore population.
B = Desired level of non-sterility (10^{-6}).

F_{bio} = D121 (log A - log B)
= 1.9 (log (2.367×10^6)- log (10^{-6}))
= 1.9 (6.3741-(-6))
= 1.9 X 12.3741 min.
= 23.5 min.

The Biological F_0 (or F_{bio}) value to achieve a 12-log reduction required minimum 23.5 minutes exposure temperature. If sterilization cycles contain minimum F_0 value ≥23.5 minutes, therefore we can claim 12 log reduction or over kill.

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