Factors and Principles

The Science of Sterilization

Donna Swenson

How well a product is sterilized by a specific sterilization process depends on many factors. A thorough understanding of the scientific principles underlying sterilization is necessary to understand the importance of each step in the cleaning, disinfection, and/or sterilization process. This article describes these factors and principles, which include microbial growth and bioburden, resistance, and lethality; sterility assurance level; spore log reduction; probability of a nonsterile unit; and lethality values.

In a blog I subscribe to, a member recently wanted to know at what point during a vacuum steam sterilization cycle the 12-spore log reduction actually occurs, in order to achieve a 10^{-6} sterility assurance level (SAL). One would assume the answer is that a 12-spore log reduction would take 12 times as long as it would take to achieve a one-spore log reduction. However, this is incorrect. To understand what a spore log reduction is, one needs a basic understanding of logarithms and several scientific concepts upon which all sterilization processes are based. The following descriptions use steam sterilization as an example, but these concepts can be applied to any sterilization process.

A wide variety of factors influence the rate at which microorganisms grow. In the presence of adverse conditions, bacteria may not be able to grow at all. Nutrients and water are necessary for all life to grow. Different types of microorganisms require different nutrients to survive, and without moisture, microorganisms will either die or enter a protective state (for example, by forming bacterial endospores and protozoan cysts). Under ideal conditions, microbial cells can grow at very rapid rates. The time it takes for a cell to reproduce, its generation time, varies between species—from as little as 10 minutes to 24 hours or more. A bacterium that can reproduce once every 20 minutes will produce more than two million cells in just seven hours (Table 1).

Time (min)	Number of divisions	Number of bacteria
0	0	1
20	1	2
40	2	4
60	3	8
80	4	16
100	5	32
120	6	64
140	7	128
160	8	256
180	9	512
200	10	1,024
220	11	2,048
240	12	4,096
260	13	8,192
280	14	16,384
300	15	32,768
320	16	65,536
340	17	131,072
360	18	262,144
380	19	524,288
400	20	1,048,576
420	21	2,097,152

Table 1. The exponential growth of a bacterium that can reproduce once every 20 minutes: In two hours, there will be 64 bacteria cells, and in seven hours, more than two million bacteria.

About the Author

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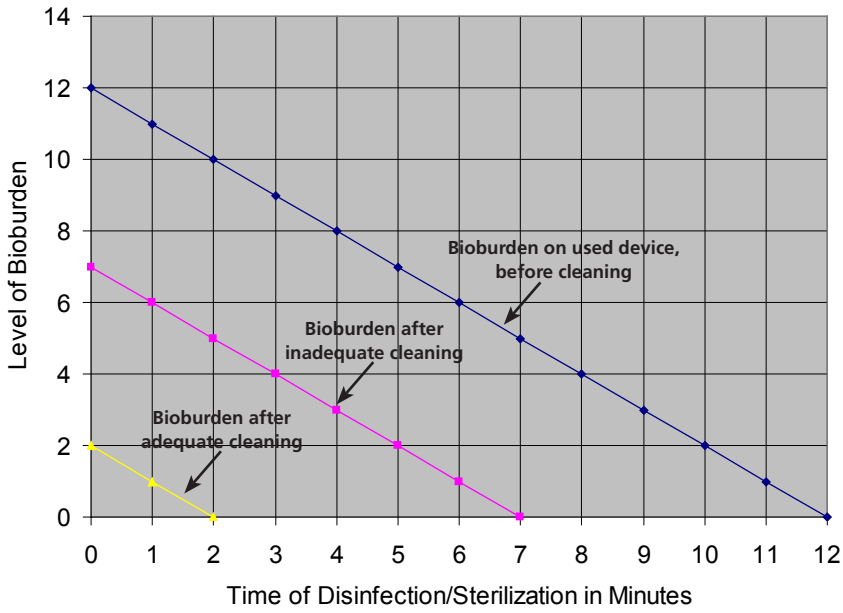

Figure 1. Chart of length of sterilization time vs. logarithmic bioburden on a used medical device before cleaning, after inadequate cleaning, and after adequate cleaning.

The total number of viable microorganisms present on a device is known as bioburden, and if this is too high, a disinfection or sterilization process will not be effective. Initial cleaning processes are therefore designed to remove bioburden and microbial load, after which disinfection and sterilization processes are designed to inhibit or kill any microorganisms remaining on the device. If a sufficient number of microorganisms are not removed during the cleaning process, the disinfection or sterilization process will fail.

Figure 1 shows how disinfection and sterilization processes are capable of inhibiting or killing a specific number of microorganisms per unit of time. From the graph, it can be seen that before the device is cleaned (data in blue) the amount of the bioburden present means that 12 minutes of exposure to a sterilization process is required in order to sterilize the device.

Microorganisms differ greatly in their ability to resist destruction by physical or chemical means.

After acceptable cleaning (data in yellow), bioburden has been reduced such that only two minutes of exposure to the specified sterilization process is needed to sterilize the device. Note that four minutes of the sterilization process is sufficient to sterilize the well-cleaned device, but not the device that has been inadequately cleaned (data in pink), as the bioburden in the latter is too high for the specified sterilization process to be effective.

Microorganisms differ greatly in their ability to resist destruction by physical or chemical means. Vegetative bacteria are generally considered the least resistant to destruction, and bacterial endospores are the most resistant to destruction. Some cellular structures, such as mycobacterium's slime layer, provide some protection to the cell.

Bacterial spores are resistant to extremely high or low temperatures, extremely low humidity, and most chemicals; and can only be destroyed by sterilization processes. Vegetative bacteria, viruses, fungi, and mycobacteria can usually be destroyed by chemical or physical disinfection methods. Depending on the resistance capabilities of the particular organism, efficacy of specific chemicals will vary.

There are a number of physical and chemical means to kill microbes. The most common physical sterilization methods include moist heat, dry heat, and radiation. The most common chemical methods include ethylene oxide, hydrogen peroxide, ozone, and peracetic acid. Irrespective of method, microbial death is measurable as a logarithmic function.

A logarithm is the factor by which a fixed number, the base, must be raised to produce that number. For example, the logarithm of 10,000 to base 10 is four, i.e., 10x10x10x10 (in other words, the number of times the base needs to be multiplied by itself to produce the number). A logarithmic function follows a straight line curve. Microbial death, or sterility assurance, uses a logarithmic base of 10. This means that application of a process for x number of minutes will produce a one-log reduction in the number of microorganisms present, or a 90% reduction in the population.

SAL is a microbiological term used to describe the "probability of a single viable microorganism occurring on an item after sterilization." The term SAL takes a quantitative value, generally 10^{-6} or 10^{-3}. When applying this quantitative value to assurance of sterility, a SAL of 10-6 has a lower value but provides a greater assurance of sterility than a SAL of 10^{-3}, according to ANSI/AAMI/ISO TIR11139:2006 *Sterilization of healthcare products.*

The probability of a nonsterile unit (PNSU) is the probability that viable microorganisms are present on a product after sterilization. This

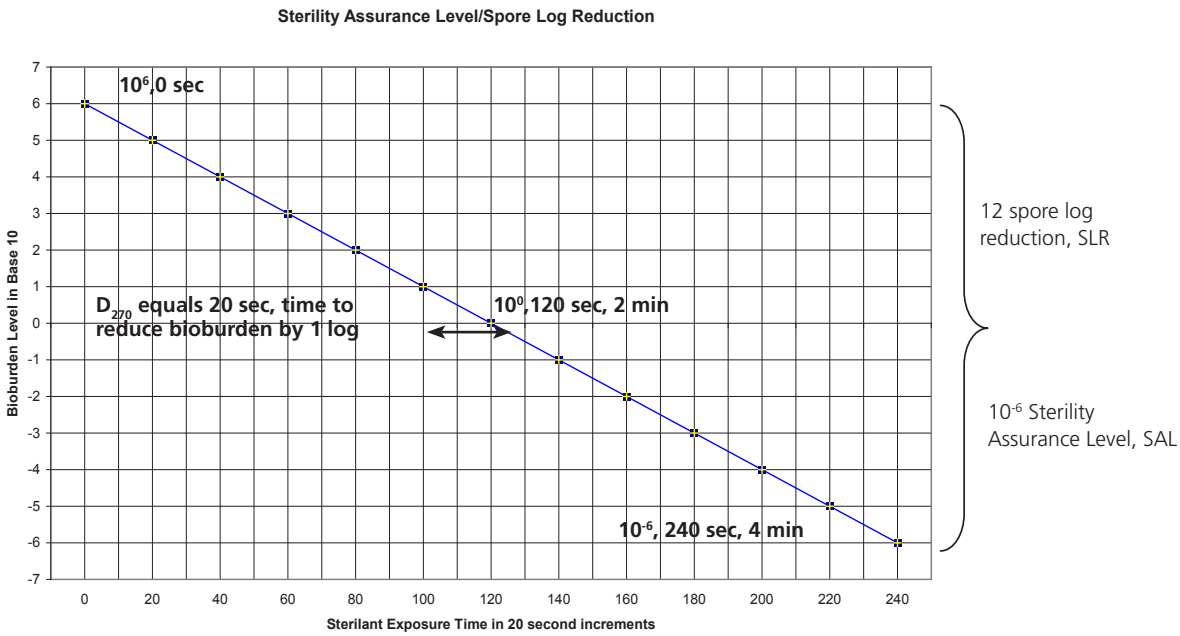

Figure 2. Sterility Assurance Level and Spore Log Reduction

could be one or many microorganisms. A sterilization process with a SAL of 10^{-6} means that there is a probability that one-in-a-million products sterilized by that process will not be sterile. This is the most common SAL used for the sterilization of invasive medical devices.

It is impossible to prove that all microorganisms have been destroyed by a given sterilization process. Whether a device is single-use or reusable, the bioburden present on the device must be reduced prior to the sterilization process to a level that the sterilization process is capable of killing, within the parameters being used by that process. As we have seen, microorganisms killed by disinfection and sterilization processes are measured logarithmically. This means that a 12-spore log reduction is needed to reduce a population of one million organisms to a SAL of 10^{-6}.

Spore log reduction is the log of the initial number of spores, minus the log of the spores surviving the sterilization treatment. Therefore, a microbial population of one million bacteria reduced to 100,000 bacteria would be reduced by one log, or 90%. A six-spore log reduction is needed to reduce one million bacteria to one. To then reduce the microbial population to a 10-6 SAL requires an additional six- or a 12-spore log reduction (Table 2).

In microbiology, the time needed for a particular processing condition to reduce a microbial population by 90% (one log) is known as decimal reduction value (D-value or D). Biological indicators containing spores resistant to a particular sterilization process are used to test that process. For example, *Geobacillus stearothermophilus* is used to test the steam sterilization process because this organism is a heat loving bacterium. D-value can be used to measure a sterilant's efficiency at reducing the specific microorganisms present on a biological indicator.

Typically, the sterilization condition being measured is given as a subscript when noting the D-value for a particular organism, e.g. D270°F (132°C) = 20 seconds. Usually, each lot

For a sterilization process to be effective, the following three conditions must be present:

- Conditions lethal to microorganisms
- Sufficiently low bioburden on device
- Adequate contact of sterilant with all surfaces of the device

If any of these three conditions are not met, then sterilization will not occur.

Number of microorganisms	Log of microbial population	Spore log reduction (from original)
1,000,000	106	0
100,000	105	1
10,000	104	2
1,000	103	3
100	102	4
10	101	5
1	100	6
0.1	10-1	7
0.01	10-2	8
0.001	10-3	9
0.0001	10-4	10
0.00001	10-5	11
0.000001	10-6	12

Table 2. Sterility Assurance Level and Spore Log Reduction

of a biological indicator is given a distinctive D-value. The D-value of *Geobacillus stearothermophilus* is about two minutes at 250°F (121°C) and about 20 seconds at 270°F (132°C).

Therefore, after exposure to moist heat at 270°F (132°C) for two minutes, it is possible that one organism of *G. stearothermophilus* out of one million on a biological indicator could still be alive. These recommendations are minimum acceptable parameters. Depending on factors outside the manufacturer's control, such as packaging, loading configuration, and cleaning, these recommendations may not be adequate (Figure 2).

The question posed in the blog was: At what point during a vacuum steam sterilization cycle does the 12-spore log reduction actually occur in order to achieve a 10^{-6} SAL? Using the saturated steam sterilization process, and if the reference microorganism is *G. stearothermophilus* (with a D value of D270 = 20 seconds), the answer is that it will take four minutes to achieve a 12-spore log reduction, if one million bacteria were present at the beginning of the sterilization process, and if ideal conditions for sterilization are present.

This method uses a biological indicator to test the steam sterilization process. If a different indicator organism is used, or if the sterilization conditions change, time to achieve this spore log reduction will also change. There are many variables, the most important of which include adequate cleaning, level of bioburden present, microbial resistance, microbial lethality values, device configuration, sterilization temperature, steam quality, loading configurations, and time needed for steam to penetrate all surfaces. The correct answer to the question is therefore, "It depends!" ■

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